

# Flaming Fabaceae

—Using an Alcohol Flame

to Break Seed Dormancy

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## ABSTRACT

Soaking Fabaceae seeds in alcohol and then passing them through a flame for 2 to 3 s is a quick and easy way of promoting germination of at least 8 species of Hawaiian legumes. In addition to stimulating germination, the technique effectively sterilizes seeds for in vitro germination for micropropagation or for germination in the greenhouse.

## KEY WORDS

Micropropagation, greenhouse, threatened and endangered, recalcitrant, legume

## NOMENCLATURE

ITIS (2002)

In 1991, the Lyon Arboretum on O‘ahu initiated the Rare Hawaiian Plant Program utilizing micropropagation as a tool for plant genetic conservation. The mission of this project was to prevent further extinction of Hawaiian plant species, propagate plants for use in approved restoration and reintroduction projects, and initiate and maintain an in vitro germplasm collection of the “critically endangered” plants included within the Genetic Safety Net List (GSNL). The GSNL generated by the Hawai‘i Rare Plant Restoration Group is a collection of plant taxa identified as “critically endangered” and given top management priority.

Plant micropropagation is a technology that has been developed and redefined continuously over the past 30 years and has received an increasing amount of interest as a propagative method for plant genetic conservation (Dodds 1991). In Hawai‘i, micropropagation is commonly used to propagate many Hawaiian plant taxa, including our most “critically endangered” for germplasm storage, ex situ use, and restoration and reintroduction projects. Micropropagation is especially suitable when: 1) the plant species are difficult to propagate using conventional propagative methods; 2) viable plant propagules are rare due to inbreeding depression or difficulty in accessing plants; 3) plants have very small, recalcitrant, or immature seeds or spores; or 4) only poor quality propagules are available due to unhealthy parent stock.

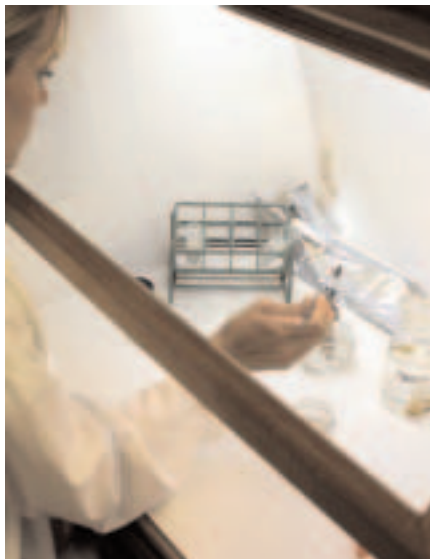


Figure 1. Flaming the alcohol off a *Canavalia* seed enhances germination.



Figure 2. A *Canavalia napaliensis* seedling about 2 wk after treatment.

In Hawai'i, a total of 13 native genera with 16 endemic species occur within the family Fabaceae (Wagner and others 1999). The fruit is usually a legume and typically possess hard, smooth seeds. Physical dormancy is present, caused by an impervious seed coat that requires scarification to initiate the germination process (Janick 1979).

At the Lyon Arboretum we work with very small amounts of Fabaceae seeds and have found that germination can be expedited as well as enhanced by quickly passing alcohol-coated seeds through a flame and allowing the alcohol to burn. Flaming is apparently effective in weakening the hilum, which then allows water imbibition to occur. The heat also efficiently sterilizes seeds for in vitro seed sowing or even for propagation in a clean greenhouse seed tray. So far, we have successfully propagated 8 endemic Hawaiian species (Table 1) with the following procedure.

### FLAMING PROCEDURE

1. Soak seeds in a 10% Clorox™ bleach solution for 15 min, providing gentle agitation.
2. In a transfer hood, pour 95% ethyl alcohol (you may substitute with 70% isopropyl alcohol) into a small beaker or fireproof container (you may also do this as a “non-sterile” technique on the

bench top if you plan to germinate the seeds in a greenhouse). Keep a petri dish or some other cover close at hand in case you set the alcohol on fire. Smothering the flame by covering the beaker will extinguish the fire.

3. Place the seeds into the beaker and let them soak for approximately 1 min (do not rinse off bleach solution).
4. With forceps, extract seeds one or two at a time and pass them briefly (2 to 3 s) through a flame (Figure 1).
5. Extinguish seeds by dropping them into sterile water (if you plan to germinate seeds in the greenhouse, you need only clean water). You may also extinguish the seeds in vitro by placing them directly onto the agar surface in your culture vessel.

Seed germination in vitro does not require any special media formulation. In the laboratory, we use a half-strength Murashige and Skoog (MS) medium (Murashige and Skoog 1962) without hormones, solidified with Phytogel®, and pH adjusted to 5.7 to 5.8. After extinguishing the seeds on the agar, the culture vessels with seeds are kept at approximately 22 °C (72 °F) under a 14-h light and 10-h dark cycle. In the greenhouse, we sow seeds into trays containing a 3:1 (v:v) perlite:vermiculite seed mix and place them under 50% shade with prevailing Hawaiian temperature conditions.

Within 24 h seeds swell up to 33% greater than their original size and within 3 to 4 d germination is visible. With *Canavalia napaliensis*, we had a 7.5-cm-long (3-in) seedling in just 2 wk (Figure 2). One main advantage of this technique is that it saves us time—without flaming, these seeds normally take at least 6 mo to germinate in our shadehouse.

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TABLE 1

*Species in the Fabaceae successfully germinated using an alcohol flame treatment.*

Scientific name	Common name
<i>Acacia koa</i> Gray	koa
<i>Caesalpinia kavaiensis</i> Mann.	uhiuhi
<i>Canavalia kauaiensis</i> Sauer.	Kaua'i jackbean
<i>Canavalia napaliensis</i> St. John	Mākaha Valley jackbean
<i>Erythrina sandwicensis</i> O.Deg	wili wili
<i>Sesbania tomentosa</i> Hook. & Arn.	'ohai
<i>Sophora chrysophylla</i> Seem	māmāne
<i>Vicia menziesii</i> Spreng.	Hawai'i vetch
<i>Vigna o-wahuensis</i> Vogel	O'ahu cowpea

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