Jamie L. Whitten Plant Materials Center

Coffeeville, MS

Vol. 14 No. 1 Technical Report April 1999

Evaluation of Sulfuric Acid Scarification for Improving Germination of Yellow Lotus Seed

Janet Grabowski

ABSTRACT

Yellow lotus is an attractive flowering wetland plant that has several potential uses. It can be propagated both vegetatively and by seed; however, vegetative propagation may not be feasible for some plantings due to the large size of the plant. Seed requires scarification for proper germination and an alternative to the available mechanical scarification methods is needed. Scarifying the seed by soaking in concentrated sulfuric acid for one hour was tested. This method provided superior germination percentages compared to mechanically scarifying the seed with a file in one of the two treatment years. Additional refinement of soaking intervals may provide more consistent improvements in germination. The seed responded negatively to cold stratification following the scarification treatment and should be planted without further treatment. After germination, water levels on the germination bench should be maintained so that the leaves will be held above the surface of the growing medium to avoid the seedling mortality that was exhibited in this experiment.

INTRODUCTION

Fig. 1. Yellow lotus flower

Nelumbo lutea (Willd.) Pers., known variously as yellow lotus, American lotus, water chinquapin, or pond nuts, is a spreading perennial wetland herb with flowers and leaves arising directly from large, fleshy rhizomes. It has large (up to 2 foot wide), round, bluish-green, glaucous leaf blades held on long, stiff petioles, with some of the leaf blades floating on the water surface and others emergent above the water surface. The large, solitary flowers are held above the leaves on stiff stalks; the petals are pale creamy yellow in color, with numerous darker yellow stamens surrounding a golden yellow, enlarged, flat-topped receptacle (Figure 1). As the fruits mature, the recptacle becomes brown, to blue or black in color and contains numerous embedded hard, rounded, gray to black, indehiscent, nut-like fruits (Godfrey and Wooten, 1981). Hereafter, these indehiscent fruits will be referred to as seed. Yellow lotus is native to eastern and central portions of the US and southern Ontario. Sacred lotus or East Indian lotus, *Nelumbo nucifera* Gaertn., is a similar species, native to southern Asia and Australia, that is often cultivated in the US; it can be

Janet Grabowski is a Biologist at the Jamie L. Whitten Plant Materials Center, 2533 County Road 65, Coffeeville, Mississippi 38922-2652. Phone: (662) 675-2588; FAX: (662) 675-2369.

USDA NRCS Natural Resources Conservation Service

Homer L. Wilkes, State Conservationist
Jackson, Mississippi

Plant Materials Program

distinguished from yellow lotus by its larger plant size and pink or sometimes white flowers (Bailey Hortorium, 1976).

Because of their showy flowers and leaves, both lotus species have been recommended for planting in water gardens (Dobbs, ND) and due to their rhizomatous nature they are also recommended for shoreline plantings (St. Johns River Water Management District, 1996) However, the large size and widely spreading nature of yellow lotus has also earned it a reputation as a weedy plant in some situations, and research has been done to determine chemical control methods (Brunson, 1998). Yellow lotus was once an important Native American food source and sacred lotus is widely grown in the Orient for its edible rhizomes and seed (Bailey Hortorium, 1976). Yellow lotus does have some wildlife benefits; the seed is sometimes eaten by waterfowl, and the plants will attract several bird species, also the rhizomes may be eaten by beaver and the plants provide shade and shelter for fish (Fassett, 1957).

Yellow lotus can be propagated by division of the rhizomes (Bailey Hortorium, 1976); however, due to the large size of the plant, a fairly large planting piece is required, which complicates the planting operation (Figure 2). Yellow lotus can also be propagated by seed; however, the fruit wall or pericarp is very hard and requires scarification for proper germination (Bailey Hortorium, 1976). This hard pericarp is a very effective dormancy mechanism and seed viability can be maintained almost indefinitely. Sacred lotus seed over 500 to more than 1000 years old have been reported to germinate following scarification (Maeda et al., 1996; Shen-Miller et al., 1995). Scarifying the seed by mechanical means is difficult. Its large size precludes the use of most scarifying machines because the seed has a tendency to catch in the mechanisms and be broken or damaged, and the rounded shape of the seed makes manual scarification difficult because there is no easy way to secure the seed while sanding or filing to break through the seed coat. Besides, manual scarification is only practical for small lots of seed. There have been reports of using acid scarification to break dormancy of sacred lotus seed (Maeda et al., 1996); however, the effect of acid scarification on yellow lotus has not been as widely reported. Therefore, a study was conducted to compare germination of yellow lotus seed scarified by soaking in concentrated sulfuric acid to seed that was manually scarified, and to determine the effect of cold stratification on scarified seed.

Fig 2. Shoot with attached rhizomes

MATERIALS AND METHODS

Seed was collected in September 1996 from plants either growing in wet cells at the Jamie L. Whitten Plant Materials Center (PMC), Coffeeville, Mississippi or from the original population of plants growing in the basin of Grenada Reservoir. The original collection site was located in Yalobusha County, Mississippi and the plants were assigned the accession number 9077064. Seed was removed from the surrounding dried receptacle, manually sorted to remove seed that had visible signs of weevil feeding, and placed in a seed cooler maintained at 55°F and 45 percent relative humidity.

The study was conducted in 1997 and repeated in 1998. Seed treatments used were mechanical scarification (Mech. Scar.), done by holding the seed in a pair of vise-grips and filing the seed on one side until the white tissue of the cotyledons was visible, and acid scarification (Acid Scar.), by soaking the seed in concentrated sulfuric acid for one hour. Maeda et al. (1996) showed good germination of sacred lotus seed after a six hour acid soak, but previous work at the PMC, in which seed was periodically removed from the acid treatment and opened to examine the effect on the fruit wall, showed that a one hour soak appeared to reduce pericarp thickness

sufficiently to promote germination. Seed from both scarification treatments were also exposed to a four to five month cold stratification treatment (Strat.). There was not an untreated control, because it has been previously shown that non-scarified seed will not germinate. Each treatment consisted of 20 seed and the study was arranged as a randomized complete block with three replications. Germination containers used were 7 inch x 5-1/4 inch x 2-5/16 inch plastic bedding plant liners and the seed was planted ½ inch deep in the growing media. Growing media used was a 3:1 mix of peat moss/sand amended with commercially recommended quantities of pelletized slow-release fertilizer, dolomitic lime, Micromax micronutrient fertilizer, and Aquagro wetting agent. The sand was pasteurized before use in an electric soil sterilizer that was heated to 180°F for approximately 30 minutes. The stratification treatments consisted of planting the seed in their germination containers, irrigating until the media was thoroughly moistened, placing them in a cooler maintained at 42°F with no humidity control, and watering them as needed throughout the stratification period. For the 1997 test, seed was scarified and stratified on November 21, 1996 and moved to the greenhouse on March 27, 1997, the same day the non-stratified seed were treated and planted. In 1998, the stratification date was January 9, 1998 and the test date was April 8, 1998. The germination containers were placed on a bench where water levels were maintained at a depth of ¼ to ½ during the study period, except for short periods of time when the benches were drained and flushed to remove excess algae.

Germination counts were made when a sufficient number of seedlings were present to warrant counting, which was April, 17, 1997 and April 24, 1998, and at two additional three week intervals thereafter. In the later counts, some of the seedlings had begun to spread and were somewhat difficult to count accurately. Germination percentages were calculated for the three germination counts and the total germination for each treatment and these values were subjected to an analysis of variance. Appropriate mean separation was determined by using a Tukey's honestly significant difference test (HSD) at the five percent level of probability.

RESULTS AND DISCUSSION

The results from the 1997 test are presented in Table 1. Manual scarification significantly increased germination percentages compared to the other treatments. No visible signs of toxicity were apparent on any of the acid scarified seedlings in either year of the study. Germination was poor for both stratification treatments. The treated seed appeared to be susceptible to deterioration during the stratification period and a great deal of fungal growth was noted for both treatments and both study years. Germination counts for the scarification treatments decreased drastically by the third count. The seedlings grew in a prostrate manner and seedling mortality was due to deterioration of leaves and shoots lying on the surface of the growing medium (Figure 3). Surviving plants were infested with aphids, a problem that was probably exacerbated by plant stress. In nature, the leaves of young seedlings would have been held above the soil surface by standing water. The shallow depth of the germination bench used in these tests would not allow elevation of the water level to prevent this problem.

Table 1. The effect of mechanical and acid scarification and stratification treatments on the germination percentage of yellow lotus seed planted in 1997.

Treatment	1st Count	2nd Count	3rd Count	Total
	----------%----------			
Mech. Scar.	68a*	28a	5	67a
Mech. Scar + Strat.	0c	0b	0	0c
Acid Scar.	17b	20ab	3	22b
Acid Scar. + Strat.	8bc	7ab	3	12bc

*Treatment means in each column followed by the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$.

Table 2 lists the results of the 1998 test. In this year, germination was significantly better for the acid scarification treatment, and both scarification treatments were better than the scarification plus stratification treatments. The reason for the different response to acid scarification shown for the two treatment years is not known. The same seed lot was used in both years, so the thickness of the seed coat should not have varied significantly between study years. A clock rather than a timer was used to monitor the treatment times, so there could possibly have been a few minutes difference in the soaking interval between the two treatment years and therefore, human error could have caused the variable response. Also, there could have been some damage to the seedling caused by the mechanical scarification treatment that reduced germination percentages for that treatment. Shen-Miller et al. (1995) filed the sacred lotus seed they tested only at the proximal end near the point of attachment to the receptacle because that is the only area where the cotyledons do not adhere to the pericarp. With the crude apparatus used to secure the seed in this test, there was no way to ensure such an exacting placement of the scarification treatment. They also only filed until the pink colored testa of the seed was visible whereas the seed in this test were filed until deeper tissues were exposed. Germination counts for the acid scarification treatment did not decrease as severely by the third germination count as in the first study year, but overall seedling mortality was significant over time. The probable reasons for this were discussed above.

Table 2. The effect of mechanical and acid scarification and stratification treatments on the germination percentage of yellow lotus seed planted in 1998.

Treatment	1st Count	2nd Count	3rd Count	Total
	----------%----------			
Mech. Scar.	27ab*	0b	0b	27ab
Mech. Scar + Strat.	0b	0b	0b	0b
Acid Scar.	45a	40a	22a	45a
Acid Scar. + Strat.	3b	0b	2b	5b

*Treatment means in each column followed by the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$.

CONCLUSIONS

Acid scarification does appear to be a practical method of treating yellow lotus seed to promote germination. A soaking time of one hour appeared to be sufficient in one treatment year, but not the other. Additional testing may be required using varying soaking durations to determine the optimum periods. Once the pericarp is broken, the seed is ready to germinate immediately and it does not benefit from a cold, moist stratification period. Seedling mortality, which was exhibited throughout the experiment, would likely have been reduced if water levels on the germination bench had been increased to elevate the developing leaves and shoots above the surface of the growing medium.

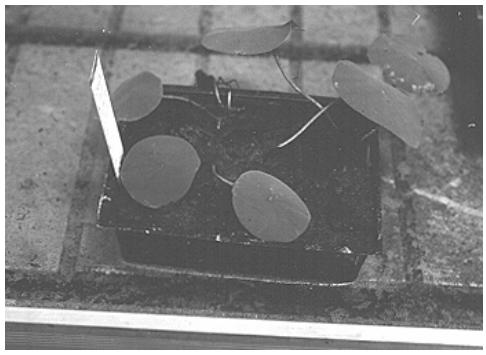

Fig. 3 Germination container with seedlings

LITERATURE CITED

Bailey Hortorium. 1976. Hortus III. Macmillan Pub. Co., New York, NY. 1290 pp.

Brunson, M.W. 1998. Aquatic weed identification and control. Miss. State Univ., Coop. Ext. Service. http://ext.msstate.edu/pubs/is1032.htm. 3 pp.

Dobbs, S. ND. Water gardens: installation and maintenance. Univ of Georgia, Coop. Ext. Service. http://www.ces.uga.edu/agriculture/horticulture/watergarden.htm. 10 pp.

Fassett, N.C. 1957. A manual of aquatic plants. Univ. of Wisc. Press, Madison, WI. 405 pp.

Godfrey, R.K. and J.W. Wooten. 1981. Aquatic and wetland plants of southeastern United States dicotyledons. Univ. of Georgia Press, Athens, GA. 933 pp.

Maeda, Y., T. Minamikawa, B.M. Xu. 1996. Metabolic activities in germinated ancient lotus seeds. J. Exp. Bot., 47(297): 577-582.

St. Johns River Management District. 1996. Shoreline Plants. StreamLines, Summer Issue. http://sjr.state.fl.us/info/streamln/su96sln3.htm. 2 pp.

Shen-Miller, J., M.B. Mudgett, J.W. Schopf, S. Clarke, R. Berger. 1995. Exceptional seed longevity and robust growth: ancient sacred lotus from China. Am. J. Bot., 82(11): 1367-1380.

NOTES