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Slow and Fast Desiccation of Single-cell Thick Fronds of Filmy Ferns

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[Abstract] Filmy ferns can desiccate and recover after rehydration to resume photosynthesis. Slow and fast desiccation rates were compared in filmy fern fronds to determine whether structural or physiological differences may occur between desiccation rates. Slow desiccation is considered to be more similar to natural conditions experienced by plants that grow under the forest canopy. A fast desiccation rate will help to understand whether slow desiccation is important for recovery and viability.
Keywords: Desiccation tolerance, Filmy fern, Desiccation, Hymenophyllaceae

[Background] The Hymenophyllaceae is a family of epiphytic pteridophytes highly endemic to shady, constantly humid forest (Figure 1). There are some species of this filmy fern group that can survive desiccation to 20% relative water content, remain in this state for an extended period, and survive following rehydration (Figure 2) (Garcés *et al.*, 2018) (See example timelapse, Video 1).

Figure 1. *Hymenophyllum caudiculatum* growing in nature under the forest canopy

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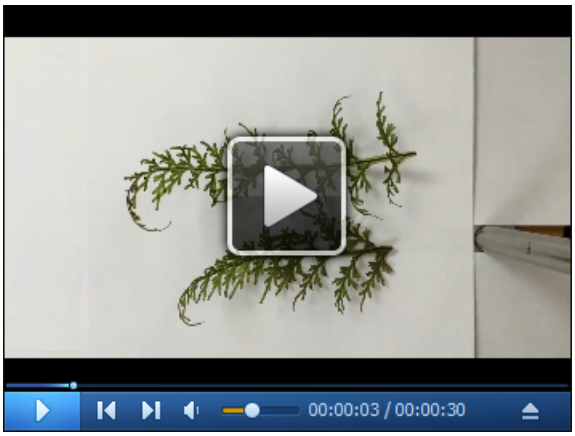
Figure 2. Detached *Hymenophyllum caudiculatum* fronds under different conditions (Modified from Garcés, 2014)**.** (Top) fresh detached, (middle) desiccated, and (bottom) rehydrated.



Video 1. Example of curling of *Hymenophyllum caudiculatum* fronds after desiccation/rehydration

Early studies of desiccation tolerance of *Tortura ruralis* examined slow or rapid dehydration on 500 mg fresh moss tissue (Dhindsa, 1987). Rapid desiccation was imposed by placing the tissue over activated silica gel granules in a desiccator (Relative Humidity [RH] of nearly 0%). Slow desiccation was administered by placing tissue samples over a stirred, saturated solution of ammonium nitrate contained in a desiccator (65% RH). A final weight of less than 20% original fresh weight was obtained in about 8 h of slow drying and in less than 30 min of rapid drying.

As ammonium nitrate is a hazardous and regulated substance in several countries, in this work, the slow desiccation solution was replaced by a saturated solution of potassium chloride (RH 75%).

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Materials and Reagents

1. Absorbent paper
2. Petri dishes (Merck, catalog number: CORM3160-150X15)
3. Silica gel 2-5 mm (Merck, catalog number: 1077351000)
   Note: Dry in an oven for 24 h at 60 °C.
4. Filmy fern fronds collected from Katalapi Park, Chile (Garcés *et al.*, 2018).
   Note: Fully expanded fronds of about 20 cm long were used in this protocol.
5. Potassium chloride (KCl) (Merck, catalog number: 1049361000)
6. Saturated KCl solution (see Recipes)

Equipment

1. Glass desiccator, amber 300 mm (W.W. Grainger, model: 5YHV5)
2. Analytical scale (Sartorius, catalog number: BP221S)
3. Forced air drying oven 50 L (Biobase, catalog number: BOV-T50F)

Procedure

1. Wash the detached fronds from fully hydrated plants by soaking in distilled water for five minutes, remove excess water carefully by blotting with absorbent paper.
2. Place the fronds in a glass desiccator in the dark at room temperature, with 500 g silica gel (fast desiccation) or over 500 ml of a saturated solution of potassium chloride (KCl), 75% RH (slow desiccation) for 24 h.
3. Weight fronds every 10 min. Weight needs to be determined very rapidly.
4. For rehydration, place the previously desiccated fronds in Petri dishes with 10 ml distilled water in the dark for 24 h. Weight fronds every 10 min.

Recipes

1. Saturated KCl solution
   Dissolve 170 g of KCl to 500 ml distilled water

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