Embryo Cap Removal and High-temperature Exposure - Stimulate Rapid Germination of Needle Palm Seeds

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Abstract. High synchrony, rate, and germination of needle palm [*Rhapidophyllum hystrix* (Pursh) H.A. Wendle & Drude] seeds were achieved only after removing the sclerotesta and embryo cap, which imposed physical dormancy. After scarification, recently harvested seeds or seeds stored for 12 months at 5C and 100% relative humidity had 96% and 98% final germination (G), with 9 to 11 days required to achieve 50% of final germination (T_{50}) at 30C. Germination temperature controlled G, T_{50} , and days between 10% and 90% of final germination ($T_{90} - T_{10}$) of scarified seeds, with respective values of 98% 9 days, and 5 days at 30C, and 18%, 31 days, and 12 days at 15C. Seeds with 36% moisture at harvest had no reduction in G until moisture was <14%. Germination of seeds with 19% moisture declined from 80% if stored at 0C to 33% if stored at -10C; no seeds germinated after storage at less than -10C.

Needle palm is a low, bushy palm, with single to multiple trunks and medium foliar texture, that reaches a mature height of 2 to 3 m. It is native to the coastal plain of the southeastern United States, with distribution from central Florida, north to Georgia, and west to Mississippi (Shuey and Wunderlin, 1977). *Rhapidophyllum* seeds require 6 months to 2 years to germinate (Clancy and Sullivan, 1988; Popenoe, 1973; Shuey and Wunderlin, 1977; Wagner, 1982). Clancy and Sullivan (1988) reported that seeds scarified after stratification required 435 days for the first seed to germinate and 530 days from sowing to 14.3% final germination (G). Wagner (1982) obtained 6.7% G 195 days after sowing and reported that seed scarification did not increase G or promote earlier germination. To our knowledge, no comparison of needle palm seed germination at various temperatures has been reported. The purposes of this research were to 1) evaluate the effectiveness of several methods of mechanical seed scarification, 2) determine the best germination and seed-storage temperatures, and 3) establish the tolerance levels of seeds to subzero temperatures or partial desiccation. To achieve these objectives, four separate studies were conducted.

Scarification of stratified and nonstrati-

fied seeds. Needle palm seeds were collected from natural stands in north-central Florida during Fall 1990 and 1991. In this and subsequent studies, recently harvested seeds were scarified mechanically by removing, with a scalpel, 2 × 2-mm portions of the sclerotesta to 1) expose the endosperm in areas away from the embryo, 2) show the embryo cap, or 3) remove the embryo cap (Fig. 1). The fourth treatment contained nonscarified seeds. Each treatment consisted of four 15-seed replications. These four treatments later were repeated using seeds stored for 12 months at 100% relative humidity (RH) and 5C. In all studies, seeds were surface-sterilized before being scarified by successive immersion in 50% (by volume) ethanol for 1 min and 2.6% sodium hypochlorite solution for 12 min, followed by three rinses in sterile deionized water (Kane et al., 1988). After scarification, seeds were immersed in 0.5% sodium hypochlorite solution for 12 min and blotted dry before being placed in moist, sterile peatmoss that had been autoclaved twice previously at 105C for 1 h. Each replication of 1.5 seeds was sealed in a heat-sealed pouch (Kapak Corp., Minneapolis). Seeds were germinated in four incubators at 30C, and germinated seeds with an emerging cotyledonary petiole were counted weekly (Fig. 2). G and average times in days or weeks to achieve 50% of final germination (T_{50}) were calculated according to Furutani et al. (1985). The design for the study was four randomized complete blocks of four treatments each. The data were tested by analysis of variance (ANOVA), and treatment means were compared using Tukey's highest significant difference (HSD) procedure.

Stratification of needle palm seeds for 12 months at 5C and 100% RH increased G only when scarification allowed the sclerotesta or embryo cap to remain (Table 1). Stratification did not increase G of nonscarified seeds nor of seeds scarified by removing the embryo cap and the sclerotesta, the latter seeds having 98% and 96% G for fresh and stored seeds, respectively. The T_{50} values for seeds recently harvested or stratified were similar (Table 1). Nonscarified seeds had higher T_{50} values than seeds scarified to expose or remove the embryo cap. Removing the embryo cap resulted in faster germination than leaving the cap intact (Table 1).

Germination temperature. Seeds in this study were scarified by removing the sclerotesta and embryo cap under sterile conditions, and scarified seeds were germinated in moist, sterile peatmoss. In preliminary studies, light did not influence the germination of scarified seeds (data not presented). During germination, seeds were kept in dark incubators (model CB-1; Stults Scientific Engineering Corp., Springfield, Ill.) at constant 15, 20, 25, 30, or 35C (± 1 C). G, T_{50} , and days between 10% and 90% of final germination ($T_{90} - T_{10}$) values were used for evaluation. A completely randomized design with four 15-seed replications of each treatment was used. Data were analyzed by ANOVA and the fitting of regression (linear, quadratic, and cubic) equations to determine if trends in G, T_{50} , and $T_{90} - T_{10}$ values were present over germination temperatures.

The temperature that yielded the highest G, lowest T_{50} , and shortest $T_{90} - T_{10}$ was 30C (Fig. 3). When modeled over germination temperatures of 15 to 35C, the trends in G, T_{50} , and $T_{90} - T_{10}$ (Fig. 3, Eqs. A, B, and C), respectively, differed in terms of the regression models fitted from the trends modeled over the 15

Fig. 1. Longitudinal section showing needle palm seed structure: S = sclerotesta, C = embryo cap, EM = embryo, EN = endosperm.

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to 30C range (Fig. 3, Eqs. A₁, B₁, and C₁). G increased quadratically from 18% to 98% as temperatures increased from 15 to 30C (Eq. A₂). At 35C, G was 92% or 6% lower than G at 30C, a result suggesting that 35C was not optimal. Over the entire 15 to 35C range, modeling G required the fit of the cubic polynomial (Eq. A₁). Between 15 and 30C, quadratic Eq. A₂ fitted G better than cubic Eq. A₁. At 30C, T₅₀ was 8 days and significantly lower than the 11 days at 35C. The trend in T₅₀ values was quadratic when measured over the entire 15 to 35C range (Eq. B₁) or over the shorter 15 to 30C range (Eq. B₂); the latter providing the better fit between 15 and 30C. The most synchronous germination was at 30C, with 12

Table 1. Final germination (G) and weeks required to achieve 50% of final germination (T₅₀) of needle palm seeds stored for 0 or 12 months at 5C and 100% relative humidity after removing portions of the testa.

Scarification	Seed stratification (months)			
	0		12	
	G (%)		T ₅₀ (weeks)	
Nonscarified	7.3 c ^{z,y}	12.3 d	11.3 a	9.0 a
Only endosperm exposed	10.5 c	56.0 c ^x	9.8 ab	6.3 ab
Only embryo cap exposed	23.2 b	79.5 b ^x	9.0 b	5.8 b
Embryo cap removed	98.3 a	96.5 a	1.3 c	1.5 c

^zMean separation in columns by Tukey's HSD at $P \leq 0.01$.

^yValues represent the means of 60 seeds during a 20-week germination period.

^xDenotes that stored seeds had a significantly ($P \leq 0.01$) higher mean G than nonstored seeds.

Fig. 2. Structure of a recently germinated needle palm seedling: EO = eophyll, PI = plumule, L = ligule, P = cotyledonary petiole, S = seedcoat, Sh = cotyledonary sheath, R = primary root, AR = adventitious root.

Fig. 3. Plots of fitted regression equations for (A) average final germination, (B) days to 50% of final germination (T₅₀), and (C) span in days between 10% and 90% of final germination (T₉₀ - T₁₀) for needle palm seeds as a function of germination temperature (T).

days required for $T_{90} - T_{10}$ at 15C, 5 days at 30C, and 7.5 days at 35C. The $T_{90} - T_{10}$ values were fitted adequately using simple linear regression equations, where the fit between 15 to 30C was better ($r^2 = 0.78$) with Eq. C₂ than the fit between 15 to 35C ($r^2 = 0.57$) with Eq. C₁. When tested using a least significant difference procedure, the $T_{90} - T_{10}$ of 7.5 days at 35C was significantly ($P \leq 0.05$) higher than that of 5 days at 30C, a result that confirmed our suspicion that 35C was too high.

Seed moisture content. Recently harvested seeds were placed in 15-seed replications, weighed, and dehydrated in open 9-cm-diameter glass petri dishes in forced-draft ovens at 40C for 6, 12, 24, 48, 72, or 96 h. After dehydration, seeds were reweighed and placed immediately in 25-ml vials, which were sealed and stored at 25C for 7 days. After storage, the dehydrated seeds were surface-sterilized and scarified as described previously and allowed to germinate in moist, sterile peatmoss at 30C. Germination of seeds whose cotyledonary petiole had emerged was counted daily and seed moisture content was recorded. Data were analyzed initially by fitting polynomial regression equations relating G to seed moisture content. The absence of trends in G that could be described easily by regression equations prompted comparisons among the germination percentages using Tukey's HSD. The initial moisture content of recently harvested seeds was defined as the weight lost from four lo-seed samples after drying at 105C for 48 h.

Needle palm seed viability can be lost through desiccation during warm storage. G was unaffected by reducing seed moisture content at harvest from 36% to 14%, but G declined when moisture content was reduced to $\leq 8\%$. Seeds with $<8\%$ moisture failed to germinate (Table 2). No changes in T_{90} or $T_{90} - T_{10}$ values occurred after seed moisture content was reduced from 36% to 8% (data not presented). Shuey and Wunderlin (1977) recommended storing needle palm seeds at low RH to prevent fungal growth, but our results indicated that excessive seed drying during warm storage would reduce seed viability.

Seed storage temperature. Each 15-seed replication of recently harvested seeds was weighed and stored in 15 × 4-cm plastic petri

dishes on wire screens supported by segments of tubing 1 cm above a chemical desiccant. Constant 22% RH was maintained in the dishes, which were sealed with Parafilm (American Can Co., Greenwich, Conn.) after 50 ml saturated potassium acetate solution was added to the bottom of each dish (Copeland, 1976). The seeds were stored for 14 days in incubators at 25C, then reweighed and placed immediately into 25-ml glass jars. The jars were sealed and immersed in a controlled-temperature polyethylene glycol-water (v/v) bath (Guy and Carter, 1984) and held at 10, 5, 0, -5, -10, or -15C. Bath temperatures were lowered 3C/h to final temperatures, held for 7 days, then increased 4C/h to 10C. After low-temperature treatment, seeds were surface-sterilized and scarified, as described previously, before being allowed to germinate in moist, sterile peatmoss at 30C. The experimental design consisted of six incomplete blocks, each containing three temperature treatments with 15 seeds/treatment. The data were tested by ANOVA and the fitting of regression (linear, quadratic, and cubic) equations.

Needle palm seeds lose viability during storage at <0 C (Fig. 4). Recently harvested seeds contained 36% moisture, which was reduced to 19% after storing the seeds at constant 22% RH for 14 days. Seeds stored at 5 and 10C had 95% and 93% G, respectively, but G was 80%, 45%, 33%, and 0% after storage for 7 days at 0, -5, -10, or -15C, respectively. A quadratic model provided the best fit of the G values for seeds stored at 10 to -5C, while including results at -10C required a cubic model fit (Fig. 4).

The results of the four studies indicate that needle palm seeds have physical dormancy imposed by the thick-walled, tightly-packed sclereid cells in the sclerotesta. Apparently, water imbibed by the outer, fleshy sarcotesta is unable to penetrate the sclerotesta and hydrate the embryo. Scarifying the sclerotesta permitted water to reach the endosperm, but resulted in only small increases in G. This result indicated that water entering at the point

of scarification was unable to hydrate the embryo, or the hydrated embryo was unable mechanically either to dislodge the embryo cap or penetrate the sclerotesta. Stratifying seeds at 5C and 100% RH for 12 months before scarification resulted in 56% G, a result possibly indicating softening of the embryo cap and sclerotesta.

Removing the embryo cap and sclerotesta from recently harvested seeds resulted in the emergence of the cotyledonary petiole from half of the germinating ($G = 98\%$) seeds in 1.3 weeks. This rapid germination without stratification indicated that seeds at harvest have mature embryos and that the embryos contain no chemical capable of inhibiting germination. A preliminary study showed that the seeds had no light requirement for germination. The results of our studies indicate that needle palm seeds have a physical dormancy imposed by the seedcoat, a result that supports earlier reports that the seeds require 6 months to 2 years to achieve 7% to 14% G (Clancy and Sullivan, 1988; Popenoe, 1973; Shuey and Wunderlin, 1977).

Emergence of the cotyledonary petiole from seeds after removing the embryo cap and sclerotesta was promoted by constant warmth (30 to 35C), with delayed and asynchronous germination below 25C. Carpenter (1988) reported that seed germination of the palms *Acoelorrhapha wrightii* Griseb. and H. Wendl. (everglade), *Coccothrinax argentata* Jacq. (silver thatch), *Sabal etonia ex* Nash (scrub palmetto), and *Thrinax morrisii* H. Wendl. (key) required constant 30 or 35C. Wagner (1982) found that seedling growth rates of several palm species were most rapid and uniform at 30C.

Needle palm plants are more hardy at <0 C than other palm species with a similar natural distribution range, but the seeds are less tolerant of cold. Seeds of *Sabal palmetto* Lodd (sabal palm) or *Serenoa repens* Bartr. (saw palmetto) lost no viability after 14 days of storage at 0 to -20C (Carpenter, 1987), but G of recently harvested needle palm seeds de-

Table 2. Effect of reduced seed moisture content during 7 days of storage at 25C on final germination (G) of recently harvested needle palm seeds.

Dehydration at 40C (h)	Seed moisture (%) ²	G (%) ³
0	36	95 ab
6	29	97 a
12	21	93 ab
24	14	88 b
48	8	12 c
72	5	0 d
96	3	0 d

²Seed moisture expressed as percent dry weight (105C, 48 h).

³Mean separation by Tukey's HSD at $P \leq 0.05$; 0% G values for 5% and 3% moisture levels were not included in the analysis of variance.

Fig. 4. Plots of fitted regression equations expressing average final germination of recently harvested needle palm seeds as a function of storage (T) at -10 to 10C for 7 days.

clined with temperatures from 0 to -10C during 7 days of storage, and no seeds germinated after storage below -10C. Needle palm seeds contain 36% moisture at harvest, while *Sabal* and *Serenoa* palms contain 12% and 15%, respectively (Carpenter, 1987). Storing needle palm seeds containing 19% moisture at sub-zero temperatures may have contributed to the decline or loss in seed viability. In our desiccation study, seeds with a moisture content <14% experienced reduced G. It is possible that the greatest cold tolerance of seeds occurs between 14% and 18% moisture content.

Literature Cited

- Carpenter, W.J. 1987. Temperature and imbibition effects on seed germination of *Sabal palmetto* and *Serenoa repens*. HortScience 22:660-661.
- Carpenter, W.J. 1988. Temperature affects seed germination of four Florida palm species. HortScience 23:336-337.
- Clancy, K.E. and M.J. Sullivan. 1988. Some observations on seed germination, and polyembryony in the needle palm *Rhapidophyllum hystrix*. Principes 32: 18-25.
- Copeland, L.O. 1976. Principles of seed science and technology. Burgess, Minneapolis.
- Furutani, S.C., B.H. Zandstra, and H.C. Price. 1985. Low temperature germination of celery seeds for fluid drilling. J. Amer. Soc. Hort. Sci. 110:153-156.
- Guy, C.L. and J.V. Carter. 1984. Characterization of partially purified glutathione reductase from cold hardened and nonhardened spinach leaf tissue. Cryobiology 21:454-464.
- Kane, M.E., T.J. Sheehan, and F.H. Ferwerda. 1988. In vitro growth of American lotus embryos. HortScience 23:611-613.
- Popenoe, J. 1973. The hardiest palms. Fairchild Trop. Gardens Bul. 28:11-14.
- Shuey, A. and R.P. Wunderlin. 1977. The needle palm, *Rhapidophyllum hystrix*. Principes 21:47-57.
- Wagner, R.I. 1982. Raising ornamental palms. Principes 26:86-101.