

EFFECT OF PRE-TREATMENTS ON SEED GERMINATION RATE FROM DIFFERENT BALD CYPRESS (*TAXODIUM DISTICHUM* RICH.) TREES

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Abstract

In the 1950s, the interest of the professional public was drawn by *Taxodium distichum* Rich., as an allochthonous coniferous species in Serbia. It can be considered a fast-growing species, one of rare coniferous species that can be favourable for the afforestation of lowlands, and especially flooded sites, on which it can achieve high productivity. There is only one registered *Taxodium* seed stand in Serbia, located near Bačka Palanka, within FMU “Palanačke Ade – Čipski Poloj”, Compartment 11, Section a, area 0.22 ha, with 111 trees. The stand is about 70 years old, $d_s=51.7$ cm, $h_s=26.5$ m. The effect of four different pre-sawing treatments on *Taxodium* seed germination percentage was studied on five trees originating from the seed stand. Germination percentage was researched on a standard sample (4 x 100 seeds per tree), during 28 days. The study data were processed using the software “Statgraph 6.0”. The summary statistics was presented, the two-way analysis of variance for treatment and tree, and LSD-test. The study results show that seed immersion in citric acid (100 or 500 ppm) during 48 hours stimulates the germination percentage in laboratory conditions. Based on the two-way analysis of variance, it was confirmed that the differences in the number of germinated seeds was the result of the applied treatments, and not of the seed origin. Taking into account that this treatment is simple, inexpensive and harmless to human health and environment, it can be more widely applied in the production of *Taxodium* seedlings.

Key words: germination percentage, seed, treatment, citric acid.

Introduction

The genus of *Taxodium* Rich. was widely spread in Europe and North America in the past. Today, it grows on flooded soils in the Southeast and coastal regions of USA, from Louisiana to Florida. Based on ecological and botanical characteristic, three species are distinctive: *Taxodium distichum* Rich., *Taxodium ascendens* Brongn. and *Taxodium mucronatum* Ten

It was introduced in Europe in 1640, and is grown mainly as ornamental species. It could also be grown in forest plantations (Vidaković 1982). In Serbia, predominant is *Taxodium distichum* in urban parks and forests (Belgrade, Novi Sad, Vršac, etc.), although *Taxodium ascendens* was noticed in a park in Banja Koviljača (Tucović and Ocokoljić 2005).

Bald cypress as introduced species in Southeastern Europe becomes subject of

interest of forest scientists since the fifth decade of the last century (Petrović 1951, Špiranec 1959). In Serbia, it could be considered as fast growing species, one of the few coniferous species for planting on lowlands, especially for flooded sites on which it can reach a great productivity (Tucović and Stilinović 1970). Bald cypress has a great role in landscape architecture where it is grown in groups or individually on wet sites where other conifers can't grow.



Fig. 1. Seed stand of *Taxodium distichum* Rich. near Bachka Palanka.

Bald cypress is mainly regenerated by seeds, although it is possible to regenerate it by cuttings or stump shoots (Vidaković 1982, Lee et al. 1976, Prenger 1985, Conner et al. 1986, Conner 1988). Natural germination rate is small, 2–15 % (Monk and Brown 1965; USDA Forest Service 1965, 1974; Faulkner 1982; Gunderson 1984; Krauss et al. 1998). Because of that, a different pre-sowing treatments are desirable for stimulation of germination (Liu et al. 2009). Information about germination rate of Bald cypress grown in Serbia doesn't exist. In Serbia, it is hard to find trees in plantations or in groups with more than 50% of sound seeds (Stilinović 1985).

Material and Method

There is only one seed stand registered in Serbia, near town of Bachka Palanka, with registration number S 01.10.01.01, which is managed by Management Unit Novi Sad (Figure 1). Seed stand is located in FMU „Palanačke Ade – Čipski Poloj“, Compartment 11, Section a, with an area of 0.22 ha, with 111 trees. The stand is of artificial origin, about 70 years old, with average DBH 51.7 cm, and average height 26.5 m. The area is flat, at 80 m above sea level. The site is not flooded but it is

under strong influence of underground water. The site belongs to the coenological group of White willow and poplar forests (*Salicion albae* Soó) on undeveloped soils.

In September 2010, an estimation of seed-crop was executed together with an evaluation and selection of plus trees (Isajev and Mančić 2001). From the selected plus trees, seeds were collected in the same year. Seed extraction was done manually right after cone collection. After extraction, seed was stored in refrigerator at 3–5 °C for 90 days.

Effect of 4 different pre-treatments (labeled with I to IV) on seed germination rate was analyzed on seed from 5 Bald cypress genotypes (labeled with 1 to 5). Untreated seeds after cold stratification were labeled as control sample (K), Table 1. Germination was tested in Seed laboratory of Institute of Forestry in Belgrade, using Krstić germination table and following ISTA protocol (1996). Statistic analysis was done in programme Statgraph 6.0.

Results

Results presented in table 2 show germination rate of bald cypress seeds between 0.8 % (pre-treatment I) and 24.4 % (pre-treatment IV). Lowest germination

Table 1. Description of the applied pre-treatment.

Treatment label	Treatment procedure
I	Soaking of seeds in 65 ml of 97 % solution of ethanol for 5 minutes
II	Soaking of seeds in 65 ml of water, for 48 hours in thermostat cabinet at 30–35 °C
III	Soaking of seeds in 65 ml of 100 ppm solution of citric acid for 48 hours
IV	Soaking of seeds in 65 ml of 500 ppm solution of citric acid for 48 hours
K	Untreated seeds – control sample

rate is noticed with use of pre-treatment I, between 0 % and 4 %, with average value of 0.8 %. The sample treated with pre-treatment II shows results between 0 % and 5 %, with average value of 1.8 %. The sample treated with pre-treatment III shows results between 9 % and 40 %, with average value of 18.2 %. The best results are shown by sample treated with pre-treatment IV, with germination rate between 12 % and 37 %, with average value of 24.4 %. Control sample (K) shows germination rate between 0 % and

Table 2. Average seed germination rate ± standard deviation from different pre-treatments and trees.

Pre-treatment	Average	Tree	Average
I	0.8±1.78 ^a	2	5.0±6.40 ^a
K	1.0±1.24 ^a	3	5.4±6.99 ^a
II	1.8±2.05 ^a	1	10.6±15.27 ^a
III	18.2±12.93 ^b	5	11.2±11.52 ^a
IV	24.4±10.26 ^b	4	14.0±19.49 ^a
P-value	0.0001		0.2521

Note: Values in the same columns followed by different letters are statistically different at $p < 0.05$.

3 % with average value of 1 %. This result indicates necessity and importance of pre-treatments. The analysis of variance shows statistically significant differences between pre-treatments. LSD test shows grouping of pre-treatments in two homogenous groups, with clear separation of pre-treatments with citric acid.

Tree 1 shows germination rate between 0 % and 37 %, with average value of 10.6 %. Tree 2 shows germination rate between 0 % and 12 %, with average value of 5.0 %. Tree 3 shows germination rate between 0 % and 16 %, with average value of 5.4 %. Tree 4 shows germination rate between 0 % and 40 %, with average value of 14.0 %. Tree 4 shows germination rate between 2 % and 27 %, with average value of 11.2 %. Results of analysis of variance show absence of statistically significant differences between trees. Results of LSD test show grouping of all trees in one homogenous group.

Discussion

Nature of dormancy of bald cypress seeds is not well-known. Bald cypress seeds show moderate dormancy, which can be overcome with cold stratification (Bonner 2008). Bald cypress seeds show dormancy of seed-coat and any treatment, which softens and weakens seed-coat improve germination rate. This is supported with bald cypress seed anatomy, which is coated with fused flakes. The cause of inhibited germination is in acidic components of resin, which surround seed (Liu et al. 2009). Oils from bald cypress cones have some alcohol extracts consisted mainly from *d*- α -pinene, some *d*-limonene, and small amounts of carvone and seskvit-

erpene (Parry 1921). Most of these resin ingredients are hardly soluble in water and because of that inhibit water consumption during germination.

The lack of understanding of nature of dormancy brings a large number of chemical and physical pre-treatments recommendations for dormancy removal. One of the pre-treatments that give good results is soaking of bald cypress seeds in concentrated sulfuric acid for a period of 4 hours (Murphy and Stanley 1975). Alternative method requires cold stratification by soaking of seeds in water at temperature of 4 °C for 90 days, or until they start to germinate. Pre-treatment with sodium hydroxide gives satisfactory results because alkaline solution can neutralize acids from seed resin and increase germination like that (Liu et al. 2009). For *Taxodium mucronatum* Ten. seeds, pre-treatment with citric acid is shown as one of the best (Denny and Arnold 2007). Compared with these pre-treatments, the use of ethyl alcohol and hydrogen peroxide has a negative impact on germination rate (Denny and Arnold 2007, Liu et al. 2009). Besides chemical treatments, warming and mechanical scarification are recommended.

Conclusions

Natural germination of Bald cypress seeds is notified as very small, from just few to 15 %, as a result of seed dormancy. Seeds contain a larger amount of resin (Liu et al. 2009), which is probably cause of decreased consumption of water necessary for germination.

Results of this research show that pre-treatment of soaking seeds in 500 mg·l⁻¹ (500 ppm) solution of citric acid,

for 48 hours provide the highest germination rate. This is consistent with results of (Denny and Arnold 2007), on seed of *Taxodium distichum* Rich. var. *mexicanum* Gordon (syn. *T. mucronatum* Ten.). According to them, in absence of stratification, the best pre-treatment is soaking of seeds in 100 mg·l⁻¹ (100 ppm) solution of citric acid for 48 hours. The difference of solution concentrations, which gives best results, are probably caused by different amount of resin in seed. Amount of resin is genetically determined and it is consequence of adaptation on different environment. Because of that, the general recommendations should be avoided and the best pre-treatment should be recommended on population level. Differences between genotypes are not noticed and germination rate is determined by pre-treatment.

Pre-treatment with citric acid is simple, inexpensive and harmless for human and environment, and therefore can be recommended for wide use in Bald cypress seed processing.

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