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Germination of *Stewartia pseudocamellia* Seeds is Promoted by Desiccation Avoidance, Gibberellic Acid Treatment, and Warm and Cold Stratification[1]

Brian A. Oleksak[2] and Daniel K. Struve[3]
Department of Horticulture and Crop Science, The Ohio State University
Columbus, OH 43210-1086

Abstract

Japanese Stewartia, *Stewartia pseudocamellia* (Maxim.), seeds were extracted from immature capsules and handled under nondesiccating conditions. Moisture content of fresh seeds was 82%. After exposing seeds for 48 hrs at room conditions seed moisture content was 56% and after 24 hrs at 80C (176F) it was 49%. Both ambient and 80C (176F) drying conditions resulted in complete loss of viability based on a tetazolium test. Exposing seeds to a 24 hr aerated water soak in 1 mM gibberellic acid (GA_3), and a 3 month warm (25C, 77F) stratification period increased seed germination only after 6 months of cold (4C, 40F) stratification compared to seeds given a 24 hr aerated water soak without GA_3 and similar warm and cold stratification periods. Best management practices for handling and germination of fresh Japanese Stewartia seeds are: (a) harvest seed capsules when they turn from green to brown, (b) maintain seeds under nondesiccating conditions, (c) treat seeds with a 24 hr aerated water soak in 1 mM GA_3 before a 3-month warm stratification, and (d) provide at least 7 months cold stratification.

Index words: seed dormancy, double dormancy, seed desiccation, sexual propagation.

Significance to the Nursery Industry

Japanese Stewartia has many desirable landscape characteristics but is considered difficult to propagate either sexually or asexually. Japanese Stewartia seeds were found to be sensitive to desiccation; viability was lost when seeds were exposed for 2 hr to room conditions. Thus, Japanese Stewartia's reputation for having difficult-to-germinate seeds may be attributed to loss of seed viability caused by desiccation before dormancy breaking treatments are initiated. Seeds germinated rapidly and at a relatively high percentage (51%) when given a 24 hr aerated water soak in 1 mM GA_3 solution followed by 3 months warm and 7 months cold stratification periods.

Introduction

Japanese Stewartia is a summer flowering tree species that was introduced to North American gardens from Japan. It attains a mature height of approximately 23 m (50 ft) with a loosely ovoid form, frequently being multi-trunked. The bark exfoliates from the trunk to reveal underlying tones of pink to cinnamon brown, offering color to otherwise bleak winter landscapes. The fall color display is equally brilliant as the light green leaves change to orange, yellow and brick red. In midsummer Japanese Stewartia bear masses of 8 cm (3 in), white fragrant flowers, reminiscent of *Camellia japonica*.

Despite these attractive attributes, Japanese Stewartia is not commonly offered in the nursery industry due to difficulties in propagation. Though softwood cuttings may root when treated with 2000 to 4000 ppm indole butyric acid (IBA), they often fail to break bud the following spring (4). Seeds are described as 'double dormant', with the embryo and its surrounding integuments suspected of maintaining the dormant state (8). The standard nursery practice for production of Stewartia seedlings is to sow seeds outdoors in the fall, then allow 2 years for seedling emergence (6).

Pretreatment to encourage higher and more uniform germination of Stewartia seeds has focused on alleviating dormancy. An understanding of viability loss in Stewartia seeds is of equal importance to dormancy constraints since the seeds have been described as 'short-lived' (5, 9).

Stewartia is a member of *Theaceae*, a family comprised of predominantly tropical and sub-tropical species (3). Seeds of warm latitude species often exhibit 'recalcitrant' storage behavior—that is, the inability to be dried to moisture contents below 30% without suffering viability loss—and short storage life (2). If Stewartia seeds are recalcitrant, then the practice of drying the capsules to extract the seeds (5, 10) needs to be reexamined. Also, the low germination percentages previously ascribed to seed dormancy may be attributed to loss of viability caused by desiccation before dormancy breaking treatments are begun. The objectives of this study were to: 1) determine the effects of drying on seed viability of Japanese Stewartia and 2) better define seed dormancy breaking treatments.

Materials and Methods

Seed collection. Capsules were collected in mid-September from 3 trees at The Dawes Arboretum, Newark, OH. The fruits appeared green and slightly pubescent with the calyx tightly furled. Seeds were removed from the capsules using a razor blade to slice a 5 mm (0.25 in) piece from the base and the tip of the capsule. The razor blade was then inserted into an exposed locule and twisted to crack the woody capsule. Each locule contained approximately 8 seeds, of which 4 to 5 were plump and purplish in color. Seeds that were brown and shriveled were discarded. Seeds were placed immediately in a closed plastic box filled with moist sand at 25C (77F). The seeds were maintained under these conditions until they were used in germination and viability tests. Prior to germination and viability tests, sound seeds were

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[2]Former graduate student.

[3]Associate Professor, to whom all correspondence should be addressed.

separated from unsound seeds by floation. Only sound seeds were used in the studies.

Seed moisture loss and seed viability test. Fifty seeds were removed from warm moist stratification conditions [25C (77F)] and high relative humidity (there was condensation on the germination boxes indicating a saturated atmosphere) and divided into 2 groups of 25 seeds to determine moisture loss when exposed to desiccation conditions. Two desiccation conditions were used: 1) drying oven: exposing seeds to 24 hr at 80C (176F) and 2) air drying: exposing seeds to room conditions on a laboratory bench for 48 hr at 25C (77F) and 50% relative humidity. Room relative humidity was measured with a Bacharach Compact Sling Psychrometer (Bacharach Instrument Co., Pittsburgh, PA). Individual seeds were weighed prior to and after the drying treatments. Seed dry weight was estimated by weighing another 50 individual seeds, placing them in a drying oven at 80C (176F) for 10 days, and recording their dry weight. Percent seed moisture content was determined by subtracting dry weight from fresh weight, dividing by dry weight and multiplying by 100.

Viability of dried seeds was compared to nondried seeds via a tetrazolium test. Immediately after drying, oven-dried and air-dried seeds were placed in separate flasks and given a 24 hr aerated water soak. Following the water soak, a sagittal cut was made to remove the woody wing and expose the embryo. Seeds from each treatment were placed in petri dishes and covered with a 10% aqueous solution of 2,3,5-triphenyltetrazolium chloride (TZ). The petri dishes were covered and maintained at 38C (100F) for 24 hr. Embryos were excised from the surrounding tissue and observed under a 10× binocular dissecting scope for staining patterns. For comparison, nondried seeds were removed from warm stratification conditions described earlier, weighed to determine fresh weight, and treated with an aerated water soak and TZ as before. Their initial moisture content was estimated as previously described.

Germination test. Another subset of 2000 seeds was selected and divided into two groups, one received a 24 hr soak in 1mM (1000 ppm) GA_3 (Sigma Chemical Co., St. Louis, MO), the other received a 24 hr aerated water soak. Upon completion, seeds were removed and placed on double layered germination paper (Anchor Paper Co., Eau Claire, WI) in two replications of 100 seeds per treatment. Each treatment was covered with a third layer of paper, rolled and sleeved in polyethylene bags to retain moisture. All treatments were then exposed to a 3-month warm (25C, 77F) stratification period. Following warm stratification, both GA_3 and water soaked treatments were placed in a cooler at 4C (40F) for cold stratification of 3, 4, 5, 6 and 7 months. Following the designated length of stratification, the treatments were returned to 25C (77F) for germination. Germination counts were taken daily for 2 weeks. Germination was defined as extension of the radicle to at least 1 cm (0.4 in).

Results and Discussion

Seed moisture loss. Moisture content of nondried warm stratified seeds was 82 ± 2%. After 48 hr of air drying at room temperature, moisture content was 56 ± 3%; after oven drying 24 hr, it was 49 ± 2%. Thus, when exposed to mildly desiccating conditions such as those encountered during harvest and cleaning, Japanese Stewartia seed may lose moisture comparable to oven drying.

Tetrazolium test of seed viability. The salt 2,3,5-triphenyltetrazolium chloride, which is used in the TZ test, is colorless when dissolved in water but forms formazan, a red insoluble pigment, when in contact with dehydrogenase enzymes associated with viable, respiring tissue. Viable embryos are stained pink to scarlet, while white, unstained tissue is nonrespiring and therefore nonviable. Embryos removed from nondried seeds were stained completely red while the cotyledons and hypocotyl regions appeared pink. The radicle stained brightest red with the most intense red appearing near the tip. Embryos removed from oven-dried seeds were unstained, indicating that the high desiccation conditions were sufficient to destroy embryo viability. Embryos removed from air-dried seeds were also unstained, though a slight pink blush color was noted near the radicle

Table 1. Germination of Japanese Stewartia seed following a factorial treatment combination of a 24 hr aerated water soak or a 24 hour aerated water soak in 1mM GA_3 and 3 to 7 months cold (4C, 40F) stratification after a 3-month warm (25C, 77F) stratification period.

Aerated water soak (mM GA_3)[z]	Cold stratification (months at 4C)	Germination at 24 hours (%)	Final germination (%)	T_{50}[x]
0	3	0a[y]	0a	na[w]
	4	0a	0a	na
	5	1a	1a	1
	6	9b	15b	1
	7	2a	6b	2
1	3	0a	0a	na
	4	0a	0a	na
	5	1a	1a	na
	6	17c	23c	1
	7	40d	51d	1

[z]Seeds were given either a 24-hr aerated water soaked or a 24-hr aerated water soak in 1 mM GA_3 before a 3-month warm (25C, 77F) stratification period followed by various cold stratification periods.

[y]Germination percentages are the means of two 100-seed replications. Means within a column followed by different letters are significantly different from each other at the $\alpha = 0.05$ level using the Student-Newman-Keuls test.

[x]T_{50} days to reach 50% of final germination percentage.

[w]na is not appropriate due to the low percent germination.

tip. Because formazan is insoluble, it remains compartmentalized within viable cells allowing a more informative estimate of damage within embryonic organs (7). Embryos removed from air-dried seeds sustained additional physical damage as evidenced by broken cotyledons.

Seed germination test. A 24 hr aerated water soak in GA_3 significantly increased germination only after exposure to at least 6 months cold stratification following a 3-month warm stratification period (Table 1). After 6 months cold stratification, seeds given a 24 hr GA_3 soak had 23% germination, while those receiving an aerated water soak without GA_3 had 15% germination. The differences were greater after 7 months cold stratification, 51 vs 6%. Germination was rapid; 40% germination occurred within the first 24 hr at 25C (77F) and 51% germination occurred within 72 hr. Time to 50% final germination was 1 or 2 days (Table 1). These results suggest that synchronous seed germination is possible for Japanese Stewartia, although germination completeness is relatively low.

Cold stratification influenced embryo length and cotyledon morphological development in Japanese Stewartia embryos. The average embryonic length at the time of seed harvest in September was 2.0 mm (0.75 in). Embryos excised from seeds that received a combination of 3 months warm and 6 months cold stratification averaged 6.5 mm (0.4 in) in length, while embryos that had received only warm stratification for 9 months averaged 3.0 mm (0.2 in) in length. The warm stratified seeds remained at 25C (77F) since harvested in September. Embryos from warm stratified seeds appeared flat and somewhat spatulate. Embryos from warm and cold stratified seeds had plump cotyledons that curved backward and showed a slight invagination down the center.

The necessity for cold stratification to overcome embryo dormancy is a common requirement for plant species from temperate regions. The ability to withstand desiccation is also an important survival mechanism. With the exception of recalcitrant species, the seeds of many tree species may be dried to thresholds of 10–12% moisture; the seeds of conifers may be dried even lower (1). Seeds of Japanese Stewartia require both a warm and a long cold stratification period to alleviate dormancy, typical of many northern species, and are recalcitrant, typical of tropical tree species.

The following recommendations for germination of Japanese Stewartia seeds are: (a) harvest capsules when they turn from green to brown and are still non-dehisced; (b) maintain seeds under non-desiccation conditions at all times; (c) treat the seed with a 24 hour aerated water soak in 1 mM GA_3 before warm stratification; and (d) follow a 3 month warm stratification period with at least 7 months cold stratification.

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