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Germination of Maiapilo (*Capparis sandwichiana*) Seeds: Determining Dormancy Classification

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Abstract. **The Hawaiian archipelago's sole native member of the Capparaceae plant family, the endemic maiapilo (*Capparis sandwichiana*), is a species of conservation importance with poor natural regeneration. This study identifies important seed characteristics, such as its seedcoat water permeability, embryo development, and time to germination, to determine dormancy classification. To test for water-permeable coats, seeds were mechanically scarified and imbibed in tap water over a 48-hour time period. During imbibition, scarified and nonscarified seed masses increased at similar rates. The same seeds were then tested for germination with daily alternating regimens of 12 hours of light/dark and temperatures of 25/15 °C. The nonscarified seeds germinated at a significantly higher rate than scarified treatments, indicating the presence of water-permeable seedcoats to rule out physical dormancy (PY). To test for physiological dormancy, gibberellic acid was used in germination tests. Caper seeds were sown without (control) and with (500, 1000 ppm) gibberellic acid (GA_3) treatments to help determine dormancy classification. Mean germination for each replicate ranged between 10% (500 ppm GA_3) and 60% (1000 ppm GA_3). There was no significant difference with final germination or overall time (days) to reach 25% total germination (T_{25}) among treatments and controls. Because 1) seedcoats were permeable to water, 2) embryos were fully developed, and 3) germination took longer than 30 days, *Capparis sandwichiana* seeds most likely have nondeep physiological dormancy. These results will benefit managers and growers with a better understanding of seed characteristics for dormancy alleviation and successful germination of this threatened endemic species.**

Of the 1381 native vascular plant taxa that comprise Hawaii's native flora (Wagner et al. 2023), 878 species are considered species of conservation importance in need of in situ and ex situ management (Laukahi 2024). One Hawaiian endemic species of conservation importance, *Capparis sandwichiana,* is the only member belonging to the Capparaceae family native to the island chain. Its current population is decreasing because of many threats, including coastal development, invasive species, and poor regeneration (Caraway 2020; Culliney and Koebele 1999). In addition, Hawaiian coastal species were found to be highly vulnerable to increased salinity exposure (Walsh et al. 2023), which is predicted in Hawaii from sea level rise (Kopp et al. 2014) and increased drought (Elison Timm et al. 2015). Because of land-use history, invasive grass species, shifting climates, and increasing ignitions, wildfire is increasing in Hawaii (Yelenik et al. 2024), compounding the threats *C. sandwichiana* and other native species face.

The endemic caper of Hawai'i, known as "maiapilo" or "pua pilo" in Hawaiian (Gon 2008), is a low-growing spineless shrub with vine-like straggling branches, scattered on coral, basaltic rocks, or in soil along coastal areas or, less frequently, inland (Wagner et al. 1990). Growing in dry regions on cliffs, lithified sandstone, old lava flows, rocky gulches, and some beaches, the species was most recently assessed as Vulnerable for *The IUCN Red List of Threatened Species* under criteria B1ab (i, ii, iii, iv, v) (Caraway 2020).

The taxon is recommended by Laukahi, the Hawaii Plant Conservation Network (https://laukahi.org) for ex situ conservation and use in habitat restoration programs, as well as landscape practices. However, further research is needed on general ecology, life history, population size, distribution, trends, population genetics, and threats (Caraway 2020).

Other *Capparis* species may demonstrate similar characteristics with members of its genus. Although germination rates are relatively low (Foschi et al. 2022b, 2023b; Sottile et al. 2021), bioassays and clinical trials show that *Capparis spinosa* seeds possess bioactive constituents responsible for antioxidant, anticancer, and antibacterial effects (Nabavi 2016). Early Hawaiians used *C. sandwichiana* medicinally for healing fractured or broken bones (Neal 1948). Similarities with the Hawaiian caper may extend to other areas, such as various health-promoting effects and cultivation protocols, adding further value to seed dormancy and germination research.

Although Chau et al. (2019) observed 100% germination, few tests have been published to evaluate its anecdotally low rates of germination. Evaluating seed characteristics of *C. sandwichiana* can give conservation managers and growers valuable information to increase the success of seed banking and production. To increase germination, a better understanding of *C. sandwichiana* seed biology is required.

In general, seeds can be dormant or nondormant, and there are five types of seed dormancy: 1) morphologically dormant seeds imbibe water and germinate within a month but have an underdeveloped embryo; 2) morphophysiological seeds also imbibe water, but germination takes more than a month with underdeveloped embryos that are physiologically dormant; 3) in physiological dormancy, seeds imbibe water and have fully developed embryos, but germination takes longer than a month, and cold stratification is often required to increase growth; 4) with physical dormancy, the embryo does not have enough "push power" to overcome the restraint of covering layers; and 5) in combinational dormancy, seeds do not imbibe water and have developed embryos with both physical and physiological dormancy (Baskin and Baskin 2004).

Studies show that seeds of other *Capparis* species may have physical dormancy, physiological dormancy, combinational dormancy, or nondormancy (Baskin and Baskin 2014). Much research is available on the closely related common caper bush, *Capparis spinosa*, grown agriculturally as a culinary ingredient and medicine. This research suggests that physical dormancy is imposed by its endocarp (Sozzi and Chiesa 1995) but dormancy alleviation is also influenced by physiological limitations, as shown in experiments with sulfuric acid pre-soaking (Bahrani et al. 2008) and treating with gibberellins (Foschi et al. 2020, 2022a; Sottile et al. 2021).

However, there are very few seed germination procedures published for *C. sandwichiana*. Lilleeng-Rosenberger's (2005) publication "*Growing Hawaii's Native Plants. A Simple*

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Step-by-Step Approach for Every Species" suggests soaking seeds in tap water for 3- to 5-d before sowing. According to the Reforestation, Nurseries, and Genetic Resources "Native Plant Network Propagation Protocol Database," maiapilo seeds should be pretreated with a 24 h soak in cold tap water and sown immediately (Martz and Starr 2003).

The objective of this study was to determine seed dormancy classification for *C. sandwichiana*. If seeds have characteristics of physiological dormancy, 1) they will imbibe water, 2) have an embryo that does not grow inside the seed before germination, and 3) require more than 4 weeks to germinate (Baskin et al. 2006). Because GA_3 and other gibberellins can break dormancy and promote germination (Baskin and Baskin 2016), our results may further indicate the presence of physiological dormancy (Baskin and Baskin 2014) as inferred with other species of the genus, such as *Capparis erythrocarpos*, *Capparis ovata*, *Capparis pyrifolia*, *Capparis scortechinii*, and *C. spinosa* (Bahrani et al. 2008; Orphanos 1983; Piotto and Di Noi 2003; Sozzi and Chiesa 1995). This is important information for conservation managers and horticulturists to increase germination rates for this threatened species with poor regeneration.

Materials and Methods

Dormancy classification. Knowing the type of seed dormancy is essential for successful propagation, so a flowchart (Fig. 1) was followed to identify dormancy classification (Baskin and Baskin 2014; Wilkinson et al. 2014). After initial seed collection and extraction, an imbibition experiment was conducted to detect if seedcoats were nonpermeable to water (physical dormancy). Next, seeds were examined to determine if embryos were underdeveloped (morphological dormancy). Last, germination experiments were administered to find out if seeds do not germinate within 30 d (physiological dormancy).

Seed collection and extraction. For all the following experiments, fruits of *C. sandwichiana* were collected when the berries were starting to split as an indicator of harvest maturity (as reported for *C. spinosa*, Foschi et al. 2020) from Māhā'ulepū Heritage Trail/Makauwahi Cave Reserve in south Kaua'i (four plants) and Pu'u Ka Pele Forest Reserve in west Kaua'i (one plant) between 1 Nov 2021 and 2 Nov 2022. They were then stored in partially sealed zip-top plastic bags to continue maturation (Lilleeng-Rosenberger 2005) in ambient conditions [~50% relative humidity (RH) at 25 °C] at the National Tropical Botanical Garden (NTBG) Seed Laboratory until seeds were manually extracted within 3- to 5-d from the fetid, orangish-yellow fruit under running tap water (~25 °C) with a metal strainer inside of a bowl. Seeds that floated or appeared underdeveloped were not used in the experiments, as directed in caper seed selection for commercial use (Foschi et al. 2022a, 2023a), except for the sinker/floater experiment "*Effects of GA_3 (on 18-d-old seeds) and germination of floating seeds*" (see later in this article). The extracted seeds were then blotted on paper towels to remove most of the orange pulp residuals from the outer seedcoat. Seeds in all experiments were placed on coffee filter paper to dry for 2- to 3-d, then stored in envelopes in the same conditions on a shelf at the NTBG Seed Laboratory until experiments began 1 to 3 weeks later (see later in this article; Table 1). The one exception (NTBG Accession # 20210545) had seeds stored (in the same conditions as described previously) for ~1 year (371 d) before being used in experiments.

Imbibition. This experiment was the first step to following the key to seed dormancy types (Fig. 1). The effects of mechanical scarification on imbibition mass indicate if a seedcoat is permeable, or not permeable to water (physical dormancy). Two fruits of one individual *C. sandwichiana* plant (NTBG Accession #20210517) were collected at an elevation of 17 m (coastal low shrublands) from Makauwahi Cave Reserve in south Kaua'i at an elevation of 10 m on 1 Nov 2021. The stems of these fruits were set in a beaker of tap water, until the maturing fruits turned yellowish-orange in color 4 d after collection. The extracted, then dried seeds from the two fruits were combined and stored at ambient laboratory conditions (~50% RH at ~25 °C) for ~3 weeks until used in experiments.

To test the effect of mechanical scarification on imbibition, three replicates each of 50 mechanically scarified (individually with a single-edge razor blade) and of 50 nonscarified (control) seeds were placed on seed germination paper (Anchor Paper Company, St. Paul, MN, USA), moistened with distilled water, in 60-mm-diameter petri dishes on a laboratory bench. After 0, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 24.0, and 48.0 h, the surfaces of the seeds were blotted dry, weighed to the nearest 0.001 g, and returned to the moist seed germination paper in petri dishes. The amount of water imbibed was determined as actual increases in seed weights and converted to percentage increase.

Embryo development. This next step to following the key to seed dormancy types (Fig. 1) was to determine if embryos are fully developed, or underdeveloped (morphological dormancy). To visually assess the development of embryos, seeds of *C. sandwichiana* (NTBG Accession # 20220025) were used in this study from one fruit (280 seeds total) collected from Makauwahi Cave Reserve "Rock Hill" in south Kaua'i at an elevation of 11 m on 14 Jan 2022. To determine if embryos are fully developed or underdeveloped (having morphological dormancy), 100 seeds were submerged in 10 mL vials with tap water for 24 h on 23 Jan 2022.

Seeds were then tested for viability using the tetrazolium chloride (TZ) staining technique with (1%) 2, 3, 5-Triphenyl-2H-Tetrazolium Chloride (AOSA 2010). Using a single-edged razor blade, the outer seedcoats were first bisected to expose the embryos. The seeds were submerged in the 1% TZ staining solution (with four replicates of 25 each placed in individual glass vials) and incubated for 3 hours at 30 °C. Seeds were then removed, and the outer structures surrounding the embryos were excised. The embryos were then observed under a dissecting microscope to confirm full development by determining viability and vigor with the color and extent of stained tissue. Embryos were evaluated to consider a seed as viable (staining proceeded gradually and uniformly from the exposed surface inward where changes in color intensity were gradual without distinct boundaries) and weak but viable (stained grayish red or brighter red) (ISTA 2010).

Germination. All seeds were used in experiments between 30 Nov 2021 and 23 Nov 2022 (with the exception of NTBG Accession # 20210545). To assess dormancy classification based on germination time (Fig. 1), the following replicates were placed in 60-mm-diameter petri dishes on seed germination

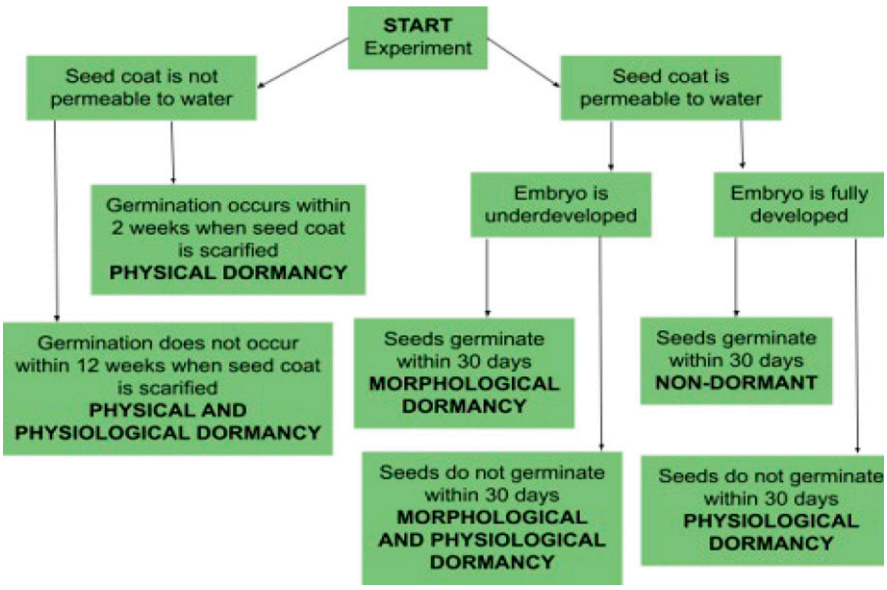

Fig. 1. Key to seed dormancy types. Knowing the type of dormancy is essential to successful seed propagation. Adapted from illustration by Jim Marin in Tropical Nursery Manual (Wilkinson et al. 2014).

Table 1. Summary of methods shows the types of experiments (imbibition and/or germination) conducted for the five seed collections. All seeds were harvested from Kaua'i populations (south or west) between Nov 2021 and Nov 2022. The duration of germination experiments lasted from 82 to 204 d.

NTBG accession	Expt. type	Population	Collection date	Sow date	Duration (d)	No. of treatments (w/ control)	No. reps per treatment	No. seeds per replicate
20210517	Imbibition	South	1 Nov 2021	26 Jan 2022	2	2	3	50
	Germination	South	1 Nov 2021	26 Jan 2022	175	2	3	50
20210545	Germination	South	16 Nov 2021	22 Nov 2022	204	2	5	20
20220025	Germination	South	14 Jan 2022	26 Jan 2022	133	3	4	20
20220070	Germination	South	16 Feb 2022	11 Mar 2022	82	3	3	15
20220431	Germination	West	2 Nov 2022	23 Nov 2022	154	2	3	20

paper (Anchor Paper Company) moistened with gibberellic acid (GA_3; PhytoTech Laboratories, Lenexa, KS, USA) for treatment concentrations of 500 and 1000 ppm GA_3, as demonstrated effective in commercial caper studies (Foschi et al. 2020, 2022a, 2022b, 2023b). A solution of 0.1% Plant Preservative Mixture (PPM^{TM}; Plant Cell Technology, Washington, DC, USA) in distilled water was added to prevent the germination of bacteria and fungi spores. Petri dishes were then sealed with plastic paraffin film to retain moisture and inhibit airborne contamination. Seeds were exposed to 12 h light [~45 $\mu mol \cdot m^{-2} \cdot s^{-1}$ cool white (4100 K) fluorescent light]/12 h dark in a germination chamber with daily simultaneous alternating temperature regimes of 25 °C in light/15 °C in dark. The seeds were observed every 7 d for up to 7 months to monitor germination. All nongerminated seeds used in germination experiments were tested for viability using the TZ staining technique.

Effects of mechanical scarification on germination. After completion of the imbibition test, the same seeds used in the imbibition study (NTBG Accession #20210517) were placed in the germination chamber at the previously discussed conditions and monitored for 25 weeks.

Effects of GA_3 (on 9-d-old seeds) and germination. Seeds of *C. sandwichiana* (NTBG Accession # 20220025) were used in this study from one fruit collected from Makauwahi Cave Reserve "Rock Hill" in south Kaua'i at an elevation of 11 m on 14 Jan 2022. On 26 Jan 2022 (9 d after seed extraction), four replicates of 20 seeds each, with an additional treatment of 500 ppm GA_3 (refer to methods in the previous section "Germination"), were placed in the chamber for 4 months (133 d) to monitor germination.

Effects of GA_3 (on 18-d-old seeds) and germination of floating seeds. Seeds of *C. sandwichiana* (NTBG Accession #20220070) were used in this study from one fruit collected on Māhā'ulepū Heritage Trail in south Kaua'i at an elevation of 14 m on 16 Feb 2022. Seeds were processed based on the methods described previously. Seeds that floated or seeds that sank but appeared underdeveloped were separated from seeds that settled to the bottom. These were set aside and germinated alongside seeds that sank, treated with 0 and 1000 ppm GA_3. On 11 Mar 2022 (18 d after extraction), a germination test for floating seeds and seeds that sank (with or without 1000 ppm GA_3) was conducted. Each treatment consisted of three replicates with 15 seeds each. The germination test followed the procedure discussed above but monitored for 82 d.

Effects of GA_3 (on 15-d-old seeds) and germination of soaked seeds. Seeds of *C. sandwichiana* (NTBG Accession # 20220431) were used in this study from two fruits collected from Pu'u Ka Pele Forest Reserve in west Kaua'i at an elevation of 59 m on 2 Nov 2022. On 23 Nov 2022 (15 d after seed extraction), each replicate (three replicates of 18 seeds from larger fruit and three separate replicates of 20 seeds from smaller fruit) was placed in the germination chamber for ~5 months (154 d). An additional three replicates were placed in sealed glass vials of tap water for 30 d and then transferred to the germination chamber in petri dishes for the same period.

Effects of GA_3 on germination of 1-year-old seeds. Seeds of *C. sandwichiana* (NTBG Accession # 20210545) were used in this study from two fruits collected from Makauwahi Cave Reserve "Rock Hill" in south Kaua'i at an elevation of 11 m on 16 Nov 2021. Seeds were processed based on the methods described previously. They were then stored at ambient laboratory conditions (~47% RH at 23 °C) in one breathable envelope on a shelf at the NTBG Seed Laboratory for ~1 year (367 d after extraction) until used in experiments. On 22 Nov 2022, five replicates of 20 seeds each (with treatments of 0 and 1000 ppm GA_3) were then placed in the germination chamber for ~7 months (204 d) and observed weekly to determine if dormancy had been broken after dry storage.

Data analysis. For imbibition tests, the amount of water taken up was determined as actual changes in seed weights and converted to percentage increase: $[(Wi - Wd)/Wd] \times 100$, where Wi and Wd = masses of imbibed seeds and dry seeds, respectively. Data were analyzed in (software version) using Statistix 10.0 (1985-2013 Analytical Software) to conduct a two-sample *t* test. A linear design scatter plot was created in R version 4.1.3 (R Core Team 2023) with RStudio version 2022.12.0 (RStudio Team 2022) using the ggplot2 package (Wickham et al. 2016) to explore data with 95% confidence error bars. The time-series model compares differences between the mass percent increase of scarified and nontreated seeds in relation to time (hours) of imbibition.

For final germination (%), parametric [analysis of variance (ANOVA)] and nonparametric (Kruskal-Wallis) analyses were conducted. These two analyses provided a robust assessment by validating results across methods that differ in their assumptions about data distribution given our small sample sizes. Data wrangling, analyses, and visualization were conducted in R version 4.1.3 (R Core Team 2023) and RStudio version 2022.12.0 (RStudio Team 2022) with packages ggplot2 (Wickham 2016), survminer (Kassambara et al. 2024), MASS (Venables and Ripley 2002), and ggpubr (Kassambara 2023). Boxplots were generated for final germination while 1-Kaplan-Meier curves and 95% confidence bands were

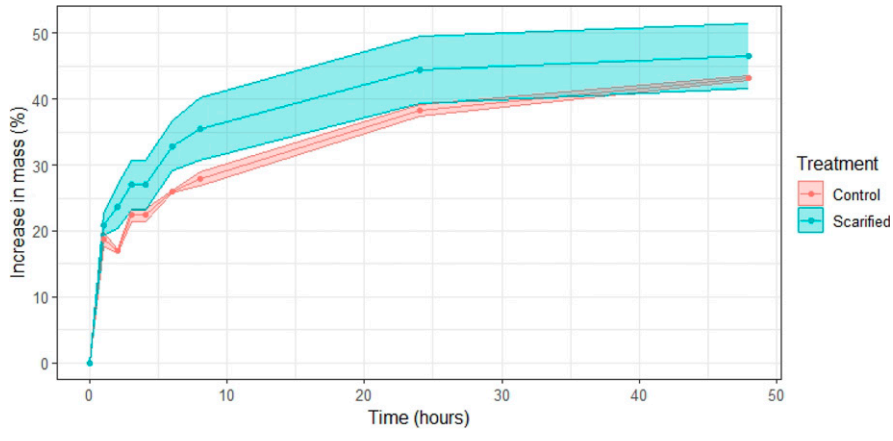

Fig. 2. Time course series shows seed mass increase (%) related to imbibition time (hours) of mechanically scarified and nonscarified *Capparis sandwichiana* seeds for the "Imbibition test" during the 48-h period.

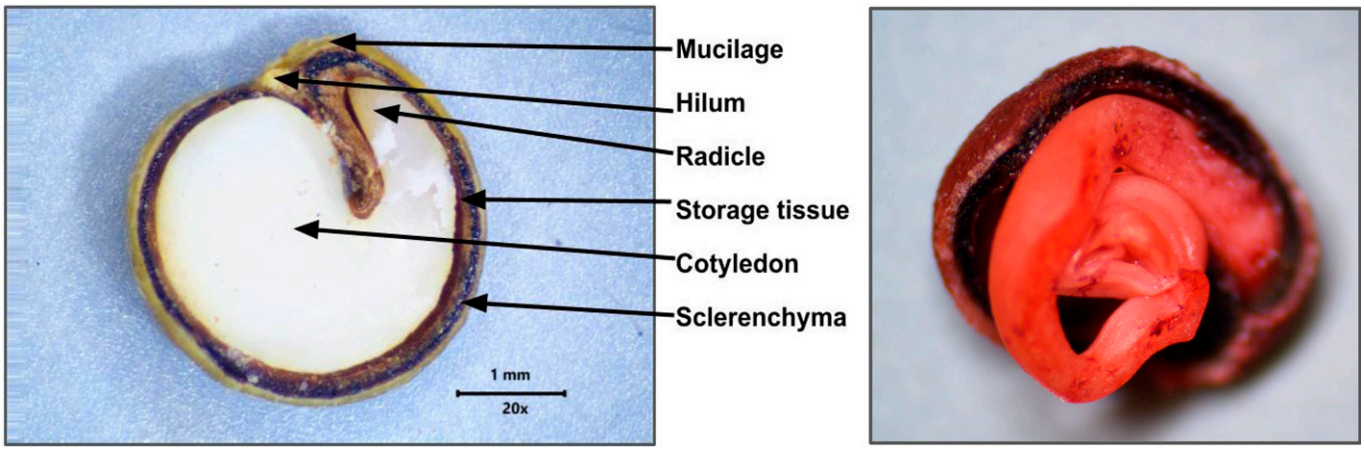

Fig. 3. Both the unstained (left, labeled) and TZ-stained (right) embryos (shown magnified ×20) were fully developed and filled their entire outer structure for *Capparis sandwichiana*.

generated for the survival analysis in relation to time (days) of germination. Further analyses were performed using 1) log-rank test to compare survival distributions of two samples (*Effects of GA_3 and germination of fresh seeds)*, 2) Tukey's honestly significant difference (HSD) to interpret statistical significance of the difference between means *(Effects of GA_3 and germination of floating seeds)*, and 3) Dunn's all-pairwise comparisons test *(Effects of GA_3 on germination of year-old seeds)*.

Results

Imbibition. The amount of water imbibed was determined as actual increases in seed weights and converted to percentage increase. The mass of mechanically scarified seeds increased by 20.9% in 1 h and 46.4% after 48 h, whereas the mass of nonscarified seeds increased by similar rates of 18.73% in 1 h and 43.27% in 48 h. There was not a significant difference between percent increase in mass (P value = 0.268) as a result of the water-permeable seedcoat (Fig. 2).

Embryo development. To determine if embryos were underdeveloped (indicating morphological dormancy), seed embryos were evaluated with the maximum area of stained tissue permitted to consider a seed as viable (ISTA 2010). Both the unstained and TZ-stained embryos were fully developed and filled the entire outer seedcoat (Fig. 3).

Germination

Effects of mechanically scarified and nonscarified seeds on germination. Overall average mean germination occurred at ∼15 weeks with a low total germination rate of 6%. A mean of 3% (±3.712 se) of the mechanically scarified seeds germinated, and 9% (±3.712 se) of the nonscarified seeds germinated. There was a significant difference (P value = 0.0395) in germination of treated and nontreated seeds. Scarified seeds germinated between 3 and 20 weeks, for a mean of 12 weeks. Control seeds germinated between 2 and 25 weeks, for a mean of 16 weeks.

Effects of GA_3 (on 9-d-old seeds) and germination. Neither final percent germination (ANOVA P value = 0.847) nor the curve of germination (Log-rank tests based on Kaplan-Meier germination models; P value = 0.72) indicated significant differences between treatments or controls over the 120-d period. Average final germination across all treatments and control was 27.5 (±0.083 se)%. Mean final germination ranged between 20.0 (±0.060 se; 500 ppm GA_3) and 33.75 (±0.082 se; control)%. Mean time to overall germination across all treatments and control was observed at 63.11 d, and ranged between 28

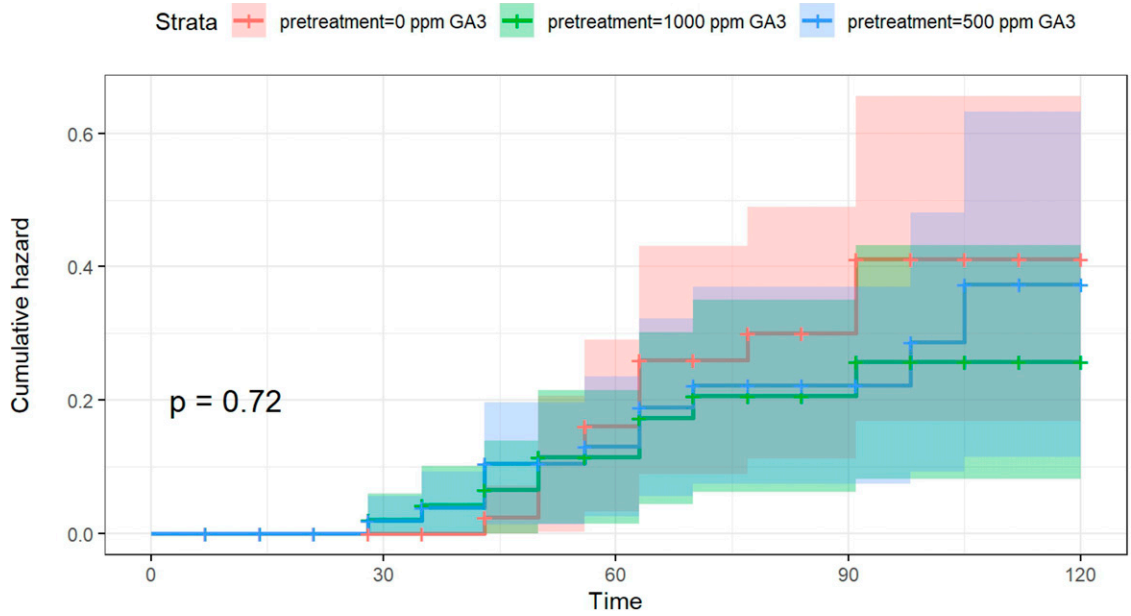

Fig. 4. Time-to-event (days) survival analysis shows comparisons for 0 ppm GA_3 (pink), 500 ppm GA_3 (blue), and 1000 ppm GA_3 (green) treatments in the germination experiment "Effects of GA_3 (on 9-d-old seeds) and germination" for *Capparis sandwichiana*, with the corresponding P value indicating no significant difference among treatments during the 4-month period.

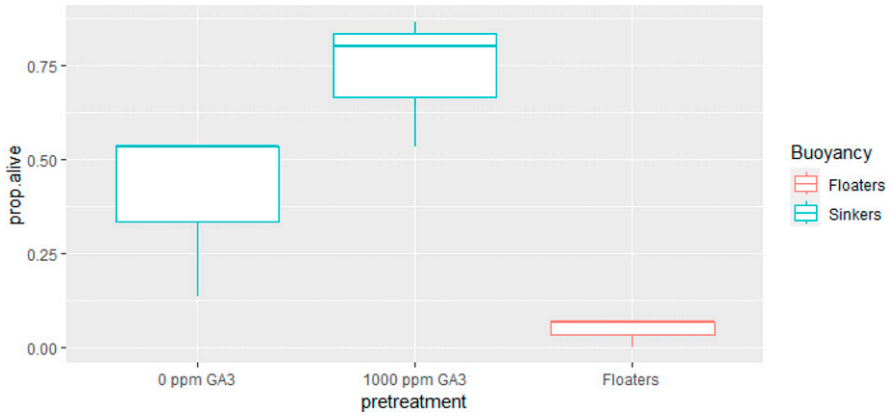

Fig. 5. Boxplots show final total germination (proportion alive) for sinking seeds (blue) with 0 and 1000 ppm GA_3 treatments and nontreated floating (red) seeds for the germination experiment "Effects of GA_3 (on 18-d-old seeds) and germination of floating seeds" for *Capparis sandwichiana* after 82 d.

(±0.018 se; 500 ppm GA_3), 28 (±0.02 se; 1000 ppm GA_3), and 105 (±0.091 se; 500 ppm GA_3) days. Mean time to 25% germination (T_{25}) across all treatments and control was observed at ~63 (±0.72 se) days (Fig. 4).

Effects of GA_3 (on 18-d-old seeds) and germination of floating seeds. There was a significant difference (P = 0.013) with the germination rate between floating and nonfloating seeds using ANOVA and post hoc Tukey's HSD (Fig. 5). Germination of floating seeds was low at 4.44% (±0.312 se), so it was discarded from the overall final analysis. There was no significant difference between final germination of GA_3 treatment and control (Fig. 6). Average final germination across treatments was 56.67% (±0.16889 se). Mean germination ranged between 40 (±0.211 se; control) and 73.33 (±0.171 se; 1000 ppm GA_3)%.

There was no significant difference in final germination among any of the treatments or control (P value = 0.1179) over the 76-d period. Mean time to overall germination for GA_3 treatment and control was observed at 40.18 (±0.31 se) days, and ranged between 26 (±0.049 se; control) and 76 (±0.211 se; control and ±0.203 se; 1000 ppm GA_3) days (Fig. 6). Mean time to 25% germination (T_{25}) across treatments (not including floating seeds) and controls was observed at 47 d.

Effects of GA_3 (on 15-d-old seeds) and germination of soaked seeds. There was a significant difference (P = 0.00026) with the germination rate between 1000 ppm GA_3 and soaking treatments using ANOVA and post hoc Tukey's HSD. Average germination across all treatments and control was 83.09% (±6.3676 se) over the 203-d period. Mean final germination ranged between 80.18 (±0.146 se; control) and 86.67 (±0.054 se; 1000 ppm GA_3)%. Mean time to overall germination for treatments and control was observed at 103.6 (±0.061 se) days, and ranged between 27 (±0.009 se; 1000 ppm GA_3) and 203 (±0.146 se; control) days. Mean time to 25% germination (T_{25}) across all treatments and controls was observed at 56 d (Fig. 7).

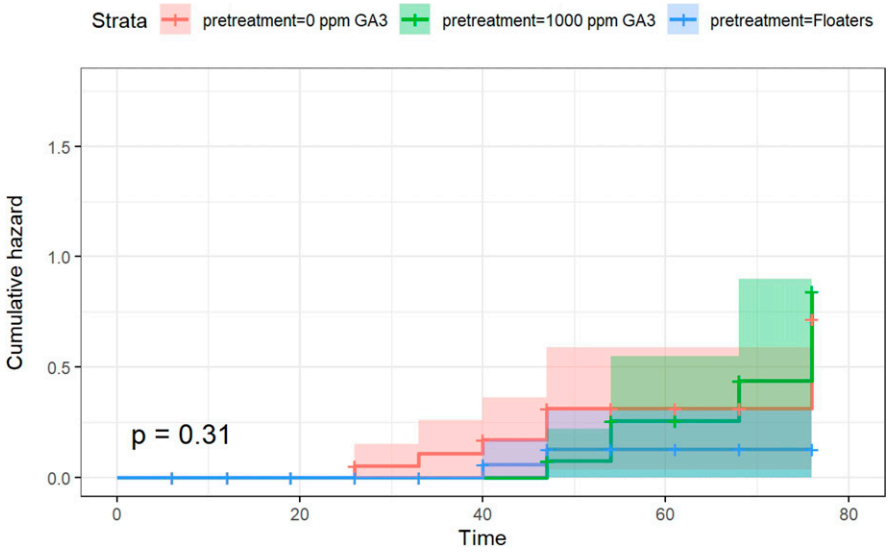

Fig. 6. Time-to-event (days) survival analysis for control (red), 1000 ppm GA_3 (green), and floating (blue) seeds shows corresponding P value indicating pairwise comparison between the control (0 ppm GA_3) and treatment (1000 ppm GA_3) for the experiment "Effects of GA_3 plus floating seeds" of *Capparis sandwichiana*.

Effects of GA_3 on germination of year-old seeds. Neither final percent germination (ANOVA; P = 0.3) nor the curve of germination (Log-rank tests based on the Kaplan-Meier models; P = 0.97) indicated significant differences between treatments or controls over the 204-d period. Average germination for the treatment and control was low at 16.5% (±0.057 se). Mean germination ranged between 12 (±0.023 se; control) and 21 (±0.024 se; 1000 ppm GA_3)%. Mean time to overall germination for GA_3 treatment and control was observed at 108.6 d; it ranged between 28 (±0.008 se; control and ± 0.008 se; 1000 GA_3) and 106 (±0.024 se; 1000 ppm GA_3) days (Fig. 8). Mean time to 25% germination (T_{25}) was never achieved.

Overall effects of GA_3 on germination. When aggregating data across all three dormancy experiments, there was no significant difference (P value = 0.51) in germination among seeds treated with GA_3 and nontreated seeds (Fig. 9). After assays ended, 29% of seeds treated with 1000 ppm GA_3 germinated, 20% of seeds treated with 500 ppm GA_3 germinated, and 34% of the nontreated seeds germinated. Seeds treated with GA_3 germinated between 27 (±0.003 se; 1000 ppm GA_3) and 175 d (±0.031 se; 1000 ppm GA_3), for an average of 93 d. Control seeds germinated between 26 (±0.003 se) and 203 (±0.086 se) days, for an average mean of 93 d.

Overall mean germination for all treatments and controls occurred in 90 d with a mean germination rate of 43%. Maximum germination reached 38.75% with control. In addition, lowest germination reached 12.5% with floating treatment. Overall time to reach 25% total germination (T_{25}) was ~204 d. Mean time to overall germination across all populations was observed at 90.47 d and ranged between 26 (±0.003 se; control) and 203 (±0.086 se; control) days. Mean time to 25% germination (T_{25}) across all populations was observed at 190 d.

When aggregating data across all three dormancy experiments, a Kruskal-Wallis one-way nonparametric ANOVA and Dunn's all-pairwise comparisons test with χ^2 approximation indicated significantly higher final mean germination among Kauai's west plant populations (36.3%) compared with the south (15.3%) (P = 0.0163, ± 4.04 se). Average germination across all sources was 18.5%. Mean final germination for south and west populations ranged between 14.5 (±3.42 se; south) and 22.5 (±3.42 se; west)% (Fig. 10). Maximum germination reached 79.5% with western populations. Lowest germination reached 13.5% with southern populations.

Discussion

Knowing the type of seed dormancy is essential to successful seed propagation. Previous research on the *Capparis* genus has indicated that seed dormancy is partly due to physiological limitations (Bahrani et al. 2008; Foschi et al. 2020; Orphanos 1983). The

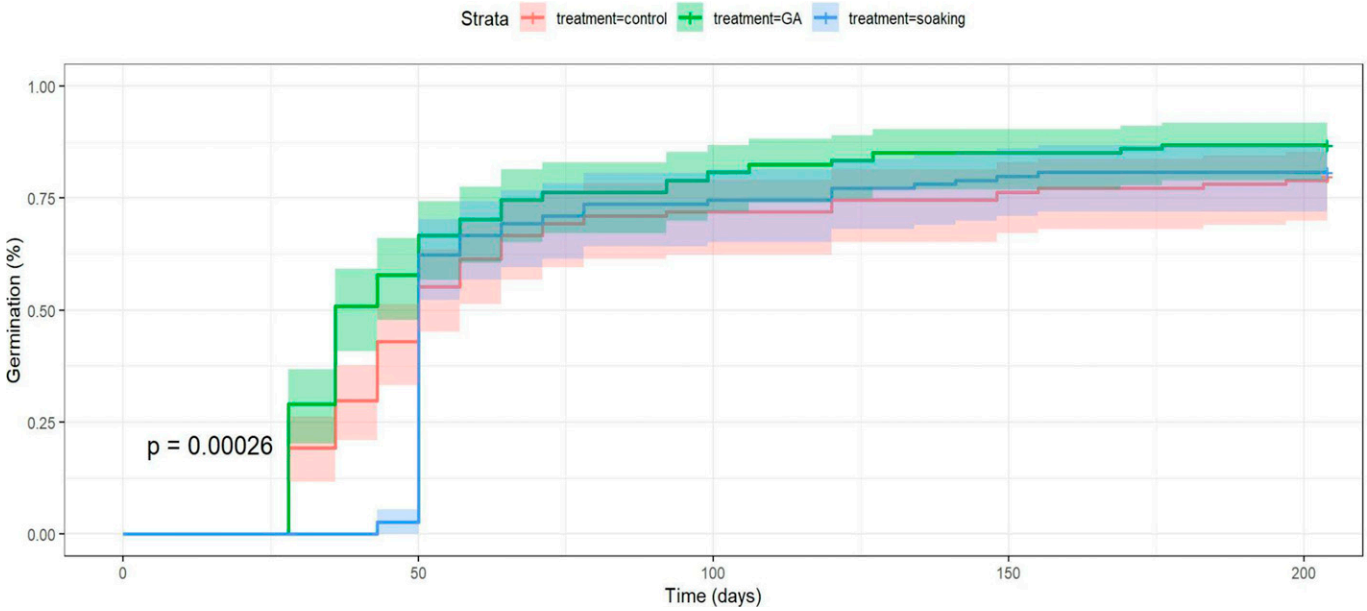

Fig. 7. Time-to-event survival analysis shows lowest corresponding *P* value (0 and 1000 ppm GA_3) indicating a significant difference ($P = 0.00026$) with the 30 d tap water soaking treatment in the germination experiment "Effects of GA_3 plus soaked seeds" of *Capparis sandwichiana* during the 7-month period.

effects of gibberellic acid (GA_3) on germination may indicate the presence of physiological dormancy in members of the genus (Baskin and Baskin 2004). Since embryos are fully developed and seedcoats are water permeable, further germination tests on *Capparis sandwichiana* were necessary to determine if seeds of this species exhibit physiological dormancy or nondormancy. Physiological or morphophysiological dormancy has been observed in several native Hawaiian species including in the families Amaranthaceae, Asphodelaceae, Asteraceae, Campanulaceae, Caryophyllaceae, Ericaceae, Euphorbiaceae, Gesneriaceae, Goodeniaceae, Hydrangeaceae, Loganiaceae, Poaceae, Primulaceae, Rosaceae, other species in Rubiaceae, Scrophulariaceae, Urticaceae, and Violaceae (Baldos et al. 2014, 2015; Baskin and Baskin 2014; Baskin et al. 2005, 2020; Opgenorth et al. 2024; Wolkis et al. 2018, 2022, 2023).

The first step for identifying seed dormancy classification (Fig. 1) is determining if the seedcoat is permeable to water (Wilkinson et al. 2014). There was no significant difference in imbibition between the scarified and nonscarified seeds, indicating the seedcoats are water permeable, thus ruling out physical dormancy. Significantly higher germination occurred among nonscarified seeds, further suggesting a water-permeable seedcoat.

The next step to identifying dormancy class is determining whether the seed embryos are fully developed, which was demonstrated in previous studies of the genus (Martin 1946; Le Maout et al. 1873) and confirmed in microscopic examination of TZ-stained and unstained seed embryos in *C. sandwichiana* (Fig. 2). Because the embryos are fully developed and filled their entire outer structure, this rules out the possibility of morphological dormancy.

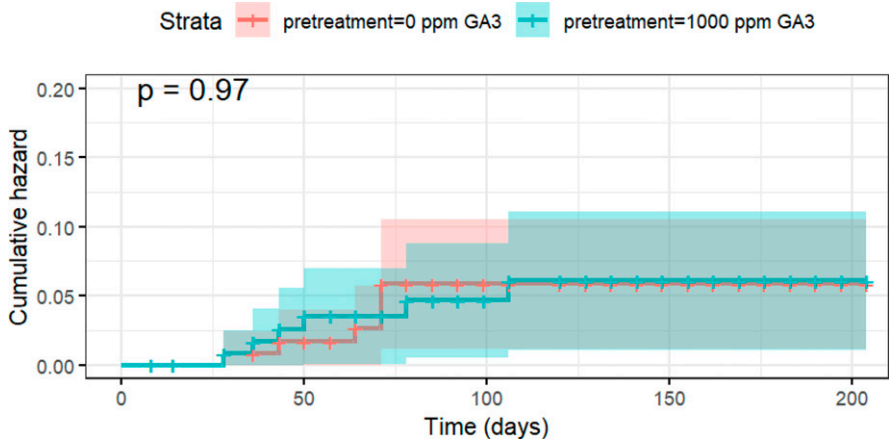

Fig. 8. Time-to-event survival analysis shows cumulative hazard proportion for germination of seeds treated with 0 ppm GA_3 (red) and 1000 ppm GA_3 (blue) in the experiment "Effects of GA_3 on year-old seeds" for *Capparis sandwichiana* during the 204-d period, with corresponding *P* value (0.97) for pairwise comparison indicating no significant difference.

The next step to dormancy identification is determining if seeds germinate within 30 d (Wilkinson et al. 2014) in optimal conditions. Germinating temperatures for experiments were relative to those where seeds were collected with annual averages of 23 °C (Giambelluca et al. 2014). In general, seeds with fully developed embryos that germinate within 30 d have characteristics of nondormancy, whereas seeds that do not germinate within 30 d have dormancy (Baskin and Baskin 2014). Because seeds in this study of *C. sandwichiana* did not germinate within an average of 30 d, they cannot be classified as nondormant. Instead, tests indicate characteristics of physiological dormancy; seeds 1) imbibe water, 2) have an embryo that does not grow inside the seed before germination, and 3) require more than 4 weeks to germinate (Table 2).

Further germination tests are necessary to determine the level of physiological seed dormancy that most likely applies (nondeep, intermediate, or deep). Deep physiological dormancy is unlikely, because native coastal Hawaiian species do not naturally experience long periods of cold temperatures (i.e., cold stratification). Future experiments testing warm stratification may find further evidence of nondeep physiological dormancy. Ecological differences between individual plants and entire plant populations (Fig. 10), as well as genetic, environmental, and seasonal variations, add to the complexity of classifying seed dormancy, which may include intermediate levels.

In our studies, there was not a significant difference in germination of nontreated seeds and those treated with GA_3, as well as the varying concentrations of gibberellic acid (Fig. 7). However, some replicates treated with GA_3 showed increased rates of fungal development, which may be a factor in low germination rates. The orange-colored mucilage surrounding the testa may increase chances

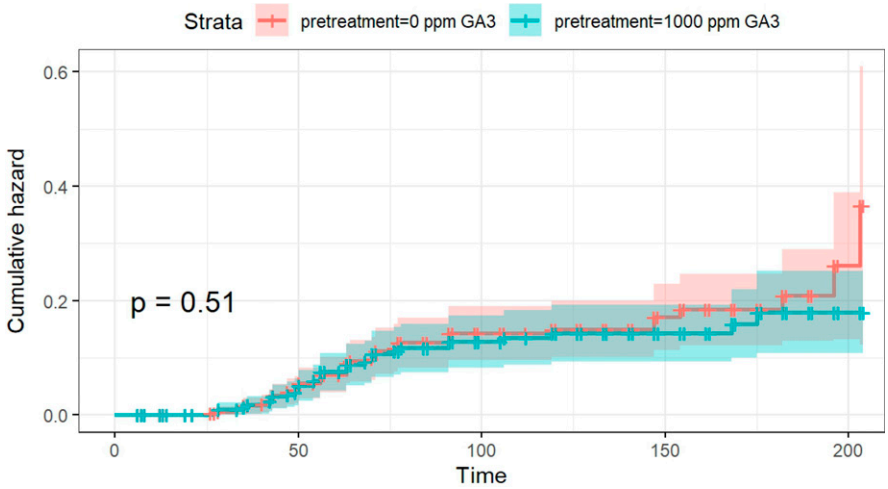

Fig. 9. Time-to-event (days) survival analysis shows overall germination (%) for GA_3 treatments, 0 ppm (red) and 1000 ppm (blue), for all four experiments on *Capparis sandwichiana* seeds, with corresponding *P* value (0.51) for pairwise comparison indicating no significant difference.

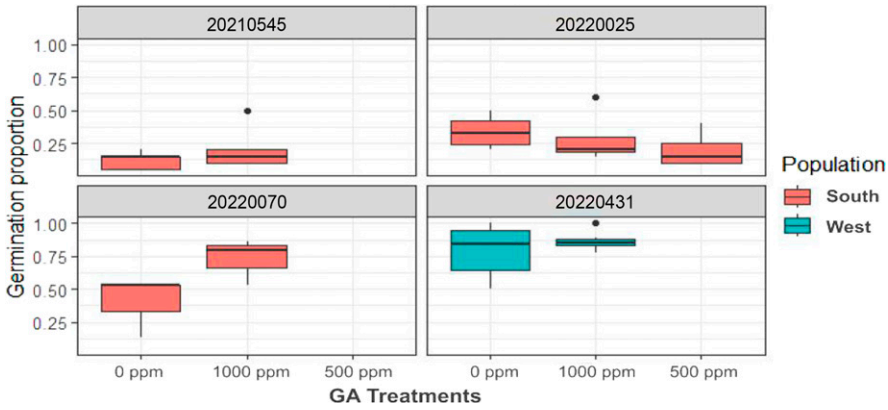

Fig. 10. Total final germination proportions compare GA_3 treatments of two populations, Māhā'ulepū Heritage Trail/Makauwahi Cave Reserve (south; red) and Pu'u Ka Pele Forest Reserve/Polihale (west; blue) for four accessions of *Capparis sandwichiana* seeds.

of fungal development in nongerminated seeds, inhibiting germination by not allowing embryos to absorb oxygen (Orphanos 1983).

In initial experiments analyzing imbibition mass and scarification, seeds placed in petri dishes in the germination chamber showed evidence of white and gray mold growth within the first week. However, seeds used in the following studies were thoroughly blotted with paper towels to remove existing pulp and did not show immediate signs of extensive mold. Soaking seeds may further remove fruit pulp (Culliney and Koebele 1999) and soften seedcoats (Lilleeng-Rosenberger 2005), preventing mold growth and priming seeds for more rapid, uniform emergence. Although all petri dishes included a 0.1% solution of Plant Preservative Mixture (PPM^{TM}) in distilled water to help prevent the germination of both bacteria and fungi spores, improved pathogen abatement methods may help increase future germination rates.

Additionally, GA_3 treatments in the year-old seed experiment showed no evidence of after-ripening, and overall seed viability for *Capparis* generally declines over time (Foschi et al. 2022b). Although the fruits of *C. spinosa* also contain many seeds, they germinate slowly and with very low percentages due to their non-deep physiological dormancy (Foschi et al. 2023b). In seeds of *C. sandwichiana,* specifically viability was observed to decline below 70% of maximum in fewer than 5 years (Chau et al. 2019). However, sampling seeds at designated intervals and determining P_{50}, defined as the time for viability to decline to 50% (Hay et al. 2022), should be explored to fully understand seed longevity. Further studies determining desiccation and freeze tolerance may contribute to ex situ conservation of this species. All of these results will benefit conservation managers and *Capparis* growers with a better understanding of seed characteristics for higher germination rates of Hawaii's vulnerable endemic species in need of ex situ and in situ conservation.

Table 2. This summary table shows results for all germination treatments (including the control) with mean final germination (%), final germination overall (%), and mean time (days) to 25% germination (T_{25}).

NTBG accession	Expt. type	Treatment	Mean final germ. (%; se)	Overall final germ. (%; se)	Mean *T*25 (days; se)
20210517	Germination	Control	9 (±3.712)	6 (±3.712)	—
		Scarification	3 (±3.712)		—
20210545	Germination	Control	12 (±0.023)	16.5 (±0.057)	—
		1000 ppm GA3	21 (±0.024)		—
20220025	Germination	Control	33.75 (±0.082)	27.5 (±0.083)	63 (±0.72)
		500 ppm GA3	20 (±0.060)		
		1000 ppm GA3	28 (±0.02)		
20220070	Germination	Control	40 (±0.211)	39.26 (±0.118)	47 (±0.97)
		1000 ppm GA3	73.33 (±0.171)		
		Floaters	4.44 (±0.169)		
20220431	Germination	Control	80.18 (±0.146)	83.09 (±6.368 se)	56 (±0.67)
		1000 ppm GA3	86.67 (±0.054)		
		30 d soaking	81.77 (±0.044)		

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