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An Acid-Bath Technique to Break Seed Dormancy in Common Sunflower, *Helianthus L. annuus* (Asteraceae)

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ABSTRACT

The phenomenon of seed dormancy is widespread in plants and serves to prevent all or most of a given population from germinating at the "wrong" time, e.g., during an unusually mild fall in an area subject to typically harsh winters. Seed dormancy is an effective survival strategy in many plant populations, but may greatly complicate efforts to establish large cohorts of seedlings (groups of similar age or developmental stage) needed for research and other purposes. In an effort to break seed dormancy in common sunflower, *Helianthus annuus* L. (Asteraceae), we conducted experiments designed to compare germination times and overall germination success among groups of field-collected *H. annuus* seeds subjected to several treatments. Overall germination success during a 14-d period posttreatment was lowest among untreated controls and groups soaked in a disinfecting solution for 15-h (1.2% and 2.6% germination, respectively; $P<0.05$) and was greatest among cohorts subjected to the aforementioned soaking-disinfecting solution for 15-h followed by additional soaking in HCl at concentrations of 0.1M and 1.0M for 55 min (87.2% and 65.9% germination success, respectively; $P<0.05$). These results suggest that the conventional soaking-disinfecting solution followed by an acid bath may provide researchers with an effective and inexpensive means of generating large cohorts of plant seedlings for field and laboratory research and other applications.

Additional index words: : enhancing germination success, germination times.

The phenomenon of seed dormancy is widespread among the seed plants (gymnosperms and angiosperms) is a survival mechanism acquired during evolution that allows seeds to postpone germination until favorable conditions occur. Some species can produce seeds with different degrees of dormancy in order to create a seed bank that will allow the species to survive even under conditions that are seemingly optimal for plant growth. While this strategy effectively prevents most or all members of a given population from germinating at the "wrong" time, e.g., during an unusually mild fall in an area characterized by typically harsh winters, the existence of seed dormancy may also hinder efforts to establish cohorts of similar-aged plants for use in field and laboratory experiments (Chandler and Jan 1985, Maiti 2006a). For example, germination times for seeds of common sunflower (*Helianthus annuus* L.) collected from a population in the Lower Rio Grande Valley (LRGV) of Texas ranged from few days to several weeks after they were collected and maintained under controlled laboratory conditions (Gandy 2010).

Two types of seed dormancy have been documented in plants. Coat-imposed dormancy is imposed on the embryo by the seed coat and surrounding tissues, and embryo dormancy is inherent in the embryo and is not influenced by the seed coat or surrounding tissues (Maiti et al. 2006a). Seed dormancy in *H. annuus* appears to be related primarily to factors associated with seed coat permeability to water, hence the common designation as seed coat dormancy (Maiti et al. 2006a; Hernandez and Orioli 1985). Seed coat dormancy may be overcome by several methods including soaking the seed in water, exposure to various chemicals including plant growth hormones, seed coat scarification and embryo excision (Maiti et al. 2006a, Maiti et al. 2006b, Dagustu and Ozer 2014, Nasreen et al. 2015). When used alone, most of these methods have proven to be either inefficient (water soaking, exposure to gibberellic acid), potentially damaging to seeds (embryo extraction) or impractical due to the extremely small size of *H. annuus* seeds (scarification

and embryo extraction) (Fig. 1).

In an effort to develop a practical method to synchronize the germination of large numbers of *H. annuus* from field-collected seed cohorts, we conducted a study designed to compare time required for germination and overall germination success among seed cohorts of *H. annuus* subjected to 1) no treatment, 2) conventional soaking in distilled water for 15h followed by a disinfecting procedure, 3) conventional soaking in distilled water for 15h, treatment with either 0.1M or 1.0M HCl for 20 min, followed by a disinfecting procedure, and 4) conventional soaking in water for 15h, treatment with either 0.1M or 1.0M HCl for 55 min, followed by a disinfecting procedure.

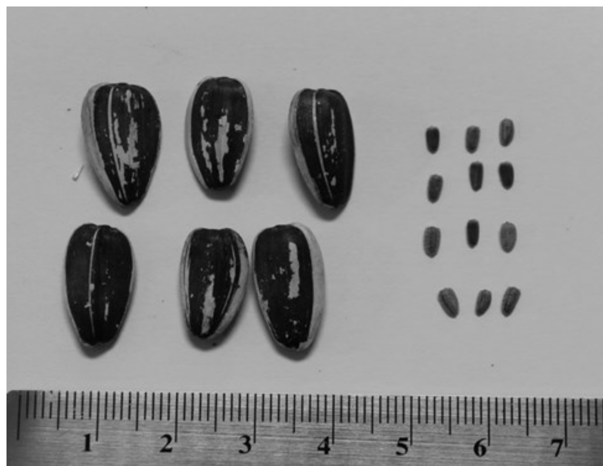

Fig. 1. Relative sizes (in mm) of cultivated sunflower seeds (left) compared to seeds of wild *Helianthus annuus* (right).

MATERIALS AND METHOD

Seeds of *H. annuus* were field collected from several areas in Hidalgo County, TX during 2008. Initally a standardized germination test in Petri dishes was conducted by placing approximately 80 seeds per Petri dish in four replications. This group of seeds was used as a control. The remaining seeds were soaked overnight for a period of approximately 15 hrs as recommended by Maiti et al. (2006a) in unsterilized distilled water, designating this procedure as soaking treatment. Independently of other treatments, and in order to avoid disease infestation, in a laminar flow hood all seeds were treated with a solution of 70% ethyl alcohol + 0.02% (v/v) Triton X-100 and distilled water for 5 minutes followed by immersion in a solution of a commercial hypochlorite solution diluted to 50% (v/v) + 0.02% (v/v) Triton X-100 with deionized distilled water for 10 minutes. Alternation of three rinses with sterilized distilled water followed the application of each solution. This was established as the disinfecting treatment.

The efficacy of a solution of hydrochloric acid (HCl) and distilled water as a dormancy breaking agent was also evaluated. Two different concentrations of HCl (0.1M and 1.0M) were applied to the seeds for a period of 20 minutes and 55 minutes, respectively, followed by three rinses with distilled water prior to the disinfecting treatment were tested. These groups of sunflower seeds were designated as chemical treatments.

To evaluate the germination activity of the sunflower seeds, the control group and treatments were placed in Petri dishes with a sterilized filter paper located at the bottom of each dish. Approximately 80 sees were placed within each Petri dish, and sterilized distilled water was used to dampen the filter paper. The Petri dishes were covered with the lids and sealed with parafilm. All treatments were replicated four times.

Petri dishes were placed in randomized order under a 14 hr light/ 10 hr dark cycle at a temperature of $20° \pm 2^{o}$ C in the laboratory. Seeds germinating by the 7th day and total number of seeds germinating prior to 15th day were counted and recorded. The seedling emergence data was analyzed using one-way analysis of variance (ANOVA) and means were separated using Tukey pairwise means comparison test (Sokal and Rohlf, 2000).

RESULTS AND DISCUSSION

Cumulative germination success of *H. annuus* seeds subjected to the various treatments during a 2-week period is summarized in Table 1. Overall germination success was lowest among the controls and the cohorts subjected to conventional soaking (15h) + disinfecting solutions (1.2% and 2.6%, respectively; $P>0.05$). Cohorts subjected to the conventional soaking solution (15h) and soaking for an additional 20 min in disinfecting solution with HCl added in two concentrations (0.1M and 1.0M) resulted in a substantial increase in overall germination success ($P<0.05$), although no differences among cohorts exposed to the two acid concentrations were evident (13.7% and 13.5%, respectively; $P<0.05$). The greatest overall germination success occurred among cohorts subjected to the conventional soaking treatment (15h) followed by soaking for an additional 55 minutes in disinfecting solution with HCl added at concentrations of 0.1 and 1.0M (87.2% and 65.9%, respectively; $P<0.05$). Of the six treatments evaluated in this study, the most effective means of breaking seed dormancy in
H. annuus appears to be soaking in water for 15h fol-

Table 1. Germination activity of wild sunflower seeds (*Helianthus annuus*) in response to different treatments.

Treatment	Avg. Number of seeds	Avg. No. of emerged seedlings on 7th day*	Avg. No. of emerged seedlings on 14th day*	Avg. Total germination (%)*
Control	85	0a	1a	1.2 a
Soaking (15h) + Disinfecting	86	1a	3a	2.6 a
Soaking (15h) + Disinfecting + 0.1 M HCl (20min)	89	6b	14b	13.7b
Soaking (15h) + Disinfecting + 1 M HCl (20min)	89	7b	12b	13.5b
Soaking (15h) + Disinfecting + 0.1 M HCl (55min)	87	51c	75c	87.2c
Soaking (15h) + Disinfecting + 1 M HCl (55min)	82	37d	56d	65.9d

*Means within columns followed by the same letter are not significantly different at 5% probability level (analyzed by ANOVA; means separated by Tukey pairwise comparison test).

lowed by additional soaking for 55 minutes in a disinfecting solution with HCl added at a relatively low concentration (0.1M).

Data collected during the study demonstrated the feasibility of using a practical and effective method to break seed dormancy thus enhance germination success in *H. annuus* seeds. Although the findings and established procedure were only in relation to sunflower seeds, the investigation opens the possibility to the potential use of the procedure with other coat-imposed dormancy seed species. This in turn, would greatly facilitate the use of these plant species for research purposes

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