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Full Length Research Paper

Seed germination enhancement for bald cypress [*Taxodium distichum* (L.) Rich.]

Guodong Liu[1], Yuncong Li[1]*, Marion Hedgepeth[2], Yongshan Wan[2] and Richard E. Roberts[3]

[1]Tropical Research and Education Center, University of Florida, 18905 SW 280th Street, Homestead, Florida 33031.
[2]South Florida Water Management District, 3301 Gun Club Road, West Palm Beach, Florida 33406.
[3]Florida Park Service, P. O. Box 1246, Hobe Sound, FL 33475.

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Bald cypress has been retreating in the estuary ecosystem of the Loxahatchee River Watershed in southeastern Florida because of the negative impacts of anthropogenic activities. Seed germination of bald cypress is an important means to increase the arbor population in the vegetation community and accelerate restoration of the wetland ecosystem. The objective of this research was to find an effective method to enhance seed germination of bald cypress, an ecologically and economically important tree species. Bald cypress seeds were hand collected under mature bald cypress trees in southeastern Florida, and kept under refrigeration at 4°C prior to the commencement of the study. The seeds were chemically treated in either 1% NaOH, 95% ethyl alcohol, 0.03 to 0.3% H_2O_2, or 1% HCl, or mechanically cut into two halves, or heated on a burner for 3.0 s. After treatment, all of the seeds were sown in Park's seed-starting trays with 72 Cells with Pro Mix BX growth medium. The results showed that soaking the seeds in a 1% NaOH solution for 5 min and then in H_2O for 24 h had the best germination rate of approximately 50%. However, heating the seeds on a gas burner was the least effective, resulting in almost no germination. Unlike the results seen in other species, H_2O_2 did not enhance germination in the seeds. Therefore, 1% NaOH was shown to be the best treatment because it could neutralize the acidity of the seed resin. Our study has verified that acidity was the chief limitation for germination of the seeds.

Key words: *Taxodium distichum*, germination enhancement, acidity of seed resin, germination rate.

INTRODUCTION

Bald cypress (*Taxodium distichum* [L.] Rich.) is a keystone species in ecology in Florida and commonly called bald cypress, cypress, southern-cypress, swamp-cypress, red-cypress, yellow-cypress, white-cypress, tidewater red-cypress, or gulf-cypress. It is a deciduous conifer growing on saturated and seasonally inundated soils of the Southeastern and Gulf Coastal Plain of the United States. Its range extends westward into Texas and northward into Illinois and Indiana (Kennedy, 1972). The trees are especially prized for their wood, of which the heartwood is extremely rot and termite resistant because a biochemical called cypressene (resin) is believed to act as a natural preservative heartwood. However, resin takes many decades to build up in the wood, making lumber cut from old-growth trees much more resistant to decay than lumber from younger trees (Sternberg and Wilson, 2004). For this reason, cypress wood, particularly from old-growth, virgin trees growing in the deep swamps, has long been favored in the building construction, fences, planking in boats, river pilings, furniture, interior trim, cabinetry, sills, rafters, siding, flooring and shingles, garden boxes, greenhouses, and many other uses (Brown, 1981). However, second-growth baldcypress lack the decay resistant heartwood of the old-growth trees (Compbell et al., 1960; Choong, 1986). At what age or size decay resistance develops is unknown, but wood from trees at least 63 years old is susceptible to rot (Choong, 1986). In Florida, as much as forty two million cubic feet of cypress timber has been harvested each year (Brown, 1995).

The unusual and pleasing appearance of bald cypress, its knees, buttressed base, massive bole, and irregular

*Corresponding Author Email: yunli@ufl.edu

Table 1. Methods used to improve seed germination for experiment 1 (Thirty seeds were used for each treatment).

Treatment I.D	Treatment
Control (CK)	Soaked in distilled water for 24 h
1	Soaked in 95% ethyl alcohol for 5 min and then in distilled water for 24 h
2	Soaked in 1% NaOH solution for 5 min and then in distilled water for 24 h
3	Treatment 1 plus treatment 2
4	Soaked in 1% HCl solution for 5 min and then in distilled water for 24 h
5	Treatment 1 plus treatment 4
6	Burned for 3.0 s over the flame of a standard laboratory gas burner and then in distilled water for 24 h
7	Cut the seeds into two halves with scissors and then in distilled water for 24 h

crown often festooned with Spanish moss, has led to its introduction as an ornamental in many parts of the world (USDA Forest Service, 1974; Brown and Montz, 1986). It is also of ecological importance for wildlife. Wild turkeys, squirrels, evening grosbeaks, wood ducks, other waterfowl and wading birds all eat its seeds as a part of their diet (Martin et al., 1951; Brunswig et al., 1983; Hamel 1983). Riverine swamps of bald cypress cause flood waters to spread out, slow down, and infiltrate the soil. Thus, these stands reduce flooding damage and act as sediment and pollutant traps (Wharton, 1977).

Bald cypress is of great ecological and economic significance in Florida (Williston et al., 1980; Black et al., 1993; Brown, 1995). However, these tough, tolerant, and somewhat idiosyncratic trees are at the heart of a fast-disappearing ecosystem. Over flooding and salinity has accelerated their disappearance. Actually, our recent research shows that this tree species can tolerate either 8‰ salinity (NaCl) or 100% root submergence but can't do both at the same time. Additionally, Monk and Brown (1965) found that bald cypress could not grow in soils with pH below 5.5. Thus, low pH of the resins might be the main factor retarding germination of the seeds.

Based on its irreplaceable function in the region's environment and economy, the species should be artificially multiplied and protected. Even though bald cypress is one of the few conifer species that sprouts and hence is capable of vegetative reproduction (Lee et al., 1976; Prenger, 1985; Conner et al., 1986; Conner, 1988), seed reproduction is the basic way for the species to be reproduced (USDA Forest Service, 1974). The seeds' natural germination is well documented (Monk and Brown, 1965; USDA Forest Service, 1965, 1974; Faulkner, 1982) and very low. For example, Gunderson (1984) found that only 2.1% seeds sown on logged and burned sites in the Corkscrew Swamp Sanctuary in Florida germinated. However, Krauss et al. (1998) got much greater germination rates. They examined the germination rate of bald cypress seeds held at salinities of 0, 2, 4, and 6 parts per thousand (ppt) seawater treatments. Mean germination rate under the seawater treatments were 26.3, 22.9, 15.4 and 10.2%, respectively. The mean germination across all salinity regimes was 14.2%. Similarly, Faulkner's germination rate was 15.5% (Faulkner, 1982). In general seeds from brackish water vegetation were greater success in germination rate than freshwater sources (Krauss et al., 1998). The results of the above studies showed that the greatest germination rate was about only one germinated out of 4 seeds. The germination rates of other plant seeds were improved by dilute acids and hydrogen peroxide because dilute acids could induce de-dormancy of the seeds and hydrogen peroxide could greatly ameliorate bioavailability of oxygen for the seeds (Durham and Wellington, 1961). Nevertheless, there is little information about their effects on germination of bald cypress seeds available based on our accessibility to related literature. Obviously, the seeds' artificial germination is still poorly understood. Therefore, the objective of this research was to

(1) Study the effects of chemical factors such as acidity, alkalinity, hydrogen peroxide, and organic solvent and of physical factors such as heat and mechanical cut on germination of bald cypress seeds.
(2) Compare and find out the best among the different treatments.

MATERIALS AND METHODS

Bald cypress seeds are different from regular seeds and are embodied in scales. They have acidic resins surrounding the seeds. Therefore, different chemical and physical factors were employed to dissolve the resins and enhance the seed germination. The chemical and physical factors included dilute hydrochloric acid, sodium hydroxide, hydrogen peroxide, and organic solvent as ethyl alcohol, heat, and mechanical cut. The seeds were hand collected from the ground under mature bald cypress trees in southeastern Florida and kept under refrigeration at 4^{o}C prior to experiments for four months. Then two experiments were conducted as follows.

Experiment 1: This experiment was started in 9 cm plastic dishes with three replicates on August 2, 2004. Treatments were described in Table 1.

After treatment, all of the seeds were sown in Park's Seed-Starting Trays with 72 Cells with Pro Mix BX growth medium. Then seedlings were counted daily and the seedling heights were mea-

Table 2. Methods used to improve seed germination for experiment 2 (Thirty six seeds were used for each treatment).

Treatment I.D	Treatment
Control (CK)	Soaked in distilled water for 24 h
A	Soaked in 4% NaOH solution for 24 h and then in distilled water for 24 h
B	Soaked in 2% NaOH solution for 24 h and then in distilled water for 24 hr
C	Soaked in 0.5% NaOH solution for 24 h and then in distilled water for 24 h
D	Soaked in 0.3% H_2O_2 + 95% alcohol for 5 min and then in distilled water for 24 h
E	Soaked in 0.03% H_2O_2 + 95% alcohol for 5 min and then in distilled water for 24 h

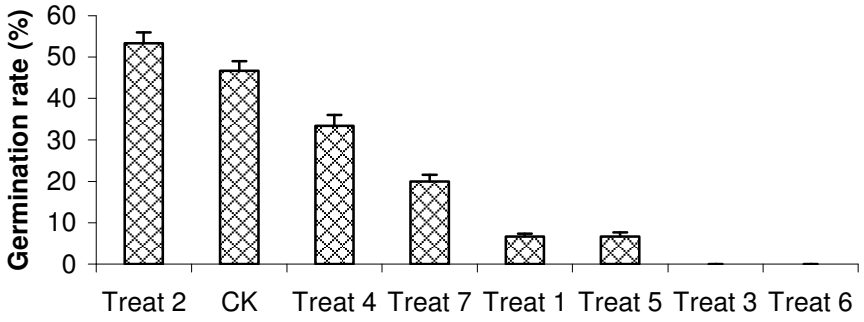

Figure 1. Germination rates (±SD) of bald cypress seeds treated with various treatments in Experiment 1.

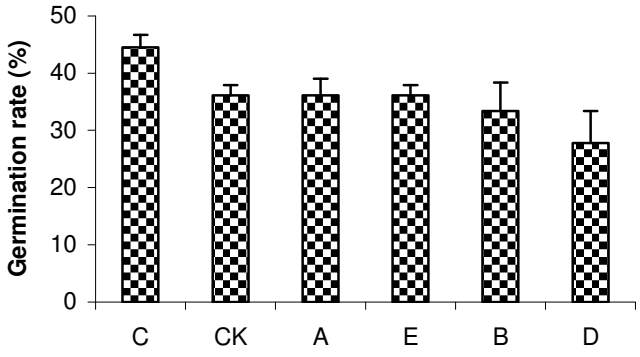

Figure 2. Germination rates (±SD) of bald cypress seeds treated with various treatments in Experiment 2

ured weekly or biweekly. Then seedlings were transplanted individually into one-gallon plastic pots on October 14, 2004.

Experiment 2*:* This experiment was started in 9 cm plastic dishes with three replicates on November 22, 2004. The treatments and methods are shown as follows (Table 2).

After treatment, seeds were sown in 6 Park's Seed-Starting Trays with 72 Cells with Pro-Mix BX growth medium. Then seedlings were measured as described in the experiment 1 and then again transplanted into one-gallon plastic pots with Pro-Mixl growth medium on January 3, 2005.

RESULTS AND DISCUSSIONS

Treatment 2 (Soaked in 1% NaOH solution for 5 min and then in distilled water for 24 h) was the only treatment performed better than the control (CK) in Experiment 1 (Figure 1). Similarly, there was only Treatment C in Experiment 2 performed better than the CK (Figure 2). The better-performing treatment in both Experiments 1 and 2 was that treated with dilute sodium hydroxide. This suggested that dilutes alkaline solution promoted germination of the seeds. The pH of bald cypress seeds extracted with 95% alcohol was measured in our lab and the value was 5.36±0.14 because the acidic resins contained in the seeds possibly inhibit germination of the seeds. Alkaline solution could neutralize the acid of seed resins and improve the germination rates. These two separate experiments showed that the seeds treated with dilute NaOH solution germinated better. This may imply that the NaOH solution neutralized the acidity from the resins wrapping the seeds because the resins have high acid numbers (Coppen and Hone, 1995). Actually, Odell (Parry, 1921) examined the oil from the cones of bald cypress and found that the alcohol-extractant contained 85% of *d*-α-pinene, 5% of *d*-limonene, small amounts of carvone, and a sesquiterpene, which is probably cypressene, and probably some isovaleric acid with a pK_a of about 4.84. A majority of the components in the resins of cypress seeds are resistant to water. This may markedly inhibit the absorption of water when the seeds are soaked in water. The acid in the resins is almost as strong as acetic acid with pK_a of 4.76. Additionally, backwater streams are generally acidic with pH 4.0 to 6.0 (FNAI and DNP, 1990)

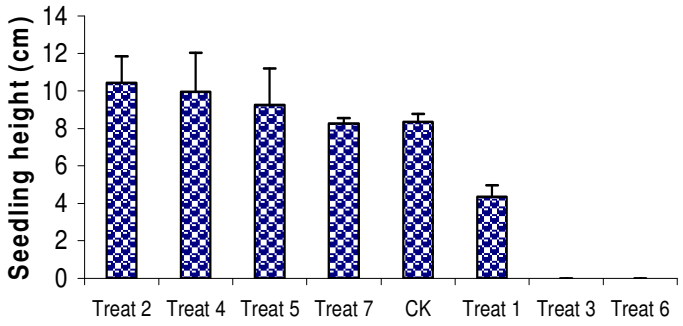

Figure 3. Bald cypress seedling heights (±SD) in Experiment 1 on the 30th day after emergence.

This may strengthen acidity in the cone of bald cypress.

These factors may be reasons for the low germination rate of the seeds. Therefore, the alkaline solutions accelerated the seed germination in this study. This may attribute to neutralization of the acidity in the resins and facilitation of dissolving of the resins (Parry, 1921). If this is the determining factor accelerating seed germination, such findings can greatly benefit the nursery industry of bald cypress. Consequently, this may be able to enhance the restoration of the species. However, ongoing and larger scale research is needed to verify these findings.

The results on this research showed that bald cypress seeds did not have the problem of dormancy after a few months' storage in refrigeration. Additionally, bald cypress is very tolerant to hypoxic stress. Thus, its seeds might be also able to bear very low-oxygen conditions. Therefore, no additional oxygen was required during the germination of the seeds. Obviously, hydrogen peroxide did not work on the germination of the bald cypress seeds in this research.

Soaking bald cypress seeds in alkaline solutions is an easy process and can be handled by any workers and it will greatly benefit the nursery industry of bald cypress because cuttings and air layering are currently the main methods for propagation of bald cypress. Accordingly, this will accelerate the restoration of the species in conservation area.

The height of Treatment 2 in Experiment 1 was the tallest and markedly taller than that of the CK.

However, Treatment 1 was much shorter than that of the CK. The seeds were heated on the flame of a gas burner in Treatment 6 showed no growth. Treatment 1 was where the seeds were soaked in 95% alcohol for 5 min. Both alcohol and heating might impact on seeds negatively (Figure 3).

Conclusions

The germination rate of bald cypress seeds could be artificially changed. Physical treatments of mechanical cutting or heating impacted the germination of the seeds negatively. Chemical treatments with acid, base, and hydrogen peroxide could be categorized into two: positive and negative groups. Either of ethyl alcohol, 1% HCl or 0.03 to 0.3% H_2O_2 didn't improve the germination. The effects of NaOH solution depended on the concentration. Higher concentration (over 1%) of NaOH didn't increase the germination. However, dilute NaOH solution (0.5 to 1.0%) enhanced germination of bald cypress seeds and growth of its seedlings. Therefore, dilute NaOH solution can be, based on this research, applied to the increase of seed germination and seedling growth of bald cypress.

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