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Protocol to determine seed dormancy, imbibition, viability, longevity and germination responses to temperature

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We use this protocol and it's working

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Abstract

We study the seed traits and germination strategies of arid plant species to investigate their regeneration strategies and classify seed dormancy. Seeds of eight keystone species were germinated under three dirunal temperatures (30/20C, 25/15C and 17/7C) for 30 days. We also tested for decline in seed viability across 24 months in dry aging, and relieved physiological dormancy in *Atriplex* through an after-ripening treatment.

Materials

MATERIALS

- Sodium Hypochlorite Solution
- Gibberellic Acid 3 (Quick-Dissolve™) **Gold Biotechnology Catalog #**G-120
- 2,3,5-Triphenyltetrazolium chloride (TTC; Tetrazolium chloride) **Gold Biotechnology Catalog #**T-112
- Sulfuric Acid (H2SO4)

Safety warnings

Gibberllic Acid andTetrazolium is a highly flammable liquid and vapor and can be toxic if swallowed or inhaled. Use product in ventilated aread and wear gloves to avoid skin contact. Sulphuric Acid can cause permenant blindness if it makes direct contact with the eye and may cause death if ingested. Sodium hypochlorite is less hazardous, but can still cause severe eye damange and skin burns. Protective clothing, eyewear and gloves should be worn when using these acids.

Seed viability

1 Test for seed viability: Place seeds in a petri dish, soak with a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TZ), and incubate seeds under diurnal temperatures for at least 48 hours. Extract seeds from the incubator and perform longitudinal dissections under a microscope to record seed viability. Seeds that remain unstained or only partially stained, are recorded as non-viable (assuming they respond to TZ treatment), and seeds that stain bright red or pink are recorded as viable. Not all species have seeds that respond to TZ staining and, to ensure accurate TZ interpretation, results are compared to tests for viability by germinating seeds on filter paper moistened with distilled water, and at diurnal temperatures (assuming seeds are non-dormant).

Seed longevity

2 Test for seed longevity: We tested for initial seed viability using step one. Excess organic matter was removed from the seed batch, and seeds were stored in paper bags, under constant dark and air-conditioned temperatures (10-20°C) and low humidity (<50%). Temperature and humidity were monitored and recorded frequently. To test for viability loss with aging, 100 seeds were extracted at 0, 3, 6, 12, 18 and 24 months, and tested for viability using the TZ methods in step one.

Seed imbibition

3 For imbibition tests, four replicates of 25 seeds were weighed, placed on moist filter paper, and incubated under three diurnal temperatures of 30/20°C, 25/15°C and 17/7°C, under a 12 hr light/dark schedule. Seeds were weighed when dry at beginning of experiment and, after 5 min on moist filter paper, seeds were removed and patted dry with a paper towel to absorb surface moisture and re-weighed. To determine increases in seed weight, seeds were re-weighed at 10 min, 30 min and at 1, 2, 3, 6, 9, 24, 48, 72, 96, 120, 144, 168 and 192+ hrs.

Classifying dormancy and germination responses to temperature

4 Classifying dormancy and germination responses to temperature: Prior to germination treatments, seeds of all species were surface sterilised by soaking in 1% sodium hypochlorite for one minute, then rinsed for 40 seconds with double distilled water. For each species, four replicates of 25 seeds each were used. Seeds were placed in 90-mm diameter petri dishes on filter paper moistened with distilled water and incubated at a 12/12-hr light/dark regime at daily alternating temperatures of 30/20°C, 25/15°C and 17/7°C). To determine the effects of gibberellic acid (GA_3) on seed germination, species

were incubated at 30/20°C and the filter paper was moistened with a 350 ppm GA_3 solution. Seed germination (when the radicle emerged to at least half of seed size) was recorded daily for 30 days, or until germination ceased for four consecutive readings across all treatments. Seeds that germinated within days without pre-treatment were classified as non-dormant. If seeds imbibe water and show a positive response to GA_3, but do not germinate within 30 days, they are physiologically dormant. If seeds do not imbibe water or germinate, they are physically dormant. Our protocol to determine dormancy adheres strictly to the methods outlined in 'Baskin & Baskin (2014) Seeds: Ecology, biogeography and evolution of dormancy and germination, 2nd ed, San Diego, Academic Press'.

After-ripening to alleviate physiological dormancy

5 To alleviate physiological dormancy in *Atriplex*, we applied an after-ripening treatment by storing seeds (according to step 2 to assess seed longevity) for one year, under constant dark and air-conditioned temperatures. After-ripened seeds were then exposed to the same germination treatments outlined in step 4. Additional tests were performed to alleviate physiological dormancy, including the move-along method and soaking seeds in boiling water and 90% H_2SO_4. All of these treatments to relieve dormancy, including the after-ripening treatment, follow the methods outlined in Baskin & Baskin (2014; see step four for reference details).