# The Recovery of Hawaiian Plant Species Using Embryo and Ovulo Culture©

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**The Lyon Arboretum Rare Hawaiian Plant Program's mission is to prevent further extinction of Hawaiian plant species, propagate plants for restoration and reintroduction projects, and initiate and maintain an in vitro germplasm collection primarily of the "critically endangered" plants. Two techniques routinely used to establish in vitro cultures of seed-derived explants are embryo and ovulo culture. Embryo culture is the isolation of an immature or mature embryo and its growth within a sterile culture. Ovulo culture is the establishment of an embryo with its endosperm. In many cases, embryo and ovulo culture can eliminate seed germination inhibitors, shorten the breeding cycle, and prevent embryo abortion. Some of the Hawaiian genera that have benefited from embryo and ovulo culture are *Alectryon, Hesperomannia, Kokia, Nestegis, Ochrosia, Pritchardia,* and *Tetraplasandra*.**

## INTRODUCTION

In 1991, Lyon Arboretum initiated the Rare Hawaiian Plant Program (RHPP) utilizing micropropagation as a tool for plant genetic conservation. The objectives were to assist in the prevention of further extinction of Hawaiian plant taxa, to propagate plants for use in restoration and reintroduction projects, and to initiate and maintain an in vitro germplasm collection of the "critically endangered" plants included in the Genetic Safety Net Listing (GSNL). The GSNL, generated by the Hawai`i Rare Plant Restoration Group (HRPRG), is comprised of approximately 150 critically endangered Hawaiian taxa, some having 20 or fewer plants in the wild. The Lyon Arboretum Micropropagation Laboratory is the designated in vitro propagation facility for the "critically endangered" Hawaiian plant species collected by the State of Hawai`i Department of Land and Natural Resources as well as the other members of the HRPRG. Hawai`i Rare Plant Restoration Group serves to identify the critically at-risk Hawaiian plant species and to develop and initiate collection strategies for landowners and propagators by identifying deficiencies in sampling and ex situ germplasm inventories.

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). It is especially utilized when: (1) species are difficult to propagate using conventional propagative methods, (2) viable propagules are rare due to inbreeding depression or difficulty in collecting plants, (3) plants have very small, recalcitrant, or immature seeds or spores, (4) plant numbers are low, reducing the amount of material available for propagations, and (5) propagules are of poor quality due to unhealthy parent stock. One of the goals for RHPP is to acquire and propagate adequate representations of all the critically at-risk Hawaiian plant taxa, especially those included

in the GSNL (Sugii and Lamoureux, 1998; Hawai`i Rare Plant Restoration Group, 1999). Vegetative propagules as well as mature and/or immature seed are collected from these individuals and sent to the laboratory for in vitro propagation. Two techniques used routinely for the establishment of seed-derived explants in in vitro cultures are embryo and ovulo culture.

## EMBRYO CULTURE

Embryo culture entails the excision of an immature or mature embryo from the seed and its growth in vitro, with the goal of obtaining a viable plant. Many native Hawaiian plant species exhibit impaired reproductive capabilities, which result in premature seed abortion. In these cases, embryo culture has been useful in rescuing immature embryos and producing viable plants (George, 1993 and Dodds, 1991). Embryo culture can also be useful in overcoming seed dormancy and shortening the germination time (Bridgen, 1994).

***Kokia cookei.*** Immature fruits are washed in water then sterilized in 10% Clorox + Tween 20 solution for 1 h, then dipped into 95% ethanol and briefly flamed. The fruit are cut open and the embryos are excised. The seed are carefully sliced open and the embryo removed. Embryos are cultured on half-strength Murashige and Skoog medium (MS) (Murashige and Skoog, 1962).

***Pritchardia* spp.** Immature fruit of *Pritchardia* are washed in water then carefully cracked open with a hammer or pliers. The embryos are excised and sterilized in a 10% Clorox + Tween 20 solution for 5 min. The embryos are placed onto half-strength MS medium. Germination time is significantly shortened through embryo culture.

## OVULO CULTURE

Ovulo culture is the establishment of an embryo with its endosperm and may or may not require the removal of the seed coat. It is especially useful when embryos are difficult to dissect due to various reasons such as small seed size, or the culture medium for embryo culture is complicated. It may not be suitable if the maternal tissue or the endosperm exhibits an inhibitory action on the development of the embryo (Pierik, 1987).

***Tetraplasandra* sp.** Immature fruit are washed with water then sterilized in a 10% Clorox + Tween 20 solution for 1 h. The fruits are then dipped into 95% ethanol and briefly flamed. The fruit are cut open and the immature seeds excised and placed onto half-strength MS medium. Excessive browning of the culture medium usually occurs and the seeds are transferred to fresh medium after 1 week. One additional subculture may be necessary before seed germination.

***Hesperomannia arbuscula.*** The immature achenes are cleaned and washed with water then soaked in a 10% Clorox + Tween 20 solution for 30 min. The Clorox solution is decanted and the achenes are rinsed with sterile water. The seeds are sliced open and the ovule is extracted. Ovules are placed onto half-strength MS medium. Germinated embryos are transferred to third-strength MS medium containing 0.2 $mg \cdot L^{-1}$ IAA.

***Cyanea truncata.*** Intact immature fruit are washed in water then soaked in a 10% Clorox + Tween 20 solution for 30 min. The fruit are dipped into 95% ethanol and flamed briefly. In a sterile glass petri-dish, the ends are cut off and the fruit

are sliced open to release the small seeds. Seeds are immersed in sterile water then pipetted into half-strength MS petri-dishes. Excess water on the surface of the medium is pipetted up and discarded.

## CONCLUSION

The Lyon Arboretum Micropropagation Laboratory plays an integral role in the preservation of Hawaiian plant species through in vitro germplasm storage. Due to its location, propagative methodology and affiliations, the laboratory is the recipient of one of the largest and most diverse collections of Hawaiian plant taxa collected throughout the Hawaiian Islands. Embryo and ovulo culture have proved to be viable micropropagation techniques in the recovery of many native Hawaiian plant species especially those that have lost their ability to produce viable seed. The seedlings that are recovered through embryo and ovulo culture are placed into germplasm storage and, when possible, multiplied and ultimately restored in the wild when appropriate management of their habitat can be undertaken.

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