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The Influence of Minimal Cultivation Techniques on Growth Rate of *Robinia pseudacacia* L. Seedlings

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Abstract: The seed provenance, the type of substrate and its properties, as well as the watering regime in the first years after sowing are among the most important characteristics affecting the production of containerized seedlings. The objective of this study was to analyse the growth of black locust (*Robinia pseudacacia* L.) seedlings on different types of substrate (six mineral and two organic) with different textures and pHs under three different regimes of available water content (no-limiting, medium drought, severe drought) over a period of five months (May–October 2023), using seeds from three sources located in the southeast, south, and west of Romania. The highest seedling emergence rate (73.7%) was obtained for the medium seeds with 18.492 g weight (1000 seed weighs) (Bucharest provenance). Direct sowing in the field and containerized seedlings both showed a trend of growing in height during unlimited water and of growing in diameter during low watering. The lowest survival rate of seedlings (86.7%) occurred in the mineral substrate in the case of rendzina with additional sand (pH 8.70), and for the organic substrate, the lowest survival rate (87.0%) occurred for the peat MKS 3 substrate (pH 4.54).

Keywords: seed provenance; organic substrate; mineral substrate; available water content; severe drought; greenhouse conditions; field conditions

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1. Introduction

The black locust (*Robinia pseudacacia* L.) is the most widely used exotic species in Romanian afforestation works, occupying approximately 5% of the forested area (250,000 ha) [1,2]. Its cultivation began in the second half of the 19th century due to its ease of cultivation in the nursery and its rapid growth as an ornamental tree and later for afforestation [3–7]. In Romania, the first seedlings were grown in Băilești (Dolj County), so that they could later be planted on a large scale on degraded and eroded lands [8].

Despite all of the failures recorded in many cases, black locust remains a fast-growing forest species of great economic importance. Black locust produces valuable wood, suitable for multiple purposes, being one of the species frequently used in degraded land afforestation projects [9]. Its wood is heavy, hard, elastic and resistant to humidity variations, superior to oak in terms of mechanical properties. Due to its rapid growth, which is determined by favourable conditions in Romania, black locust has significance as a forest crop.

The ability to produce an adequate number of quality seedlings is therefore a great challenge. It is essential to use the most suitable techniques and materials for obtaining a large number of seedlings in a short period of time. The production of containerized seedlings in sheltered spaces (greenhouses) is practiced more and more in the current context of climate change for many species of deciduous and coniferous trees, due to the following advantages: avoiding mechanical and physiological damage, planting seedlings earlier, a high degree of survival after planting, and growth rate at a more sustained pace, etc. [10]. The growth rate specific for sheltered spaces was compared with seedlings produced in natural conditions [11–17].

While black locust is not conventionally sowed in containers, research was initiated on this species due to the numerous benefits associated with producing containerized seedlings [18–21]. An important factor for the production of high quality containerized black locust seedlings is the use of the appropriate size of containers [22].

Substrates have been the subject of several studies over time for coniferous and deciduous species [23–30]. The influence of the substrate on the growth of containerized black locust seedlings was addressed in several studies. The most used substrate for seedling production is peat, used as such or in different mixtures. Enriched peat (pH 6) or stabilized environment peat (pH 4) [20,31] is used with perlite and vermiculite. Black locust survives for a while in extreme conditions due to its great vitality, being affected only after the complete exhaustion of the soil. Therefore, coarse, sandy, airy, permeable, neutral or slightly acidic soils are favourable for growing black locust seedlings, while clayey, compact, battered, carbonate-rich soils are contraindicated for its cultivation [32].

Evaluating the provenance of seeds is a useful way to improve the nitrogen-fixing capacity and therefore the growth rate of black locust [33]. The seed's provenance, genotype and the heterogeneity of the environment of origin potentially influence the seed germination capacity [34] and the quality of containerized seedlings [35]. The genetic correlation coefficient highlights that the propagation of black locust could become extremely efficient through appropriate selection [21]. The importance of seed provenance to obtain high-quality *R. pseudacacia* L. containerized seedlings was analysed using seeds from two sources (Greek and Hungarian provenance) [20]. Previously, it was demonstrated that Cyprus seeds had higher survival rates compared to those collected from Greece, the seedling characteristics also being different [19].

Climate seasonality is one of the most important environmental variables that can determine tree growth fluctuations [36], an aspect less studied in the case of seedlings. In the case of soil water limitations, the treatments are differentiated by the amount of water [37,38] or by different irrigation time intervals [39,40]. Plant trait adjustments to balance water supply with water demand are essential for maintaining the survival of stands under water stress conditions [41,42]. Another way of adaptation of black locust to water stress is directly related to the plasticity of their leaf morphology, size [43] and phototropism [44], the leaves being smaller in plants that are subjected to water stress for a longer period of time [37]. A direct influence of water stress, apart from on leaf morphology, can also be observed on seed germination and root anatomy. The final seed germination percentage decreases with increasing water deficit conditions and the seedlings' roots are thinner [45]. The degree of defoliation affects the amount of black locust biomass, but not its physiology [46]. The stress caused by dry periods around values close to the withering point does not particularly affect the functionality of the plants, but rather affects the amount of biomass and their growth [37].

The growth capacity and tolerance of European black locust seedlings to drought was analysed in 2011 in a large experiment [47] that entailed the collection of seeds from 45 descendants from 7 countries (Bulgaria, Germany, Greece, Hungary, Italy, Romania and Slovakia).

The degree of survival of black locust seedlings in different conditions of water stress was also analysed [48], finding a weaker survival of the black locust in conditions of lower humidity. Due to the decrease in soil water availability during the progressive drought that occurs over the course of a day [49], the black locust seedlings also suffer different physiological changes at the level of transpiration (T) and xylem water potential. Maintaining adequate soil moisture and the administration of phosphorus-based fertilizers in suitable quantities can significantly improve the photosynthetic capacity of black locust seedlings and the efficiency of their resource use, and their photosynthetic capacity is therefore being maximized by the administration of phosphorus-based fertilizers in moderate drought conditions (at values of water retention capacity in the field around 55%–60%) [50]. The allocation mechanisms of non-structural carbohydrates in *R. pseudacacia* L. seedlings under different levels of drought [51], and the degree of adaptability of black locust to different levels of humidity [50,52–58], are very important in the current context of climate change because it is expected that many regions of the world will face increased desertification and this is one of the species frequently used in degraded land afforestation projects.

Considering all of aspects mentioned above, the purpose of this study was to compare the growth rate of black locust seedlings produced in natural conditions and sheltered spaces in order to establish minimal technologies to obtain a viable planting material for use in future afforestation or other greening projects. The research aim was to consider the practice of sowing in greenhouses, knowing that black locust is conventionally sowed directly in restoration sites or in the open field (natural conditions) of nurseries.

2. Materials and Methods

2.1. Study Site, Seed Provenance and Plant Material

This study was conducted in four sites: Sânpetru (Brașov) was the site for the greenhouse experiment, and other three sites for the field observations regarding the growth of seedlings in natural conditions are shown in Figure 1.

Figure 1. Geographical origin of *Robinia pseudacacia* L. seeds and location of the field determinations: O.S. Săcueni (North-West of Romania); O.S. Bucharest (South of Romania); O.S. Hanu Conachi (South-East of Romania) and the location of greenhouse Sânpetru-Brașov (Central).

The seeds were harvested during the end of October and the beginning of November 2022, for the Romanian seeds: O.S. Săcueni (Bihor County), O.S. Bucharest (Ilfov County), and O.S. Hanu Conachi (Galați County) (Table 1). All three seed sources are mentioned by the National Catalogue of Forest Genetic Resources [59].

In the greenhouse, the temperature was measured with the help of special sensors for temperature monitoring. Therefore, the average temperatures recorded during the seedlings growth were: 18.99 °C (May), 21.71 °C (June), 23.99 °C (July), 23.21 °C (August), 18.94 °C (September), and 14.0 °C (October). Also, a suspended shading system was installed above the plants to avoid overheating.

Table 1. Administrative, geographic, and climatic conditions of local seed sources and field conditions for conventional sowing.

Administrative Location	CODE	Latitude Longitude	Altitude (m a.s.l.)	Average	
				Temperature Min.–Max. (Mean) (°C)	Rainfall (mm)
O.S. * Săcueni (Curtuișeni), Bihor County	A1	47°32′ N 22°10′ E	140	5.7–15.9 (10.7)	617.6
O.S. * Bucharest (Râioasa), Ilfov County	A2	44°33′ N 25°54′ E	110	5.0–16.6 (10.9)	585.0
O.S. * Hanu Conachi, Galați County	A3	45°34′ N 27°35′ E	15	5.9–15.5 (11.6)	421.4

* O.S.—Romanian acronym for administrative delineation of a forest district.

The harvested seeds were stored in the refrigerator (at approximately 4 °C), in paper bags until spring. In April 2023, before sowing, the main qualitative indices of the seeds were determined in the laboratory (seed purity, weight of 1000 seeds, germination capacity, and number of seeds per kg) (Table 2) [60].

Table 2. The qualitative indices of the seeds used for obtaining black locust seedlings.

Provenance	Purity (%)	1000 Seed Weighs (g)	Germination (%)	Number of Seeds per Kilogram
O.S. Săcueni	98.46	20.471	85	48,800
O.S. Bucharest	97.76	18.492	87	54,100
O.S. Hanu Conachi	98.09	18.151	84	55,100

Because black locust seeds display exogenous dormancy due to the structure of the seed coat, the germination was stimulated by mechanical scarification before sowing, and the permeabilization of the integument was performed manually, with the help of a scalpel [61].

Sowing was carried out on the 10th of May (the seedling emergence rate (%) according with the Supplementary Materials) was conducted with, in all eight types of substrate, in HIKO V−250 type deciduous containers. Each container was 352 × 216 × 160 mm in size and with 18 growth cells. The volumetric capacity of a growth cell is 250 cm^3.

Three seeds were sown in each cell, according to the suggested sowing rates for seedling production in containers [62]. The sprouting of the seedlings was monitored for three weeks periodically, and starting on the 10th of June (one month after sowing), only one seedling (the most vigorous) was left in each container cell (also, the first measurement of the heights of all of the plants was performed). The second height measurement was carried out after one month (on the 10th of July), which coincided with the beginning of the differentiation of the watering regimes, according to the three established levels (low, medium, and high watering). Daily or every two days, depending on the need, the necessary amount of water was administered for each individual treatment with the help of a sprinkler. The differentiated watering was performed for a period of three months. In October, at the beginning of the vegetative rest, the last measurement of the seedlings (height and diameter) was conducted.

In parallel, the same sourced seeds were sown in the field conditions located in O.S. Săcueni (Bihor County), O.S. Bucharest (Ilfov County), and O.S. Hanu Conachi (Galați County) in the randomized blocks. In the field conditions, the seedlings' height and base diameter were measured in 54 individuals according to the 3 × 54 model (3 sites with natural conditions). In case of the field plantations, the base diameter and the height of the plants at the end of the growing season (October) were measured. These three field plantations are installed in the natural conditions of the sources of the seeds (without water regime differentiation). These results were used to compare the growth of black locust containerized seedlings.

2.2. Substrate

The physical and chemical characteristics of the soil for sowing in natural conditions are presented in Table 3.

Table 3. The characteristics of soils used for obtaining conventionally sown black locust seedlings in natural conditions as a field practice.

Indices \ Sites		O.S. Săcueni	O.S. Bucharest	O.S. Hanu Conachi
Texture		Sandy loam	Silty clayey loam	Sandy loam
Coarse sand (%)		44.346	4.417	7.677
Fine sand (%)		41.054	29.283	55.432
Silt I (0.02–0.01 mm) (%)		1.4	10.8	0.2
Silt II (0.01–0.002 mm) (%)		3.7	21.6	21.2
Clay (%)		9.5	33.9	15.5
pH		5.21	7.02	7.67
Organic matter content (% H)		3.36	2.59	3.52
Carbon content (% C)		1.95	1.50	2.04
Total nitrogen content (% N)		0.05	0.05	0.09
C/N ratio		40.54	27.90	23.93
Bases exchange capacity		8.0	28.8	90.8
Hydrogen exchange capacity	(me/100 g)	4.0	3.6	0.4
Total cationic exchange capacity		12.0	32.4	91.2
Base saturation degree (% V)		66.67	88.89	99.56

The greenhouse experiment included monitoring the influence of two organic substrates and six mineral substrates. The organic substrate consisted of: peat MKS 1 (namely B1) (pH 6, fine structure, 65–140 N%, 75–170 P_2O_5%, 120–270 K_2O%, 0.5–0.9 EC%) and peat MKS 3 (namely B2) (pH 4.54, medium structure, 65–140 N%, 75–170 P_2O_5%, 120–270 K_2O%, 0.5–0.9 EC), both of which are of commercial origin and were used in a mixture with 10% perlite.

The forest soils (rendzina, dystric cambisol and eutric cambisol) were collected from the Brașov area, from the top 50 cm. Their mixture with sand was in a proportion of 1:2. Table 4 presents the main characteristics of the mineral substrates determined according to the national and international standards (using three replicates for each substrate type) in the Soil Science Laboratory from the Faculty of Silviculture. The soil pH was determined using the potentiometric method in a suspension solution of water and soil [63]. For the determination of soil organic matter and carbon content, the modified Walkley–Black method was used [64]. The Kjeldahl method was applied for the total nitrogen content [65]. The bases exchange capacity was determined by treating the soil with a hydrochloric acid solution [66]. The hydrogen exchange capacity was determined by applying the Kappen method and using a potassium acetate solution [66]. The total cationic exchange capacity and the base saturation degree (%V) were computed with the help of the two previous indexes. The granulometric composition was determined by means of sieving and pipetting [67]. In all types of substrate, an equal amount of granulated coated fertilizer (Osmocote) [47] was incorporated with gradual release (for a period of 5 to 6 months), based on NPK (15%/9%/11% + microelements), respecting the recommended dose for normal fertilization (4 g/L). Before starting the experiment and sowing in containers, all of the six types of mineral substrates were sterilized with basamid granules (a nematicide frequently used in nurseries, which in humid conditions decomposes into a sterilizing gas with nematocidal, insecticide, herbicide, and fungicide effects) according to the recommended doses, respecting the time interval between treatments and sowing, namely of 30 days. The Creson test was applied to check the proper ventilation of the soil before sowing. To the other two organic substrates, a superficial layer of perlite was added over the growing medium in order to protect the seeds during the germination period from the sun, reduce the development of algae and fungi, and also allow water to penetrate better into the medium.

Table 4. The characteristics of mineral substrates used for obtaining black locust seedlings within a greenhouse.

Indices	Codes of Mineral Substrates					
	B3 *	B4 *	B5 *	B6 *	B7 *	B8 *
Texture	Silty loam	Sand	Sandy clayey loam	Sand	Sandy loam	Sand
pH	6.91	8.70	5.85	8.84	5.44	8.34
Organic matter content (% H)	3.70	1.22	2.93	2.20	1.48	1.56
Carbon content (% C)	2.15	0.71	1.70	1.28	0.86	0.90
Total nitrogen content (% N)	0.20	0.02	0.17	0.15	0.02	0.15
C/N ratio	10.91	31.71	9.76	8.61	51.63	5.98
Bases exchange capacity (me/100 g)	96.4	32.4	20.0	26.4	11.6	24.4
Hydrogen exchange capacity (me/100 g)	2.4	0.4	6.0	2.0	5.6	0.4
Total cationic exchange capacity (me/100 g)	98.8	32.8	26.0	28.4	17.2	24.8
Base saturation degree (% V)	97.57	98.78	76.92	92.96	67.44	98.39

* B3—Rendzina; B4—Rendzina + sand; B5—Dystric cambisol; B6—Dystric cambisol + sand; B7—Eutric cambisol; B8—Eutric cambisol + sand.

2.3. Substrate Watering Regimes

In the first two months, in the greenhouse, watering was performed uniformly, using a high amount of water (100%). Starting from 10 July 2023, for a period of four months, each of the mineral substrates was subjected to three different watering regimes: high watering (70% of available water capacity—AWC); medium watering (50% of AWC); or low watering (30% of AWC), namely C1, C2, and C3, respectively [38]. The watering regime was established according to the hydro-physical indicators of the substrate. For the soil moisture level, the range of available water content was determined in the case of each type of substrate depending on the substrate texture, field capacity, wilting point and bulk density [68].

In the case of the two organic substrates, several preliminary tests were performed, and the amount of water required for each of the three levels was established according to the dripping point.

Watering was generally conducted manually, by aspersion, daily or every two days, depending on the texture of the substrate and the temperature in the greenhouse, according to the recommendations of Time-Saver Standards for Landscape Architecture [69] for the production of containerized seedlings.

2.4. Experimental Design and Statistical Analysis

In the field conditions, the measurements were conducted for 54 seedlings for each site, resulting in 162 measurements. The seedlings sowed in natural conditions were measured in October. The field samples resulted from direct sowing in the natural conditions of each provenance of the seeds: O.S. Săcueni, O.S. Bucharest, O.S. Hanu Conachi, which are existing forest nurseries.

In the greenhouse, the experiment was applied in 54 replications according to the $3 \times 8 \times 3$ model (3 seed provenances, 8 types of substrates, 3 watering regimes, and 54 seedlings for each treatment–repeats), resulting in 3888 measurements of the seedling growth rate for June, July, and October (Figure 2). The first determination of seedling height was undertaken in June, the second one in July, and the third in October. In October, along with seedling height, the diameter was also determined.

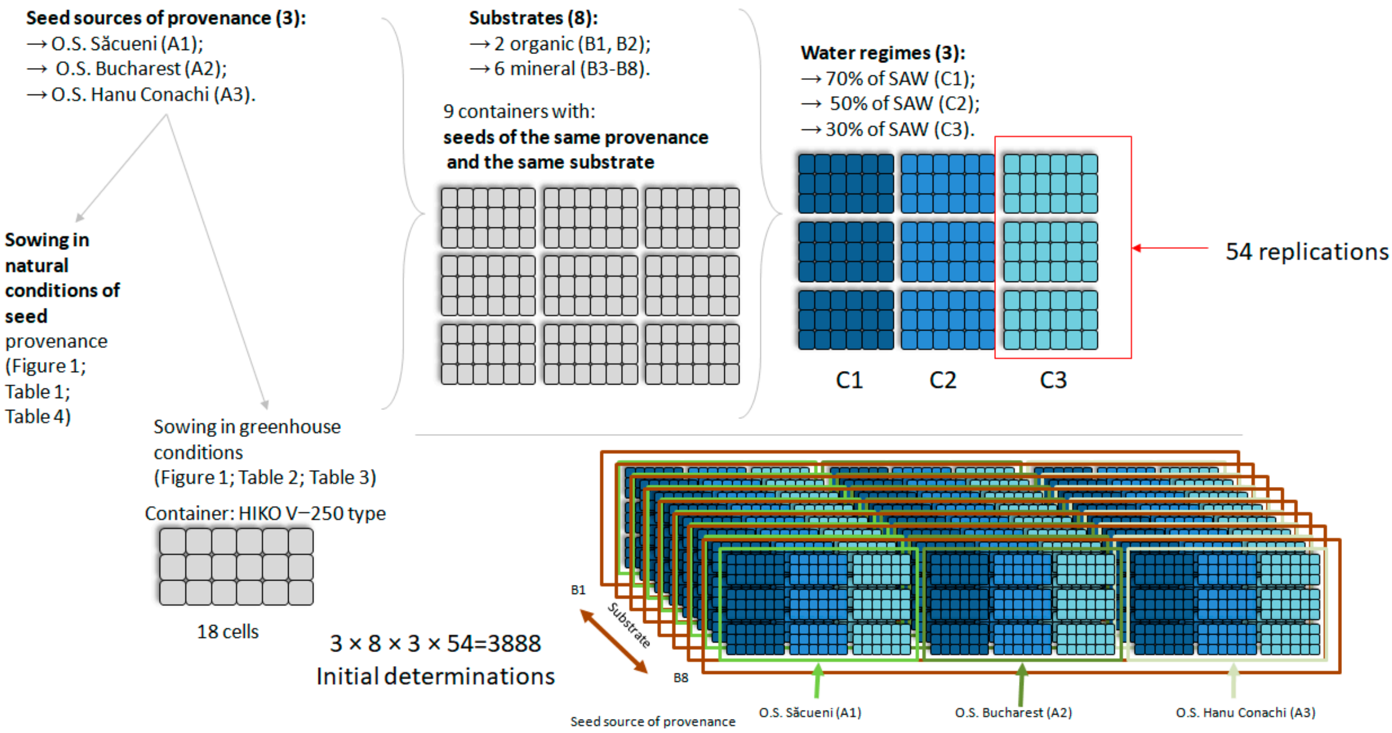

Figure 2. Experimental diagram for black locust seedlings greenhouse trials with a randomized complete block design: the main block is the substrate, second block is the provenance of the seeds; the third block (container) is the water regime; the cells of containers are the replication of the measurements.

The dead seedlings were included in the ANOVA (with 0.00 cm for each replicate). The height and diameter of the seedlings was statistically processed by applying the Duncan test for comparing the mean values (PoliFactLNK software). Different letters denote significant differences (Duncan test, $p < 0.05$).

3. Results

3.1. Plant Growth Characteristics

Data were analysed for the seedlings' height determined before (first and second measurement) and after (third measurement) differentiating the water regime. Thereby, statistical processing was applied using one-way analysis of variance (ANOVA) (Table 5).

Table 5. The analysis of variance for the statistical interpretation of seedlings' height (cm) from the greenhouse trials.

Factors	Degrees of Freedom	*F*-Value No. of Measurement 1st	2nd	3rd
Seed provenance (A)	2	41.908	4.238	34.944
Substrate (B)	7	198.973	60.844	16.747
Interaction A × B	14	32.472	7.908	2.16
Watering regime (C)	2	19.556	4.658	3.486
Interaction A × C	4	18.457	1.079	0.738
Interaction B × C	14	26.765	4.309	1.875
Interaction A × B × C	28	23.664	2.646	0.679

Table 6 presents the mean height values obtained for the seedlings as an interaction effect between provenance and substrate. O.S. Săcueni, O.S. Bucharest, and O.S. Hanu Conachi seedlings in peat MKS 3 were shorter than in peat MKS 1. O.S. Săcueni and O.S. Hanu Conachi seedlings were the longest in eutric cambisol substrate and the shortest in rendzina with sand (1:2) substrate. O.S. Bucharest seedlings presented the highest height for peat MKS 1 and the lowest for rendzina with sand (1:2) substrate.

Table 6. Effects of interaction between provenance and substrate on height (cm) of greenhouse black locust seedlings for the measurements after differentiating the water regime.

Substrate \ Provenance	O.S. Săcueni	O.S. Bucharest	O.S. Hanu Conachi
Peat MKS 1	46.02	46.76	40.52
Peat MKS 3	40.97	35.97	38.91
Rendzina	45.57	40.25	38.90
Rendzina + sand (1:2)	43.18	33.94 ^{kl}	30.04 ^{l}
Dystric cambisol	50.09 ^{abc}	42.83	39.99
Dystric cambisol + sand (1:2)	51.01 ^{ab}	41.75	39.43
Eutric cambisol	53.72 ^{a}	44.04	46.35
Eutric cambisol + sand (1:2)	45.28	39.31	34.22 ^{jkl}
SD		4.67–5.82	

The means followed by different letters are significantly different according to Duncan's test ($p < 0.05$). Being 12 classes for means differentiation, the letters are presented for the extreme mean values (highest and lowest).

Data presented in Table 7 show the height means as an interaction effect between provenance and water treatments. O.S. Hanu Conachi seedlings showed the highest growth under a moderate watering regime, but the differences recorded compared with the other two treatments are not statistically significant. O.S. Bucharest seedlings presented statistically significant differences between high and low watering regimes. O.S. Săcueni seedlings recorded no statistically significant differences between water treatments.

Table 7. Effects of interaction between provenance and water treatments on height (cm) of greenhouse black locust seedlings.

Substrate \ Provenance	O.S. Săcueni	O.S. Bucharest	O.S. Hanu Conachi
High watering	48.76 ^{a}	42.39 ^{b}	38.59 ^{c}
Medium watering	46.24 ^{a}	40.12 ^{bc}	38.72 ^{c}
Low watering	45.94 ^{a}	39.31 ^{c}	38.33 ^{c}
SD		2.78–3.27	

The means followed by different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 8 shows the height means as an interaction effect between the substrate and water treatments. The seedlings with a low watering regime in dystric cambisol were significantly shorter than in the other two treatments. The longest seedlings in the low watering regime were in eutric cambisol substrate, and the shortest were in rendzina with sand (1:2) substrate. Seedlings in rendzina with sand substrate were the shortest regardless of the water treatment.

Table 8. Effects of interaction between substrate and water treatments on height (cm) of greenhouse black locust seedlings.

Substrate \ Provenance	High Watering	Medium Watering	Low Watering
Peat MKS 1	48.32 ^{ab}	42.10 ^{cdefg}	42.89 ^{cdef}
Peat MKS 3	36.59 ^{hi}	37.07 ^{ghi}	42.19 ^{cdefg}
Rendzina	44.31 ^{bcdef}	39.44 ^{efgh}	40.96 ^{defgh}
Rendzina + sand (1:2)	36.74 ^{hi}	36.14 ^{hi}	34.29 ^{i}
Dystric cambisol	46.68 ^{abc}	46.03 ^{abcd}	40.20 ^{efgh}
Dystric cambisol + sand (1:2)	44.90 ^{abcde}	44.12 ^{abcdef}	43.17 ^{bcdef}
Eutric cambisol	48.75 ^{a}	48.41 ^{ab}	46.96 ^{abc}
Eutric cambisol + sand (1:2)	39.69 ^{efgh}	40.23 ^{efgh}	38.88 ^{fghi}
SD		4.54–5.66	

The means followed by different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 9 shows the mean height of seedlings recorded as effects of seed provenance on growth. For the first (June) and second measurement (July), during a non-differentiated regime of water, the samples of O.S. Bucharest provenance presented the highest vigour for height. For the third measurement (October), after a differentiated regime of water, the best value was recorded for O.S. Săcueni provenance.

Table 9. Effects of seed provenance on height (cm) of greenhouse black locust seedlings.

Seed Provenance	June	July	October
O.S. Săcueni (A1)	4.3 ^{b}	19.34 ^{ab}	46.98 ^{a}
O.S. Bucharest (A2)	4.5 ^{a}	19.66 ^{a}	40.61 ^{b}
O.S. Hanu Conachi (A3)	4.14 ^{c}	18.73 ^{c}	38.55 ^{b}
SD	0.16–0.17	0.65–0.68	2.08–2.19

Within the column, the means followed by different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 10 shows the mean height of seedlings recorded as effects of substrate on growth. Dystric cambisol had the greatest influence at the first measurement (June). In the second measurement (July), the greatest increases were recorded for the treatments with organic substrate (MKS 1). In the third measurement (October), the greatest influence was given by the simple eutric cambisol substrate.

Table 10. Effects of substrate on height (cm) of greenhouse black locust seedlings.

Substrate		June	July	October
Organic	Peat MKS 1 (B1)	4.74 ^{b}	23.93 ^{a}	44.44 ^{b}
	Peat MKS 3 (B2)	3.53 ^{f}	18.58 ^{cd}	38.62 ^{d}
Mineral	Rendzina (B3)	3.68 ^{ef}	14.82 ^{f}	41.57 ^{cb}
	Rendzina + sand (1:2) (B4)	3.94 ^{de}	18.08 ^{d}	35.72 ^{e}
	Dystric cambisol (B5)	5.46 ^{a}	16.97 ^{e}	44.30 ^{b}
	Dystric cambisol + sand (1:2) (B6)	4.75 ^{b}	19.53 ^{c}	44.06 ^{b}
	Eutric cambisol (B7)	4.28 ^{c}	22.71 ^{b}	48.04 ^{a}
	Eutric cambisol + sand (1:2) (B8)	4.15 ^{cd}	19.33 ^{c}	39.60 ^{cd}
SD		0.26–0.30	1.06–1.23	2.70–3.13

Within the column of the means, each month followed by different letters is significantly different according to Duncan's test ($p < 0.05$).

In field growing conditions, the seedlings' height and diameter showed a very good result comparing with the traits of the seedlings sown in a greenhouse. Analysing the technology applied to the containerized seedlings, it was observed that there was a trend of growing in height due to the high watering regime and of growing in diameter due to the low watering treatment (Table 11).

Table 11. Effects of technology on growth characteristics of black locust containerized and field seedlings.

Growing Technology	Height (cm)	Diameter (mm)
Natural conditions		
Direct sowing in field	82.29 ^{a}	6.84 ^{a}
Greenhouse conditions for sowing in containers		
High watering for peat MKS 1	48.38 ^{bc}	3.59 ^{bc}
Medium watering for peat MKS 1	42.10 ^{bcd}	3.19 ^{c}
Low watering for peat MKS 1	42.77 ^{bcd}	3.45 ^{bc}
High watering for peat MKS 3	44.91 ^{bcd}	3.66 ^{bc}
Medium watering for peat MKS 3	42.76 ^{bcd}	4.12 ^{b}
Low watering for peat MKS 3	45.06 ^{bcd}	3.30 ^{c}
High watering for rendzina	44.30 ^{bcd}	3.26 ^{c}
Medium watering for rendzina	39.45 ^{d}	3.08 ^{c}
Low watering for rendzina	40.96 ^{bcd}	3.43 ^{bc}
High watering for rendzina + sand (1:2)	39.69 ^{cd}	3.74 ^{bc}
Medium watering for rendzina + sand (1:2)	43.08 ^{bcd}	3.47 ^{bc}
Low watering for rendzina + sand (1:2)	45.00 ^{bcd}	3.72 ^{bc}
High watering dystric cambisol	44.90 ^{bcd}	3.48 ^{bc}
Medium watering for dystric cambisol	46.03 ^{bcd}	3.37 ^{bc}
Low watering for dystric cambisol	40.09 ^{bcd}	3.73 ^{bc}
High watering for dystric cambisol + sand (1:2)	46.86 ^{bcd}	3.52 ^{bc}
Medium watering for dystric cambisol + sand (1:2)	44.12 ^{bcd}	3.30 ^{c}
Low watering for dystric cambisol + sand (1:2)	43.17 ^{bcd}	3.55 ^{bc}
High watering for eutric cambisol	48.69 ^{b}	3.49 ^{bc}
Medium watering for eutric cambisol	48.39 ^{bc}	3.59 ^{bc}
Low watering for eutric cambisol	47.01 ^{bcd}	3.68 ^{bc}
High watering for eutric cambisol + sand(1:2)	39.69 ^{cd}	3.61 ^{bc}
Medium watering for eutric cambisol + sand (1:2)	40.24 ^{bcd}	3.04 ^{c}
Low watering for eutric cambisol + sand (1:2)	38.88 ^{d}	3.35 ^{c}
SD	7.32–8.94	0.63–0.77

Within the column, the means followed by different letters are significantly different according to Duncan's test ($p < 0.05$).

3.2. Seedling Survival

The seedlings' survival showed significant losses for the variant with the peat MKS 3 (B2) and rendzina + sand substrate (B4) (all of the other six substrates had 100% survival rates). Figure 3 shows the results regarding the loss of seedlings according to provenance and water regime for the two previously mentioned variants regarding the substrate. For each substrate variant, there were initially 486 seedlings (54 repetitions, three provenances, three watering regimes).

The lowest survival rate (87.0%) was determined for the organic substrate (peat MKS 3) with a high watering regime (Figure 3a). Regardless of the provenance, the watering regime of 70% in the rendzina + sand substrate had a survival rate around 92%. The seedlings from O.S. Bucharest recorded the highest losses.

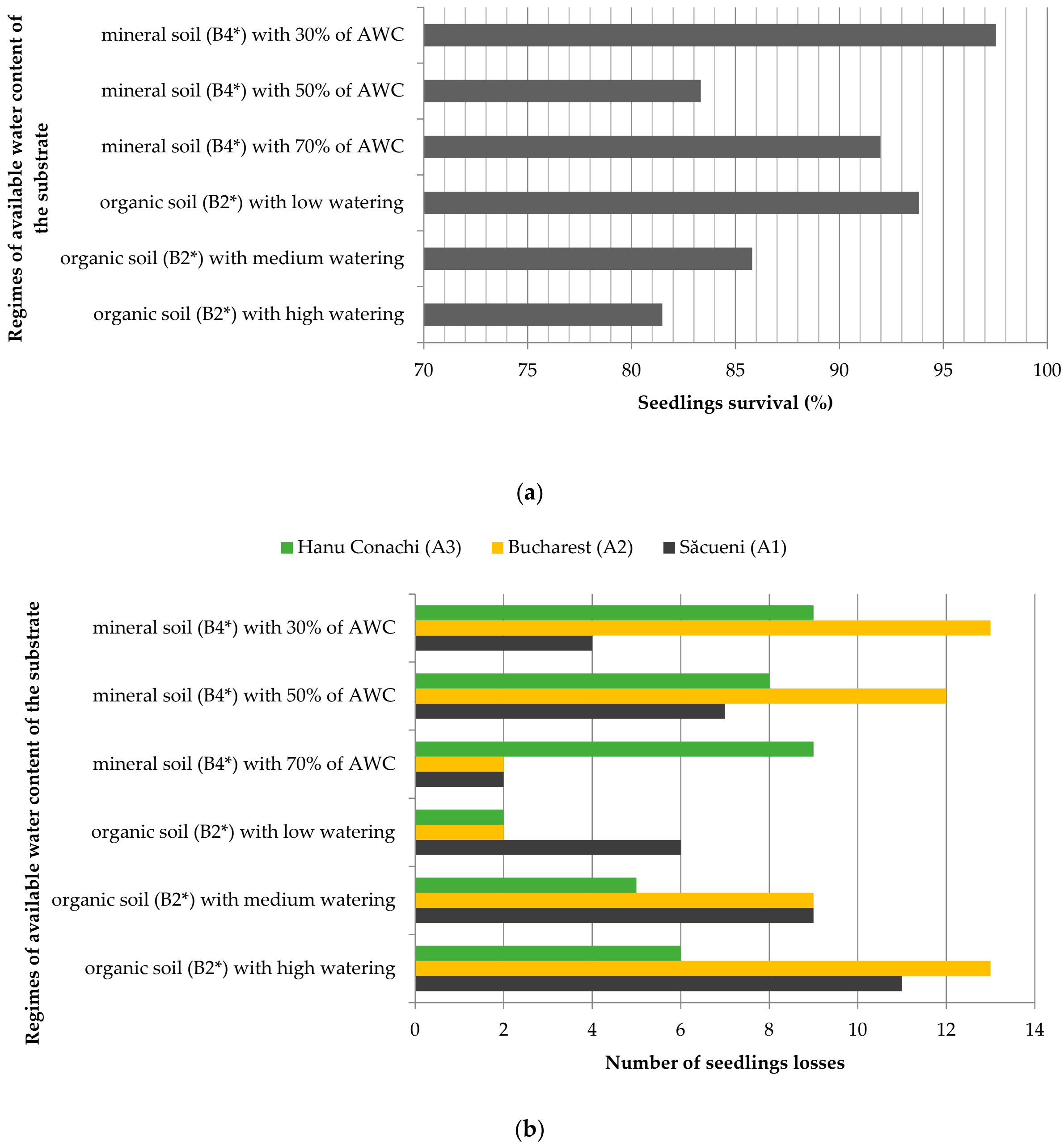

Figure 3. Seedling losses for organic and mineral substrates: (**a**) seedling survival (%) influenced by the substrate type and watering regime; (**b**) losses differentiated by the seed provenance influenced by the substrate type and watering regime. * B2—peat MKS 3 substrate; B4—rendzina with sand (1:2) substrate.

4. Discussion

The highest germination percentages are obtained from the largest seeds [70], an aspect that was not confirmed in our experiment: the seeds with O.S. Săcueni provenance (the largest seeds with a weight of 1000 seeds of 20.471 g) showed a seedling emergence percentage lower than the seeds with O.S. Bucharest provenance (1000 seeds weighing 18.492 g). The O.S. Bucharest seeds with highest germination percentages did not indicate the same good results with the peat MKS 3 substrate. The highest seedling emergence percentage was for the seeds of O.S. Bucharest provenance (73.7%) in peat MKS 1 substrate, similar to the results obtained for O.S. Sâmpetru-Almașului (73.7%) [21].

The highest values of height (H), and base diameter (D) were determined for the field samples resulting from direct sowing in natural conditions (O.S. Săcueni: H = 98.65 cm, D = 6.49 mm with 617.6 mm rainfall; O.S. Bucharest: H = 87.09 cm, D = 6.94 mm with 585.0 mm rainfall; O.S. Hanu Conachi: H = 61.13 cm, D = 7.10 mm with 421.4 mm rainfall) compared to the containerized ones (greenhouse conditions registered 43.61 cm for the mean height of seedlings, and 3.49 mm for the mean diameter). Our results showed a similar trend with the results regarding the growth of black locust seedlings from six clones [71]: the mean height of the seedlings in the field were higher (33.5 ± 9.7 cm) compared to the containerized seedlings (15.8 ± 2.2 cm) and the diameter recorded a higher mean value (4.5 ± 1.5 mm versus 4.4 ± 0.9 mm). The seedlings from O.S. Galați recorded the highest heights and diameters (H = 70.4 ± 1.4 cm, D = 5 ± 0.2 mm) [34]. The comparative growth of black locust seedlings obtained in the natural conditions of the Loess Plateau area (altitude of 1290 m and 497 mm rainfall) and with those obtained in containers (loess substrate and under different watering regimes) showed for one-year seedlings a mean value for H = 51.0 ± 5.8 cm and D = 0.65 ± 0.04 cm [71]. For this last experiment, the greatest increases in height and diameter were obtained for the seedlings from the open field treated with 0.2 mg/L brassinolide. According with our national standards, the minimum base diameter for a black locust seedling (1–2 years old) is around 4 mm [72].

In greenhouse conditions (mean temperature of 24.35 °C, 4.1 °C minimum and 44.6 °C maximum temperature), and the eutric cambisol substrate with a high watering regime showed the highest value for seedlings height (H = 48.69 cm, D = 3.49 mm), the peat MKS 3 with medium watering presented the highest value for diameter (H = 42.76 cm, D = 4.12 mm). We consider that this was possible due to the mineral substrate and the high watering treatment influencing the height of the seedlings. Our results could be compared with an experiment in which values of heights (105 ± 5 cm) were obtained in a treatment with a humidity of the substrate of 80% of the field capacity, and diameter (9.7 ± 0.9 mm) for a humidity of 60% (mean temperature of 26.4 °C) [56].

The survival rate was compromised for the organic substrate in the case of peat MKS 3 (pH 4.54 and medium structure) (survival 87.0%) and for the mineral substrate in the case of rendzina with additional sand substrate (pH 8.70, lowest humus content of 1.22% and sand structure) (survival 86.4%) comparing with 95.3 ± 8.2% survival of seedlings treated before planting with 0.2 mg/L brassinolide (which alleviates the effects of water stress and increases the drought resistance of black locust seedlings) [73]. In our research, following the comparison of seedling losses, it was found that the MKS 3 substrate, in conditions of water stress, favours better maintenance, in contrast to the rendzina + sand substrate, a variant in which the losses were higher, being relevant to treating seedlings with additional amelioration substances in this kind of substrate. In the other six types of substrate, no losses were recorded regardless of the variant throughout the experiment (May–October 2023), indicating no need for additional amelioration techniques.

Significant differences regarding the survival of the seedlings at the end of the 28 weeks of observations were recorded both between two origins (53.66% for the Greek origin and 61.6% for the Hungarian one) and between two types of substrate (in the enriched peat they survived 36.59% and in a stabilized environment 78.23%) [20]. The height of the seedlings was higher in the seedlings of Hungarian origin (34.61 cm) and in the stabilized environment the highest values were also recorded (34.35 cm) compared to the enriched peat (29.41 cm). In our research, the highest average increases were recorded in eutric cambisol substrate with a high watering regime.

5. Conclusions

The provenance of the seeds, the type of substrate, and the watering regime affected the vigour (height and diameter) and survival of the black locust seedlings in greenhouse conditions.

O.S. Bucharest seedlings, in greenhouse conditions, showed the highest heights before the water regime was differentiated. Seedlings from O.S. Hanu Conachi (the region with the lowest annual rainfall, around 420 mm) showed the lowest height both before and after watering regime differentiation. Also, after water regime differentiation, O.S. Hanu Conachi seedlings exhibited heights not significantly different regarding the watering regime treatment compared with seedlings from O.S. Săcueni (annual rainfall around 610 mm) and O.S. Bucharest (580 mm), with these preferring high watering regimes.

Seedlings from O.S. Săcueni registered the highest heights in sandy loam (pH 5.44) substrate, the characteristics of this substrate being close to the characteristics of the soil of the region of the seeds origin, but also preferring sand (pH 8.84) and sandy clayey loam (pH 5.85). Seedlings from O.S. Hanu Conachi registered similar trends as O.S. Săcueni seedlings regarding substrate effects. O.S. Bucharest seedlings exhibited the highest heights in organic substrate with a fine structure and pH 6.0.

The watering regime influenced seedling height in the organic substrate. A high watering regime was much more suitable for the organic substrate with pH 6.0 and a fine structure (MKS 1), but there was a negative influence on seedling height of the organic substrate with pH 4.54 and a medium structure. Low watering seedlings in peat MKS 3 presented a height higher than seedlings treated with high and medium watering regimes in similar substrates. Also, the watering regime showed effects regarding seedling heights in the mineral substrate. Silty loam (pH 6.91), sand (pH 8.70, pH 8.84, and pH 8.34), and sandy loam (pH 5.44) exhibited not significantly different heights, but sandy clayey loam (pH 5.85) with high and medium watering regimes generated significantly higher differences compared with the low watering regime treatment.

The medium watering regime for peat MKS 3 showed the highest base diameter of greenhouse seedlings. The seedlings cultivated in this substrate were the only ones with values over 4.0 mm, but in general, this organic substrate (pH 4.54 and medium structure) registered the most losses of seedlings. The height of the seedlings that exceeded 4.00 mm in base diameter was average (around 42.00 cm), the greatest being over 48.50 cm.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15050785/s1, Supplementary File: The seedling emergence rate (%) for seed source of provenance and substrate treatments.

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