Chemical Methods to Improve Seed Germination and Seedling Growth of Honey Locust (*Gleditsia triacanthos* L.)

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RESEARCH ARTICLE

Abstract

The present study aimed to improve the seed germination and plantlet development of honey locust (*Gleditsia triacanthos*) using two types of edaphic substrate (peat and peat+sand) and different chemical substances. Breaking physical dormancy for some forest seeds is a challenge for researchers and forest managers in order to obtain a homogeneous germination on larger areas. Germination percentage of honey locust seeds were investigated by using different chemical treatments as follows: calcium hydroxide; concentrated solution of sodium 4-nitrophenolate; solution with a content of auxines; copper sulphate; potassium permanganate and concentrated sulfuric acid. The highest percentage of seed germination was obtained when the seeds were immersed in an aqueous solution of sulfuric acid (14%), followed by 12.5% (concentrated solution of sodium 4-nitrophenolate) and 12.3% (solution with a content of auxins). The lowest values (5%) were recorded when copper sulphate was used. Regarding the different types of studied substrates, the highest percentage of germination was obtained on peat + sand. As regards to seedling development, the highest values were obtained on the peat + sand substrate, with seeds treated with sulfuric acid (15.0 cm). The concentrated solution of sodium 4-nitrophenolate and solution with auxins recorded also high values, 15.2 cm, respectively 14.9 cm. The lowest values for seedling height (11 cm) was noted when seeds were treated with potassium permanganate.

Keywords: chemical substances; germination; recipients; seedlings; seeds.

INTRODUCTION

Honey locust (*Gleditsia triacanthos* L.) is a leguminous tree from the *Cesalpiniaceae* family, originating from south-eastern part of North America, where its territory includes also the black locust, varying from South Ontario to Texas (Little, 1971; Cronk and Fuller, 1995). In the western part of the United States honey locust behaves as an invading species (Schnabel et al., 1991; Schnabel and Hamrick, 1990a), sometimes it reappears in forests (woodlands and early successional habitats) with *Madura pomifera* (Csurhes and Kriticos, 1994) and *Robinia pseudoacacia*.

Gleditsia triacanthos was introduced in large areas in Europe in 1700, presenting a high interest in parks as ornamental species, for protection of shelterbelts, along roads and fields and as barrier against wind (Ferus, 2013; Singh et al., 1991). It can be cultivated successfully in areas with air pollution (Ertekin and Kirdar, 2010), as hedges, as well as a component of flooding forests (György, 2007; Haraszthy, 2001). Although it is able to tolerate most soil types, including soils that are moderately saline, *G. triacanthos* grows most prolific on fertile, alluvial soils with a pH of 6.0-8.0 (Nas, 1980). The plant develops an extended lateral root system (Bedker and Blanchette, 1983). There is good growth in areas receiving 500 mm to 1500 mm annual rainfall (Nas, 1980)

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although the plant has been reported to survive also in areas receiving annual rainfall up to 355 mm (Mitchell, 1978).

Due to its vast territory, it is seen in many places as an invasive species (Gojdičová et al., 2002; Balogh et al., 2004), causing extreme complications in Argentinian Pampa grasslands and central natural forests, where a large effort in ecological research has been paid (Chaneton et al., 2004; De Viana and Speroni, 2003; Marco and Paez, 2000).

Gleditsia triacanthos L. is a deciduous tree with heights that can reach even 45 m in its origin country, but in Romania does not exceed normally 20-25 m. The stem is straight enough, provided with large thorns upon 10-15 cm, simple or with 1 (2-3) ramification, very rigid. The fruits of honey locust are flatten pods, often twisted, of 30-40 cm length, indehiscent (Blair, 1990; Brown and Kirkman, 1990). The seeds are brown, flatten, with very hard tegument, surrounded by a softer part and with a sweet taste, characteristics that attract animals (Vines, 1960; Burton and Bazzaz, 1991).

From ecological point of view, this species is tolerant to high temperatures (Graves et al., 1991; Graves and Wilkins, 1991; Godoy et al., 2011), resistant to drought and to the presence of salts in soil (Graves and Wilkins, 1991; Burton and Bazzaz, 1991; Burton and Bazzaz, 1995; Ertekin and Kirdar, 2010), loving the light, it presents a moderate to rapid growth speed (Burner et al., 2005). Recent studies show that it is a tolerant species to industrial and transport emissions (Benčať, 1982), relatively high resistance to low reaction of soil until pH 5 (Schindelbeck and Riha, 1988).

Generally, the reproduction through seeds is the easiest and most advantageous method of honey locust cultivation, but, without previous treatments, germination is low because of physiological rest induced by the impermeable tegument that covers the seed (Davies and Macfarlane, 1979; Geneve, 2008; Singh et al., 1991; Sîncrăian et al., 2013). In forestry practice different chemical and mechanical treatments are used, to improve the germination percentage. Masaka and Yamada (2009) obtained the highest germination rates (45.3%) through sinking of seeds in boiled water at temperatures of 60°C, for 3 hours. Also, Basbag et al. (2010) obtained the highest germination rates (25.5%) through sinking of *G. triacanthos* seeds in boiled water at temperatures of 50°C, for 30 minutes. Basbag et al. (2009) obtained germination rates between 8.39 and 29.52% for *Capparis ovata* seeds through application of different temperatures and durations.

Besides this scalding method, in practice is also enough using the chemical method, through sinking of seeds for 60 minutes in concentrated sulfuric acid, then washing in cold water, and then drying them in room at adjusted temperature, for 24 hours (Asl et al., 2011; Kheloufi, 2017).

Another successful method is the seed scarification when dried seeds are taken and placed in a metallic recipient with gravel. The recipient is strongly agitated, so that the tegument of seeds is scratched (Usberti and Martines, 2007).

The aim of this study was to evaluate the seeds germination of honey locust and the development of plants, through application of some chemical treatments, using two types of substrate (peat and peat+sand).

MATERIALS AND METHODS

The biological material

The study was accomplished in the greenhouse of University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca (Romania). Honey locust (*Gleditsia triacanthos* L.) seeds were used as biological material from trees grown in Mihai Viteazu nursery, Turda (altitude: 350 m, latitude: N46°34'15" and longitude: E23°46'45"), harvested in October 2018.

The seeds separated from pods were washed in tap water, then dried for two days at room temperature, after which they were sown. Previously, the main characteristics of the seeds (length, diameter and weight) were analysed.

The study of germination

The percentage of seed germination and the seedling growth were analyzed on two types of substrate: peat and sand+peat. The germination percentage of honey locust seeds were investigated using six chemical treatments, with different solutions (Table 1).

After each treatment, all seeds were immersed for 30 minutes in bidistilled water, and then sown in bloc type recipients, consisting each of 60 alveolae, using two types of edaphic substrate (peat and peat+sand).

For each variant 50 seeds were used in four repetitions, totally 1.200 seeds were analysed. The weight of 1.000 seeds was of 160.4 g. The germinated seeds were counted periodically starting with 7th day until 30th day, the selective criteria of germination being the radicle growth with 2 mm.

Seed germination was expressed by the percentage of germination, which indicates the proportion, in digits, of the seeds that produced seedlings considered normal, under the conditions and period specified in the SR 1908/2004 standard. At the moment of transplantation, measurements were made regarding seedlings height,

diameter and the number of leaves. The height was measured with graded ruler and the diameter was measured with sliding callipers.

Table 1. Characteristics of the two parts of the study (germination using different stimulants and germination using different types of substrate)

Variant	Treatment	Duration of treatment (seeds soaked in diluted solution)	Concentration
V1	Ca (OH) ₂ Calcium hydroxide	17 hours	20 g Ca(OH) ₂ in 0.5 l water
V2	C ₆ H ₄ NNaO ₃ Sodium 4-nitrophenolate	8 hours	1:2000 (0.75 ml active substance in one liter tap water)
V3	C ₁₈ H ₃₂ O ₅ Auxines	8 hours	1 ml active substance in 0.6 l water
V4	CuSO ₄ Copper sulfate	24 hours	1 g CuSO ₄ in 0.5 l water
V5	KMnO ₄ Potassium permanganate	24 hours	1.25 g KMnO ₄ in 0.5 l water
V6	H ₂ SO ₄ Sulfuric acid	30 minutes	concentrated sulfuric acid- 98%

Data Analyses

Analysis of variance (ANOVA) was used to compare the mean of germination affected by different types of chemical solutions and different types of substrates.

Significant differences among treatments were tested by one-way ANOVA at the 95% confidence level, and post hoc comparisons were made using the Tukey HSD test at $p < 0.05$ (Sestras, 2018).

For germination data, a two-way ANOVA, with treatments and edaphic substrate as the main factors, was performed. Significance of differences between the main factors from the ANOVA analyses were evaluated with a Student-Newman-Keuls multiple range test ($p < 0.05$).

RESULTS AND DISCUSSIONS

The characteristics of the seeds regarding the weight, diameter and length were represented as a boxplot diagram (Figure 1; Table 2). Regarding the length of the seeds, the minimum was 5.92 mm, the maximum was 9.83 mm, and the average was 7.59 mm. In terms of seed diameter, the minimum was 4.43 mm and the maximum 6.91 mm, and the seeds weighed 0.11 g (Figure 1; Table 2).

According to the literature, larger seeds have a higher germination rate because they contain more resources to support germination (Kheloufi et al., 2018; Long et al., 2015) and healthier seedlings (Kheloufi et al., 2018; Pedrol et al., 2018).

Figure 1. Boxplot representation of the seed characteristics harvested from *G. triacanthos*

Seed propagation is a method of reproduction of particular importance, because it maintains the genetic diversity of different populations. The seed is considered germinable if biochemical transformations and physiological processes can take place inside, starting the embryonic activity and determine the growth and development of the new seedling. The germination capacity of seeds can be established, first of all, according to the healthy appearance and the way in which they react to certain physical or chemical treatments (Koornneef et al., 2002).

Table 2. Characteristics of the seeds harvested from *G. triacanthos*

Statistic	Length (mm)	Diameter (mm)	Weight (g)
q1	7.32	5.35	0.10
min	5.92	4.43	0.04
median	7.59	5.70	0.11
max	9.83	6.91	0.19
q3	8.56	6.15	0.16

Regarding the germination of honey locust seeds, using peat as a soil substrate, it can be observed that superior values were recorded in V2 (10.7%) and V6 (11.3%), where the seeds were treated with concentrated solution of sodium 4-nitrophenolate and sulfuric acid. The lowest values were recorded for variant V4 (5%), where the seeds were treated with CuSO_4 (Figure 2a).

Germination of seeds using peat+sand as edaphic substrate, registered higher values in variant V6 (seeds treated with sulfuric acid) than in the other variants. Lower values were recorded for variant V4 (5%). The variants V2 (concentrated solution of sodium 4-nitrophenolate) and V3 (solution with auxins) achieved significantly superiors values comparing to V5 (KMnO_4) and V1 (calcium hydroxide) (Figure 2b).

Figure 2. The Influence of chemical treatments on honey locust seed germination, using different edaphic substrates. **(a)** peat; **(b)** peat and sand. V1-calcium hydroxide solution; V2-concentrated aqueous solution of sodium paranitrophenolate; V3-aqueous solution with a content of auxines; V4-solution copper sulfate (CuSO_4); V5-solution with a potassium permanganate (KMnO_4); V6-concentrated sulfuric acid (H_2SO_4)

Based on the literature, the seeds of several species, including honey locust are covered with a hard coat which is a barrier to water imbibition (Singh et al., 1991) and the seeds need chemical treatments or scarification.

Different studies reported a 100% germination of honey locust scarified seeds in a maximum of 60 days, while unscarified seeds, sown directly in the field germinated after two or even three years (Burton and Bazzaz, 1991).

Treatment of honey locust seeds with sulfuric acid, with concentrated solution of sodium 4-nitrophenolate, solution with auxins and solution with KMnO_4 significantly improved seed germination. These results are in agreement with Basbag et al. (2009); Singh et al. (1991); Roman et al. (2020); Asl et al. (2011).

Regarding the development and growth of seedlings obtained from seeds, the results varied considerably, depending on the treatment variant and the type of substrate (Figure 3 and Figure 4).

As regards of seedling development using peat as edaphic substrate, the seedling height recorded the highest values in variants V2, V3, respectively V6, where the seeds were treated with concentrated solution of sodium 4-

nitrophenolate, solution with auxins and sulfuric acid, while the lowest values were recorded in variant V1 (seeds treated with calcium hydroxide) (Figure 3). Regarding the seedling diameter, the variants V2, V3, respectively V6 registered high values within the studied variants. Leaf number per seedling recorded the greatest value in V6 variant (Figure 3).

Figure 3. The main characteristics of *G. triacanthos* seedlings using peat. **(a)** Seedlings' height; **(b)** Seedlings' diameter; **(c)** Number of branches/seedling. V1-calcium hydroxide solution; V2-concentrated aqueous solution of sodium paranitrofenolate; V3-aqueous solution with a content of auxines; V4-solution copper sulfate (CuSO_4); V5-solution with a potassium permanganate (KMnO_4); V6-concentrated sulfuric acid (H_2SO_4)

Regarding the development of seedlings using peat + sand as edaphic substrate, it can be observed that higher values were recorded for all the analyzed characteristics (Figure 5), compared to seedling development using peat as edaphic substrate (Figure 4).

Figure 4. The main characteristics of *G. triacanthos* seedlings using peat+sand. **(a)** Seedlings' height; **(b)** seedlings' diameter; **(c)** Number of branches/seedling. V1-calcium hydroxide solution; V2-concentrated aqueous solution of sodium paranitrofenolate; V3-aqueous solution with a content of auxines; V4-solution copper sulfate (CuSO_4); V5-solution with a potassium permanganate (KMnO_4); V6-concentrated sulfuric acid (H_2SO_4)

The height of the seedlings in variant V2 was 15.2 cm, in variant V6 was 15.0 cm, and the smallest value was registered in variant V1, as 11.0 cm. Also, for the other two characteristics, the diameter and the number of leaves, the highest values were reached in the sixth variant, seeds treated with sulfuric acid, and the lowest values were registered in the fifth variant, seeds treated with KMnO_4 (Figure 5).

Following the statistical results the seeds treated with KMnO_4 , had a medium to high germination percentage, but the seedling development recorded the lowest values, which is not recommended for forestry practice.

The treatment with the best results in terms of seed germination and seedling development was the sulfuric acid followed by concentrated solution of sodium 4-nitrophenolate and solution with auxins.

CONCLUSIONS

The present paper underlined several findings regarding seeds treatment before sowing, using different chemical substances. The choice of substances with major influence on germinative processes and their application will lead to increased germination percentage and stronger growth of honey locust seedlings.

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