Methods of Enhancing Germination of Anacua Seeds

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Abstract

Seed dormancy hampers establishment of anacua [*Ehretia anacua* (Teran & Berl.) I.M. Johnst.] in plantings for wildlife. We evaluated methods of enhancing anacua germination and causes of dormancy. Seeds were (1) scarified with 2.9 mol liter^{-1} H_2O_2 or 0.71 mol liter^{-1} NaOCl for 10, 20, or 30 minutes, or concentrated (18.0 mol liter^{-1}) H_2SO_4 for 15, 30, 60, or 120 minutes; (2) rinsed with water for 12, 24, 36, and 48 hours; (3) treated with 0.1, 1.4, 2.9, and 4.3 mmol liter^{-1} gibberellic acid (GA); (4) treated with 0.02 mol liter^{-1} KNO_3; (5) treated with dry heat (130° C) for 3, 6, 9, 12, and 15 minutes, (6) mechanically scarified; and (7) moist prechilled at 3 and 7° C for 2 or 4 weeks. Seeds were germinated in controlled environment chambers at 30° C. Germination was not enhanced by chemical scarification or rinsing. GA (1.4 mmol liter^{-1}) increased germination from 35% for controls to 61%. Mechanical scarification and dry heat enhanced germination of highly dormant seeds only. A 2-week moist prechill at 3° C increased germination of intact seeds from 6% for controls to 36%. Percent and rate of germination were similar among seed sources. Apparent afterripening requirements limited germination at 2 months after harvest to 3%. This requirement gradually broke down until at 8 months after harvest, germination had increased to 40%. Our results indicated that treatment with 1.4 mmol liter^{-1} GA or higher concentrations, moist prechilling for 2 weeks at 3° C, and storage for 8 months will increase germination of dormant anacua seeds.

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Anacua [*Ehretia anacua* (Teran & Berl.) I.M. Johnst.] is a native, semievergreen shrub or small tree of the Boraginaceae that occurs in river valleys and bottomlands in central and south Texas southward into Mexico (Vines 1960, Correll and Johnston 1970). Anacua fruits are eaten by many birds and mammals (Vines 1960), and it is highly favored as a nest site by white-winged doves (*Zenaida asiatica* L.) (Brown et al. 1977).

About 95% of the original brushland of the Lower Rio Grande Valley has been lost to cultivation and other land use practices. State and federal wildlife agencies are attempting to restore habitat for white-winged doves and other wildlife on former cropland in this region. Anacua is one of five woody plants considered to be of primary importance in habitat restoration. Other important species include spiny hackberry (*Celtis pallida* Torr.), bluewood (*Condalia obovata* Hook.), huisache [*Acacia farnesiana* (L.) Willd.], and Texas ebony [*Pithecellobium flexicaule* (Benth.) Coult.].

Low germination resulting from seed dormancy has hampered efforts to establish anacua. Alaniz and Everitt (1980) reported that the optimum temperature for anacua germination is 30° C. Seeds had to be soaked for 2 hours in concentrated sulfuric acid for appreciable germination to occur. Objectives of this study were to determine (1) additional methods of enhancing germination of anacua seeds and (2) possible causes of seed dormancy.

Methods

Anacua fruits were collected from ornamental trees in Kleberg County, Texas, and from native populations in Cameron and Hidalgo Counties, Texas. Fruits were collected in May, June, and July, 1984. Soils in the Cameron County collection site were Wil-

Table 1. Treatments tested to enhance anacua seed germination in pilot experiments. Seeds were germinated in the dark at 30° C.

Treatment	Levels tested	References
1. 2.9 mol liter^{-1} H_2O_2	10, 20, 30 minutes	(Young et al. 1981)
2. 0.71 mol liter^{-1} NaOCl	10, 20, 30 minutes	(Young et al. 1981)
3. Conc. (18.0 mol liter^{-1}) H_2SO_4	15, 30, 60, 120 minutes	(Alaniz and Everitt 1980)
4. Water rinse	12, 24, 36, 48 hours	(Young et al. 1981)
5. Gibberellic acid (GA)	0.3, 1.4, 2.9, 4.3 mmol liter^{-1}	(Mayer and Poljakoff-Mayber 1975)
6. 0.02 mol liter^{-1} KNO_3	—	(Young et al. 1981)
7. Dry Heat (130° C)	3, 6, 9, 12, 15 minutes	(Scowcroft 1978)
8. Mechanical scarification (seeds nicked with a razor blade)	—	(Kissock and Haferkamp 1983)
9. Prechilling (7° C)	2 weeks	(Young et al. 1981)

lacy fine sandy loam, a member of the fine-loamy mixed hyperthermic Udic Argiustalls and Raymondville clay loam, a member of the fine, mixed, hyperthermic Vertic Calciustolls. Soils at the Hidalgo County collection site was Rio Grande silt loam, a member of the coarse-silty, mixed (calcareous), hyperthermic Typic Ustifluvents.

The fruit of anacua is a drupe. Fruits were macerated in a fruit press to separate seeds from the flesh (Young et al. 1981). Care was taken not to damage seeds. Seeds were air-dried at 25° C and stored in cloth bags at 15° C and 40% relative humidity. Seeds of the Cameron source were used in all experiments except when sources were compared.

Seeds were germinated on blotter paper underlain by a layer of creped cellulose placed in plastic boxes measuring 13.0 by 13.5 by 3.5 cm with tightly fitting lids (Fulbright et al. 1983). The substrata were moistened with 100 ml of distilled water or the appropriate test solution and were remoistened when necessary. Seeds were treated with thiram [bis (dimethylthiocarbamoyl) disulfide] to minimize fungal growth. In pilot experiments and the afterripening experiment 4 plastic boxes containing 25 seeds each were used as experimental units for each treatment. In all other experiments, 4 plastic boxes of 50 seeds each per treatment were arranged in a randomized complete-block design and each experiment was repeated twice.

We used the term seed to refer to the anacua stone, which contains 2 seeds (Vines 1960). A seed (stone) was considered to have germinated when part of at least 1 cotyledon was visible and the radicle of at least 1 seed was 5 mm or greater in length (Kissock and Haferkamp 1983). Counts were made weekly for 14 days in pilot experiments and daily in final experiments. Rate of germination was calculated by the equation of Maguire (1962) for germination rate (GR):

$$GR = \frac{\text{number of seeds germinated}}{\text{number of days to first count}} + \ldots + \frac{\text{number of seeds germinated}}{14}$$

where GR is the sum of the quotients of the number of seeds germinated divided by the number of days for germination.

All experiments were conducted at 30° C in controlled environment chambers. In experiments where light was used, the light treatment consisted of 12 hours daily exposure of seeds to cool-white flourescent lights. Photosynthetic photon flux density averaged 26 μmol m^{-2} s^{-1}.

Nine treatments were tested in a series of pilot experiments. These treatments are described in Table 1. Chemical scarification with 2.9 mol liter^{-1} H_2O_2, 0.71 mol liter^{-1} NaOCl, and concentrated (18.0 mol liter^{-1}) H_2SO_4, and rinsing seeds in running tap water failed to increase germination. Effects of germinating seeds on substrata moistened with 4.3 mmol liter^{-1} gibberellic acid (GA), 0.02 mol liter^{-1} KNO_3, and 4.3 mmol liter^{-1} GA in combination with 0.02 mol liter^{-1} KNO_3 were determined in an additional pilot experiment because GA in combination with KNO_3 increases germination more than GA or KNO_3 alone for seeds of some species (Young et al. 1981).

Following pilot experiments, 3 final experiments were conducted. In the first experiment, effects of 1.4 mmol liter^{-1} GA, a 5-minute dry heat treatment, mechanical scarification, and light (12 hours daily) on germination were compared in a 2^4 factorial experiment. In the dry heat treatment, seeds in paper envelopes were placed in a ventilated oven at 130° C (Scowcroft 1978). Dark conditions were maintained by wrapping plastic boxes in aluminum foil. Seeds used in the experiment were harvested in late May and early June, 1984, and the experiment was conducted in November, 1984.

In the second experiment, effects of duration and temperature of moist prechilling were determined. Seeds were prechilled on moist blotters at either 3 or 7° C for either 2 or 4 weeks. Seeds were harvested in late June and early July, 1984, and the experiment was conducted in December, 1984, and early January, 1985.

In the third experiment, effects of seed source on germination were determined. Seeds from 3 sources were either untreated or germinated on substrata moistened with 1.4 mmol liter^{-1} GA to determine if differences in level of dormancy existed among sources. The experiment was conducted in late November and early December, 1984, with seeds harvested in mid-July, 1984.

Afterripening requirements were determined by germinating seeds harvested in late May and early June under dark conditions at 2, 4, and 8 months after harvest.

Data from pilot GA and dry heat experiments were analyzed by response curve analysis (Snedecor and Cochran 1967). Data from other experiments were analyzed by analysis of variance (Snedecor and Cochran 1967). Percent germination data were subjected to arcsine $\sqrt{\%} \times 0.01$ transformation before analysis of variance. Untransformed data are reported in the text. Tukey's test was used at the 0.05 level of probability to identify significantly different means when significant F values were found.

Results

Pilot Experiments

Germination increased linearly with GA concentration. Treatment with 0, 0.3, 1.4, 2.9, and 4.3 mmol liter^{-1} GA resulted in 3, 26, 26, 31, and 41% (R^2=0.50) germination, respectively. No significant (P>0.05) difference in germination was detected among seeds germinated on substrata moistened with a 4.3 mmol liter^{-1} GA solution, a 0.02 mol liter^{-1} KNO_3 solution, or a solution with 4.3 mmol liter^{-1} GA and 0.02 mmol liter^{-1} KNO_3 combined. Germination of seeds treated with 4.3 mmol liter^{-1} GA, 0.02 mol liter^{-1} KNO_3, and 0.02 mol liter^{-1} KNO_3 in combination with GA was 36, 34, and 36%, respectively, compared to 13% for controls.

Germination of 3-month-old seeds exhibited a quadratic response to duration of exposure to dry heat (130° C). Treatments with 0, 3, 6, 9, 12, and 15 minutes of dry heat resulted in 15, 39, 64, 39, 5, and 0% (R^2=0.85) germination, respectively. Optimum duration of treatment as predicted by response curve analysis was 5 minutes.

Effects of GA, Dry Heat, Scarification, and Light on Germination

Percent germination of 5-month-old anacua seeds was increased by 1.4 mmol liter^{-1} GA alone and 1.4 mmol liter^{-1} GA in combination with either mechanical scarification or a 5-minute dry heat (130° C) treatment (Table 2). Dry heat alone decreased percent germination 16% compared to untreated seeds. Mechanical scarification and light did not increase percent germination (Tables 2 and 3). The light × seed treatment interaction was not significant ($P>0.05$) (Table 3).

Table 2. Effects of dry heat (130° C) for 5 minutes, mechanical scarification, and 1.4 mmol liter^{-1} GA on mean percent germination of anacua seeds at 30° C.

Seed treatment	Percent germination	Germination rate
Control	35b[1]	1.9d
Mechanically scarified (SC)	35b	2.0cd
Dry heat (DH)	19c	1.0e
Gibberellic acid (GA)	61a	3.6a
SC + GA	53a	3.1ab
SC + DH	18c	1.0e
GA + DH	52a	2.8bc
SC + GA + DH	46ab	2.5bcd
SE[2]	3	0.2

[1]Means in a column followed by the same letter are not significantly different at the 0.05 level of probability according to Tukey's test. The light × seed treatment interaction was not significant ($P>0.05$) for germination rate. The main effect of light was significant ($P=0.047$) for germination rate. Values are averages of light and dark treatments.
[2]Standard error of the mean, 49 degrees of freedom.

Table 3. Analysis of variance for effects of light (12 hours daily), dry heat (130° C) for 5 minutes, mechanical scarification, and 1.4 mmol liter^{-1} GA on mean percent germination of anacua seeds at 30° C.

Source of variation	df	Mean square	F value	Probability of a > F
Replication (R)	7	0.04[1]		
Treatments (T)	7	0.48	23	0.0001
Error a (RXT)	49	0.02		
Light (L)	1	0.07	5	0.0574
Error b (RXL)	7	0.01		
TXL	7	0.01	2	0.1682
Error c (RXTXL)	49	0.01		

[1]Arcsine $\sqrt{\% \times 0.01}$ transformed percent germination.

Germination rate was increased by 1.4 mmol liter^{-1} GA, mechanical scarification in combination with 1.4 mmol liter^{-1} GA, dry heat in combination with 1.4 mmol liter^{-1} GA, and light (Table 2). Treatment with 1.4 mmol liter^{-1} GA alone produced the highest germination rate. Averaged over all seed treatments, germination rate was 13% higher in the light than in the dark.

Effects of Prechilling on Germination

Percent germination of anacua seeds was enhanced by moist prechilling (Table 4). Duration of prechill did not affect germination. Prechill temperatures did not affect percent germination of scarified seeds; however, percent germination of intact seeds in the 2-week treatment was higher for seeds moist prechilled at 3° C than for seeds moist prechilled at 7° C. There was a significant ($P<0.05$) mechanical scarification × moist prechilling treatment interaction. Scarification enhanced germination of seeds that were not moist prechilled, but no significant ($P>0.05$) difference in percent germination existed between intact and scarified seeds that were moist prechilled.

Moist prechilled anacua seeds germinated more rapidly than seeds that were not moist prechilled (Table 5). Duration of the moist prechilling treatment did not affect germination rate.

Table 4. Effects of moist prechilling intact and scarified anacua seeds at 2 temperatures (° C) and 2 lengths of time on mean percent germination at 30° C with a 12 hour daily photoperiod.

Length of moist prechill	Temperature	Percent germination	
		Intact	Scarified
Control	....	6 c[1]	16 b
2 weeks	3	36 a	31 a
	7	17 b	25 ab
4 weeks	3	24 ab	27 ab
	7	27 ab	31 a
SE[2]		3	

[1]Means followed by the same letter were not significantly different at the 0.05 level of probability according to Tukey's test. Main effects of moist prechilling and scarification were significant ($P<0.05$). The interaction of moist prechilling and scarification was significant ($P<0.05$).
[2]Standard error of the mean, 63 degrees of freedom.

Temperature did not affect germination rate of seeds that were moist prechilled for 4 weeks. However, seeds that were moist prechilled 2 weeks at 3° C germinated more rapidly than seeds moist prechilled 2 weeks at 7° C. Scarified seeds germinated more rapidly than intact seeds.

Table 5. Effects of prechilling intact and scarified seeds at 2 temperatures (° C) and 2 lengths of time on germination rate at 30° C with a 12 hour daily photoperiod.

Length of prechill	Temperature	Germination rate		
		Intact	Scarified	Prechill Treatment Means
Control	....	0.3	0.9	0.6 c[1]
2 weeks	3	2.5	2.2	2.4 a
	7	1.1	1.8	1.5 b
4 weeks	3	1.7	2.2	1.9 ab
	7	1.8	2.1	2.0 ab
Scarification treatment means		1.5	1.8**	
SE[2]				0.2

**Significantly different at the 0.01 level.
[1]Means followed by the same letter are not significantly different at the 0.05 level of probability according to Tukey's test. Main effects of moist prechilling and scarification were significant ($P<0.05$). The interaction of moist prechilling and scarification was not significant ($P>0.05$).
[2]Standard error of the mean, 63 degrees of freedom.

Effect of Seed Source on Germination

No significant ($P>0.05$) difference in germination existed among the 3 sources of anacua seeds (Table 6). Germinating seeds on substrata moistened with 1.4 mmol liter^{-1} GA enhanced percent germination and germination rate of all 3 sources. Sources did not differ significantly ($P>0.05$) in the degree to which percent germination and germination rate were increased by GA or in level of dormancy.

Table 6. Effects of seed source and gibberellic acid (1.4 mmol liter^{-1}) on germination of anacua seeds in the light at 30° C.

Source	Percent germination		Germination rate	
	Control	Treated	Control	Treated
Kleberg	13	48	0.6	2.4
Cameron	17	49	0.8	2.5
Hidalgo	12	42	0.5	1.9
Treatment Means	14	46**	0.6	2.3**
SE[1]	3		0.2	

**Main effect means significantly different from controls at the 0.01 level of probability according to F-values. There were no significant ($P>0.05$) differences among sources and the source × GA interaction was not significant ($P>0.05$).
[1]Standard error of the mean, 35 degrees of freedom.

Differences between the pilot experiment; the GA, dry heat, scarification, and light effects experiment; and the source comparison experiment in effects of 1.4 mmol liter^{-1} GA on germination possibly resulted from differences in seed age. Seeds used in the pilot experiment were the most recently harvested of those used in the 3 experiments and exhibited the lowest germination when treated with GA.

Effect of Length of Storage on Germination

Germination of anacua seeds from Cameron County increased with length of storage. Germination averaged 3, 13, and 40% 2, 4, and 8 months after harvest, respectively.

Discussion

Germination of anacua seeds can be enhanced by (1) germinating seeds on substrata moistened with GA solutions, (2) chilling imbibed seeds prior to exposure to warm temperatures, and (3) storage. Effects of dry heat on germination may depend on degree of dormancy. Dry heat enhanced germination of 3-month-old seeds but not of 5-month-old seeds. Seeds used in pilot tests with dry heat were harvested in early June and were germinated 3 months after harvest, while seeds used in the experiment on GA, dry heat, scarification, and light effects on germination were germinated 5 months after harvest. Further research on effects of dry heat on anacua germination is needed before it can be recommended as a presowing treatment.

Apparently, germination of anacua increases with time during storage. Duration of storage and storage conditions for maximum germination potential were not determined in this study. Further research on anacua afterripening is needed.

Dormancy of anacua seeds may result in part from a deficiency of endogenous gibberellins. Changes in apple (*Malus* sp.) seeds during afterripening include an increase in GA levels during later phases of the process (Lewak and Rudnicki 1977). Also, Mayer and Poljakoff-Mayber (1975) suggested that GA formation results from stratification in some cases. Dormancy apparently does not result from the presence of water soluble inhibitors that can easily be leached from the seeds because rinsing seeds with water did not enhance germination.

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