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STUDY OF THE POSSIBILITIES FOR IMPROVING THE SOWING QUALITIES OF SEEDS AND THE VITALITY OF SEEDLINGS FROM *Cryptomeria japonica* Don. THROUGH PRE-SOWING TREATMENT WITH ULTRASOUND

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Abstract

Recently, in the production of planting material from ornamental species of trees and shrubs from seeds, more and more attention is paid to various physical methods aimed at increasing the germination and viability of seeds in difficult to propagate species. One of these methods is pre-sowing treatment of seeds with ultrasound. The present study was conducted to determine the effect of ultrasound on the germination and viability of seeds of ornamental species of Cryptomeria (Cryptomeria japonica D. Don.). The experiments were set in the laboratory of the Department of Horticulture, Agricultural University Plovdiv. The experiment with Cryptomeria was conducted from the end of February to the beginning of August. Variants with 5, 10, 15 and 20 minutes exposure were studied. Untreated seeds were used for control. Indicators related to the growth and phenological manifestations of plants were studied. It was found that the treatment of seeds with ultrasound affects the sowing qualities of the seeds of the studied specie. Ultrasound treatment has a beneficial effect on germination in Cryptomeria, with the optimal exposure being 20 minutes - so germination increases by 11% compared to control.

Key words: *Cryptomeria, seed propagation, ultrasound treatment, germination, ornamental plant.*

INTRODUCTION

Japanese cedar or cryptomeria (*Cryptomeria japonica* D. Don.), is a coniferous evergreen tree of the *Cupressaceae* family. The plant is indigenous to Japan and southern China and endemic to Japan. It is a widely used timber species in the Far East (Dirr, 1990). *Cryptomeria* is also considered a sacred tree in Japan with great landscape value (Creech, 1984). The plant that the Japanese call Sugi is an allogamous, wind-pollinated conifer species that is frequently used for commercial afforestation in Japan. Approximatelly 45% of all the man-made forests in Japan are composed of this species (Saito O., 2009; Forster E.J. et al., 2021). The species currently is gaining popularity not only in the northeastern United States, but also in the hot and humid southeast. The tree grows very well in rich, deep, acidic, moist soil but will tolerate heavy clay during dry and wet periods (Dirr, 1990; Tripp, 1993). The plant has a pyramidal crown, reaching 15 to 18 m in height, with descending branches. There are many cultivars of Japanese cedar that have a wide range of ornamental characteristics and uses. *Cryptomeria* has no major insect or disease problems (Baker J. and R. Jones, 1987), grows rapidly and makes an excellent evergreen screen (Dirr, 1990; Tripp, 1993). In addition, the emerald green foliage exhibits little dieback or discoloration (Tripp, 1993). This species is recommended as a replacement for Leyland cypress *Cupressocyparis leylandii* [(A.B. Jacks and Dallim.) Dallim. and A.B. Jacks] which has various disease and insect problems (Baker and Jones, 1987). In Bulgaria cryptomeria is used as an ornamental tree due to the beautiful shape of the crown, high cold resistance - withstands up to - 23^{0}C - and the relative tolerance to diseases and pests (Bogdanov B., 1984). Its extremely limited distribution in our country in public and private parks and gardens is due to the lack of supply of the plant in garden centers and nurseries. The main part of the plants offered in these sites are imported from abroad and the price is quite high. These problems stem from the lack of developed technology for the production of planting material for the conditions of Bulgaria. One of the main points in the technology for the production of planting material is the question of how to propagate. The different ways of propagation should be studied in detail and the one that has the highest efficiency and can be used for mass production of planting material in the conditions of the given area should be singled out. There is a lot of information in the specialized literature about the ways of *Cryptomeria* propagation. Special attention is paid to their advantages and disadvantages. Researchers studying vegetative propagation in *Cryptomeria* are unanimous that the most appropriate method is by stem cuttings. Jull L.G. et al. (1994) demonstrated that *Cryptomeria* stem cuttings can be rooted at all growth stages, but branch order from which cuttings are prepared is critical in achieving high rooting percentages. Although popularity and subsequent demand of *Cryptomeria* have increased, little research has been reported in journals on factors influencing propagation of the species and related cultivars by stem cuttings (Doran W.L., 1957; Waxman S., 1962; Henry P.H., F.A. Blazich, and L.E. Hinesley, 1992). For centuries, Japanese cedar has been propagated in Japan for forestry by seed and stem cuttings (Brix and van den Driessche, 1977; Ohba, 1993; Still S.M. and S. Zanon, 1991), and a body of practical knowledge on propagation and culture exists in the Far East. Some research concerning stem-cutting propagation of the species has been conducted in the United States. Information regarding factors, such as growth stage (timing) and auxin treatment, have been published (Black D.K., 1972; Bogdanov B., 1984); however, much of this information is conflicting and needs to be resolved (Dirr and Heuser, 1987; Lahiri, 1975; Nakayama, 1978; Orndorff, 1974). In addition, tree forms of Japanese cedar exhibit a well-defined branch order (branch position), which may influence rooting. For some conifers, branch order is an important factor affecting adventitious rooting (Black, 1972; Bogdanov, 1984; Miller et al., 1982) and warrants study in Japanese cedar.

Cryptomeria can also be propagated by seeds (Hartmann H.T. et al., 1990). In order to obtain more and better seeds Moriguchi et al. (2004); Moriguchi et al. (2005) and Moriguchi et al. (2007) recommend that in production of seedlings of *Cryptomeria* have to abidance three main factors, namely: first the pollen contamination rates can be >30%; this may be influenced by the surrounding forest plantations; second, the self-fertilization rate in conifer seed orchards has been determined to be generally less than 5% and third, the paternal contributions of constituted clones have been found to differ significantly from the expected equal contributions; this may be influenced by the number of male strobili. Itoo, S. (1984) compared the properties of *Cryptomeria* seeds obtained from a greenhouse (indoors) and outdoors and found that seeds obtained in the open place have better germination. On the other hand, the plants obtained from these seeds vary considerably in the main observed decorative characteristics. According to Mitsch J. (1975) and Russell R.S. (1977), a short period of statification is required for normal seed germination - from 4 to 6 weeks - at low positive temperatures, after which the seeds are ready for sowing. The issues of seed propagation in most cases are related to the study of the possibilities for increasing the germination and viability of the seeds through different ways of pre-sowing treatment. Ultrasound treatment is one of these ways (Awad T. et al., 2012; Chen G. et al., 2012; Miano A.C. et al., 2015) Ultrasound technology has been used to enhance the quality of seeds in many agricultural crops. The effect of ultrasound treatment on seed quality parameters such as germination and vigour has not been sufficiently studied (Yaldagard M., 2008). This physical way of affecting the qualities of the seeds is still poorly studied, and in cryptometry there is no information about it at all. This work investigated whether ultrasound technology affected the germination and vigour of *Cryptomeria* seeds.

MATERIALS AND METHODS

The study was conducted in the period 2020-2021. The experiments were set in the laboratory of the Department of Horticulture, Agricultural University - Plovdiv.

The seeds were collected from well-developed, healthy plants *Cryptomeria japonica* var. *japonica*. Syn: *Cupressus japonica* Thunberg ex Linnaeus f., Suppl. Pl. 421. 1782; *Taxodium japonicum* (Thunberg ex Linnaeus f.) Brongniart (Fu et al., 1999) from TP State

Hunting Farm Krichim. The collection took place in early November 2020. The seeds were separated by hand. Then lightly dried at room temperature. The ultrasonic treatment was carried out in an ultrasonic bath Ultrasonic water bath NAHITA, model 620/1, manufactured by AUXILAB, S.L., Spain with ultrasonic wave frequency 220-240 v - 50 Hz and ultrasonic power 35 W. The experiment was set on February 26, 2021 at the Agricultural University of Plovdiv. *Cryptomeria* seeds were immersed in water and wrapped in gauze and treated with ultrasound for different periods of time. 180 seeds were used, which were divided into 5 groups of 36 each - one non-treated control and 4 groups treated for 5 minutes, 10 minutes, 15 minutes and 20 minutes respectively. Immediately after sonification, the seeds were sown in a peat-pearlite mixture, each in a separate cell on a 180-cell tray. The seeds are then left outdoors and grown under natural conditions. Germination began in mid-April and ended in early May 2021.

The following indicators were studied: Growth rate of the stem; Phenological observations; Seed germination (%); Plant height (cm); Diameter of the stem at the base (mm); Number of internodes (pcs.).

The experiment was reported every week after the emergence of the first plant. The experiment ended 6 months after sowing (in August). All plants of each variant were analyzed. Seed germination was reported three months after sowing.

Figure 1. Seeds of *Cryptomeria japonica* D. Don.

The height of the stem was measured from the soil surface to the top of the plant in cm. The thickness of the stem was recorded at the base, above the root collar using a caliper in mm.

The obtained data were statistically processed by analysis of variance.

RESULTS AND DISCUSSIONS

Table 1 presents the results of studies of the seed quality of *Cryptomeria japonica.* The absolute mass of 1000 air-dried seeds was 1.279 g. This indicator is influenced by the size and fulfillment of the seeds, as well as by the climatic conditions in the growing area. These data are a criterion for the ecological plasticity of a species and its suitability for acclimatization to the conditions of an area. The vitality of the seeds determines their potential ability to germinate. In the seeds used in this experiment, the vitality was 63.94%. Germination is the most important indicator of the suitability of seeds to form normal sprouts under optimal conditions over a period of time. In *Cryptomeria japonica*, seed germination was determined at 7 days - 24.44% (Table 1).

Table 1. *Cryptomeria* seed quality

Absolute mass per 1000 seeds (g)	Vitality, %	Germination, %	Germination energy, %	Embryonic root length (cm)
1.279	63.94	24.44	73.18	1.07

Germination energy indicates the percentage of normally germinated seeds under optimum germination conditions within a period shorter than that for germination. In *Cryptomeria japonica*, the germination energy was determinated for 5 days and was 73.18%, indicating that the seeds germinate jointly and give strong and viable seedlings and, respectively, more viable plants. The average embryonic root length of *Cryptomeria japonica* is 1.07 cm, which is also evidence of seed viability and usability.

The rate of stem growth in seedlings is a criterion for the viability of plants and their successful future development. The growth rate of the *Cryptomeria* seedlings was monitored from the beginning of June to the beginning of August and is presented in Figure 2. From the data becomes clear that the plants from the control species have the lowest growth rate - their initial average height is 2.50 cm and the increase in height in the first week is only 0.16 cm. In the the next three weeks, the stem increase in height, or more precisely the differences in stem height for the seven-day reporting period, is minimal - 0.02 cm in the first week, up to 0.04 cm in the third week. Transplantation was carried out in early July. Very intersting is the fact that after this period of minimal growth, from mid-July, the plants not only do not grow, but also the height of their stems begins to decline intensively - 0.15 cm in the first week of July, 0, 37 cm in the second week of the same month and 0.1 cm the third week of July. The plants then die, most likely because of the contact of the root hairs with air oxygen during transplanting.

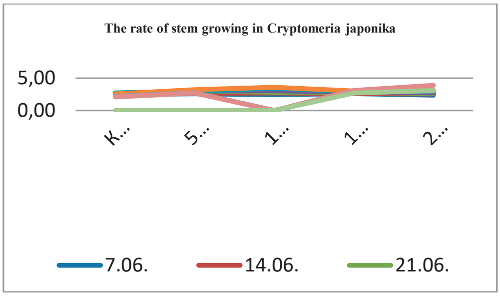

Figure 2. The rate of stem growing of *Cryptomeria japonica*

Seeds treated with different sonication exposure, give significantly taller and more viable plants, with more intense growth rate. The plants from the 5-minute exposure species have the most intensive growth rate at the end of June, with an increase in height for the seven-day period of 0.34 cm. Then follow periods of poor growth and height decrease. This process lasts until the end of July. In the beginning of August the plants die. 10 minutes of ultrasound exposure leads to the death of plants in the third week of July, but until then they are vital and growing at an intensed rate - from 0.1 cm in the third week of June to 0.25 cm in the last week of the same month. The plants with the most intensive growth rate are obtained from the seeds of 15 minutes sonication. In the third week of June - from 16 to 24 June they increase their height by an average of 0.2 cm. These plants are also characterized by an intensive growth rate in the second and fourth week of July - they grow by an average of 0.12 cm and 0.17 cm, respectively. This increase is due to the death of lower plants and an increase in average height. During the remaining weeks of the vegetation period the growth of the plants is 0.02 cm, 0.03 cm and 0.04 cm. in the second and fourth week of June and the first week of July. Here there is also a reducing in the stem height by 0.09 cm, in the third week of July. Plants received 20 minutes sonication are distinguished by the most intensive growth rate and the greatest vitality. Differences in stem height range from 0.08 cm in the third week of June to 0.87 cm in the last week of July.

Table 2. Phenological observations in *Cryptomeria*

Characteristics / Variants	Germination			
	started		masse	
	date	no. days after sowing	date	no. days after sowing
к	19.04	52	26.04	59
5	21.04	54	26.04	59
10	19.04	52	26.04	59

Table 3. Phenological observations in *Cryptomeria*

Characteristics / Variants	First stem branch			
	started		masse	
	date	no. days after sowing	date	no. days after sowing
к	28.06	122	28.06	122
5	28.06	122	28.06	122
10	21.06	115	28.06	122
15	28.06	122	28.06	122
20	05.07	129	05.07	129

Phenological observations on *Cryptomeria* seedlings are presented in Table 2. The data show that pre-sowing sonication did not significantly affect the onset of germination of cryptomeria seeds - all experimental variants except the 5-minute variant germinate 52 days after sowing on 19.04. The same applies to the mass germination of seeds, which occurs 7 days after beginning, on 26.04. or 59 days after sowing, both for treated and untreated seeds. The first stem branching occurs in plants attained from 10 minutes sonication seeds, on 21.06. or 115 days after sowing (Table 3). Next are the plants attained from seeds treated with 5 and 15 minutes sonication, which form the first stem branch 7 days later on 28.06. or 122 days after sowing.

Table 4. Phenological observations in *Cryptomeria*

Characteristics / Variants	Second stem branch			
	started		masse	
	date	no. days after sowing	date	no. days after sowing
к	-	-	-	-
5	-	-	-	-
10	05.07	129	05.07	129
15	-	-	-	-
20	-	-	-	-

Very interesting is the fact that plants growing from untreated seeds form the first stem branch within the same period. At the latest, the first stem branch is formed by the plants growing by 20 minutes seeds sonication - on 5.07., or 129 days after sowing. The second stem branch is formed only by plants from 10 minutes seeds sonication, on 5.07., 129 days after sowing, or 14 days after the appearance of the first stem branch (Table 4). The data illustrating how many of the plants form stem branches are very interesting. The highest percentage of plants forming stem branches are in the variant of 15 minutes sonication - 8.33%. Next are the plants from the variant of 10 minutes sonication - 5.55%. Plants from the variant of 5 and 20 minutes sonication, as well as untreated ones have the same percentage of plants that formed stem branches - 2.77%.

Table 5. Characteristics of the stem in *Cryptomeria japonica*

Characteristics / Variants	Stem high, cm	Number of stem branches, nb	plants with stem branches,%
к	2.72	1	2.77
5	3.23	1	2.77
10	3.60	2	5.55
15	3.10	1	8.33
20	3.90	1	2.77

In Table 5 are presenting the data regarding the height of the stem. The highest average height is reached by plants from seeds of 20 minutes sonication - 3.90 cm. The plants from seeds of 10 minutes sonication are 3.60 cm or 0.3 cm lower. followed by plants from seeds of 5 and 15 minutes, sonication respectively, 3.23 cm and 3.10 cm. The plants from the untreated variant have the lowest stem height - 2.72 cm, or 1.18 cm or 30.25% lower than the variant with the tallest plants.

CONCLUSIONS

1. Plants growing from 20 minutes seeds sonication are characterized by the most intensive and uniform growth rate.
2. Germination of *Cryptomeria* seeds is not affected by pre-sowing sonication. Germination, both initial and mass, is not affected by the duration of sonication.
3. *Cryptomeria* was found to form first and second stem branches after 10 minutes sonication. However, most plants form stem branches after 15 minutes sonication.
4. Pre-sowing 20 minutes sonication resulted in *Cryptomeria* seedlings with an average height of 3.90 cm.
5. The conclusion shows that 10 minutes pre-sowing sonication results in obtaining first and second stem branches; the duration of 15 minutes - results in obtaining the highest plant percentage of stem branches - 8.33%, and 20 minutes sonication resulted in obtaining the highest plants - 3.90 cm. We can conclude that pre-sowing sonication of *Cryptomeria* seeds is recommended in seed propagation of this species. According Aladjadjian A. (2002) the effect of under ultrasonic action is due to the mechanical energy of the ultrasonic wave which transformed into the kinetic energy of the molecules in the seed. This energy is redistributed between the molecules and transformed into chemical energy, increasing the activity of chemicals in the seeds, as a result of which accelerates their growth and development. However, other indicators should be included in further studies in order to specify the exact duration of treatment.

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