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CONSERVATION OF HAWAIIAN LOBELIOIDS — *IN VITRO* AND MOLECULAR STUDIES

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

HORTICULTURE

MAY 1996

By

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Acknowledgments

I would like to thank the staff and volunteers at the Harold L. Lyon Arboretum and the Lyon Arboretum Association for their support of the *in vitro* research. The staff of the National Tropical Botanical Garden, the State of Hawai'i Division of Forestry and Wildlife, the Nature Conservancy of Hawai'i, the U.S. Fish and Wildlife Service, John Obata, and Rick Palmer are appreciated for supplying plant material. Partial funding was supplied by the U.S. Fish and Wildlife Service, the State of Hawai'i Division of Forestry and Wildlife, the Center for Plant Conservation, the University of Hawai'i Foundation, and Sigma Xi Grants-in-Aid of Research. Thank you to Dave Lorence, Kay Lynch, Loyal Mehrhoff, Carol Nakamura, John Obata, Rick Palmer, Joshlyn Sands, and Alvin Yoshinaga for information used in this report. Special thanks to Sterling Keeley for the use of her lab and supplies and support for the RAPDs work and to Yoneo Sagawa for his knowledgeable support of the *in vitro* research.

Abstract

With over 25% of Hawai'i's Campanulaceae already extinct and many more on the verge of extinction, research in the propagation and the genetics of the remaining populations is greatly needed. *In vitro* propagation of 58 Hawaiian Campanulaceae species were attempted, through *in vitro* germination, organogenesis, or micropropagation. More than 80% of the species received as seeds were successfully germinated. No differences in germination rate or percentage was found between immature and mature seeds. Leaf explants produced viable shoots in 43% of the species, and 29% of wild-collected bud explants were successfully grown into plants. RAPDs was used to detect variability of the seedling populations of two bottlenecked species, *Cyanea asarifolia* St. John (original wild population of 15 plants) and *Delissea undulata* ssp. *undulata* Gaud. (original wild population of one plant). DNA was extracted from each species using small amounts of leaf tissue produced *in vitro* and used for the RAPDs studies. No detectable variation was found within these populations (indicating the impoverished remaining genetic information). The value of *in vitro* propagation and molecular studies of reduced populations is discussed.

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Chapter 1. Introduction

Nearly 25% of the species of Hawaiian Campanulaceae have become extinct in the century since their discovery by Western science (Wagner *et al.*1990). Many more are in danger of extinction and in need of conservation. Populations (or whole taxa) have disappeared and others exist as one or a few individuals. As the habitats of these species are threatened the need for *ex situ* conservation measures becomes great.

In an attempt to meet the needs of *ex situ* conservation of Hawaiian Campanulaceae (and other rare and endangered Hawaiian plants) a micropropagation facility was established in 1991 at the Harold L. Lyon Arboretum. When the laboratory was first established little was known about *in vitro* techniques to propagate Hawaiian plants. Even less was known about the genetic variability of seedling populations of extremely bottlenecked species. This study is an attempt to evaluate the use of *in vitro* techniques in an *ex situ* conservation propagation program and to learn if seedling variability could be detected in two bottlenecked species using molecular genetic techniques.

Hawaiian Campanulaceae

Six genera in the Campanulaceae are represented in Hawai'i. Five are endemic (*Brighamia, Clermontia, Cyanea, Delissea*, and *Trematolobelia*). The sixth (*Lobelia*) is indigenous (Rock 1919, Wagner *et al.*1990). There are two species of *Brighamia* (Lammers 1989); 22 species of *Clermontia* in two sections (*Clermontioideae* and *Clermontia*) (Wagner *et al.*1990) with six series (*Clermontioideae, Sarcanthae, Unilabiateae, Clermontia, Kakeanae*, and *Parviflorae*) (Lammers 1991); 64 species of *Cyanea* (Lammers *et al.*1993) in five sections (*Palmaeformes, Delisseoideae, Hirtellae, Genuinae*, and *Pilosae*) (Givnish *et al.* 1995); nine species of *Delissea* arranged in two sections (*Delissea* and *Macranthae*) (Wagner *et al.* 1990); 13 species of *Lobelia* in two sections of the subgenus *Tupa* (*Galeatella* and *Revolutella*) (Wagner *et al.* 1990); and four species of *Trematolobelia* (Wagner *et al.* 1990). Species in this family occur on all the main islands of the Hawaiian chain and grow in diverse environments, from dry ridges to high elevation wet forests.

In vitro Propagation

The Hungarian scientist (previously thought to be German [Nagy 1995]), Haberlandt Gottlieb, predicted in 1902 that plants could be generated from vegetative cells *in vitro* (Haberlandt 1902). His experiments failed, but later media with yeast extracts were used

allowing tomato roots to be consistently grown *in vitro*. Eventually callus (undifferentiated plant cells) was able to be maintained in culture (White 1963). Cytokinins were later found to control shoot and root regeneration (organogenesis) from tobacco callus (Skoog and Miller 1957). A method to propagate many plants from shoot tips through *in vitro* culture (micropropagation) was discovered while attempting to produce virus-free plants of cymbidium orchids (Morel 1960) and has since been used for many plant species (*e.g.*, Hartman and Kester 1983, Kyte 1987). A basic nutrient medium, now used by many researchers worldwide for *in vitro* propagation was developed by Murashige and Skoog (1962) and is commonly called MS medium (Kyte 1987). Techniques such as micro-grafting, haploid plant production from pollen, endosperm culture, protoplast culture and fusion, and embryogenesis techniques have all been established and are now used for breeding and plant production research (Bayliss 1980, Pierik 1987, Power *et al.* 1970, Schieder and Vasil 1980, Vasil 1980, Vasil and Vasil 1980). Other techniques such as the use of bioreactors for production of plant alkaloids, and *in vitro* biotransformation have found commercial uses and are actively being researched (Horsch *et al.* 1985, Pierik 1987, Scragg and Fowler 1985, Zenk *et al.* 1988).

In vitro methods are now used for conservation of endangered plant taxa as well as horticultural propagation. Reviews that cover the topic of the conservation use of *in vitro* techniques are: Engelmann 1991, Fay 1992, Withers 1991a, Withers 1991b, Withers *et al.*

1990). Among the concerns with *in vitro* propagation of rare taxa is the potential for somaclonal variation. Such variation could induce changes not represented in the native germplasm and could potentially harm native populations if left (Kidwell and Osborn 1993, Larkin and Scowcroft 1981). A new journal (*Botanic Garden Micropropagation News*, Ed: Michael F. Fay & Peter J Atkinson, ISSN 0962-7448, Royal Botanic Garden, Kew, Richmond Surrey, TW9 3AB, UK) often has at least one, if not several articles reporting the use of *in vitro* techniques on endangered plant species.

Hawaiian Campanulaceae can be easily grown from seed (Duvall 1991) and a few from cuttings (Carol Nakamura and Rick Palmer, personal communication). However, many species produce low numbers of viable seeds and some fail to produce seeds at all (Loyal Mehrhoff and John Obata, personal communication). In a greenhouse situation survival is also complicated by problems such as pests and pathogens. For rare plants the loss of a seedling is permanent loss of genetic information. If seedlings can be germinated *in vitro* and successfully cloned, chances of survival of this genetic information could be greatly increased. When so few plants remain *in situ* (the case for most Hawaiian Campanulaceae) every seedling produced is a valuable component of a conservation-oriented collection. Seeds of tropical species are notoriously recalcitrant in storage so seed storage facilities may not be a viable method of conserving populations of these endangered species (Towill and Roos 1989, Wilkins and Dodds 1983). An *in vitro* collection of a cloned seedling population is an attractive alternative.

In vitro seedling populations are of value as a source of material for experiments to learn proper or successful cultivation techniques, and for outplanting experiments. Use of clonal material ("copies" of the seedlings) can allow for experimental plant material losses without the fear of complete loss of important genetic information in the seedling populations. These clones can also be used for molecular genetic assays since only small amounts of tissue are needed to obtain DNA (Deragon and Landyr 1992, Kidwell and Osborn 1993). Greenhouse propagation of these plantlets is less hazardous and likely to be more successful than direct seeding because more individuals are available

This is the first report on the *in vitro* propagation of Hawaiian Campanulaceae. Only one other paper on micropropagation on *Campanula isophylla* Morettii, discussed techniques using on a non-Hawaiian member of the family in commercial cultivation (Brandt 1992).

RAPDs

A technique for *in vitro* DNA amplification, known as the polymerase chain reaction (PCR), was described in the mid- to late-1980s . Several good literature reviews have since been published (Arnheim *et al.* 1990, Erlich *et al.* 1991, Vosberg 1989). The technique was modified by using a thermostable DNA polymerase isolated from *Thermus aquaticus* (*Taq*), simplifying the procedure and, by allowing the reactions to be run at higher temperatures, improved the specificity, yield, sensitivity, and length of the

products that can be amplified (Saiki *et al.* 1988). This led to the procedure being used more often by researchers and opened the way for new procedures to be developed using the PCR technique.

One of those new procedures uses random primers for the amplification of template DNA. It is known as RAPDs for randomly amplified polymorphic DNA (Williams *et al.* 1990) or AP-PCR for arbitrarily primed PCR (Caetano-Anollés *et al.* 1991b, Welsh and McClelland 1990). The technique uses a synthetic, usually ten-based oligonucleotides (10-mer) to amplify segments of template DNA. The location of these sequences is random, or arbitrary, hence the name. The primer needs to be 40% to greater than 50% G+C in makeup (Akopyanz *et al.* 1992, Fukuoka *et al.* 1992, Williams *et al.* 1990). Primers are usually 10-mer but can be up to 57 nucleotides long (Crowhurst *et al.* 1991). Primers can detect as few as zero polymorphisms (Halward *et al.* 1991) to many depending on the primer:genomic DNA combination. It is usually considered best to pick primers that show fewer polymorphisms for clarity and good comparisons (Crowhurst *et al.* 1991). Two primers used together can also influence polymorphisms, resulting in a new set of polymorphisms being amplified (Klein-Lankhorst *et al.* 1991).

RAPDs work because different size fragments of DNA are amplified, depending on sequence matching of primer with template DNA, that can be separated using gel electrophoresis. Williams *et al.* (1990) postulate that the polymorphisms can be the result

of single mismatches in the primer:genomic DNA duplex at both sites defining a DNA segment, single base changes in the genome, deletions of a priming site, and insertions that render priming sites too distant to support amplification (Black *et al.* 1992). Other possible explanations of polymorphisms include polymerase slippage during replication; nontemplate-directed addition of nucleotides by *Taq* polymerase; or the amplification of *in vitro* recombinants; changes in PCR parameters such as primer/template ratios, annealing temperatures, and Mg^{2++} concentration; and DNA concentration (Lamboy 1994a, Lamboy 1994b).

Due to the random location of the primer/template, matches in types of DNA sequences amplified varies and depends on the species. For example, for *Theobroma cacao* only low copy sequences were amplified using RAPD primers (Wilde *et al.* 1992) while other researchers have found single-copy and low-copy repetitive sequences only (Crowhurst *et al.* 1991) and others found middle- to highly-repetitive sequences (Gilbert *et al.* 1990, Williams *et al.* 1990).

The sequences amplified in RAPDs are inherited in a Mendelian fashion and are dominant (Echt *et al.* 1992, He *et al.* 1992, Hu and Quiros 1991, Michelmore *et al.* 1991, Williams *et al.* 1990, Yoshimura *et al.* 1992) making them useful as genetic markers. In wheat, however, the polyploidy of the hexaploid genome has caused some problems in using RAPDs as genetic markers (Devos and Gale 1992) because the cause of these

polymorphisms is unknown. Questions of the repeatability of the results from RAPDs (Devos and Gale 1992, Hedrick 1992, Scott *et al.* 1992) have been largely resolved and this is no longer considered a problem. The fact that dominant markers result should be considered (Black *et al.* 1992, Hedrick 1992). The dominance of the markers means that heterozygotes are not detected (Chalmers *et al.* 1992b) but heterozygous individuals can be identified with RAPDs when a single primer generates at least one complementary polymorphic amplification product from each parent (Baird *et al.* 1992) and for many applications, such as the one used here, detection of heterozygotes is not a central problem.

RAPDs have been reported to show genetic variability in cultivated and *in situ* wild populations of both plants and animals; RAPD markers have been used in determining genetic variability of agricultural crops (N'Goran *et al.* 1994, Vierling and Nguyen 1992, Virk *et al.* 1995) and horticultural crops (Iqbal *et al.* 1995, Marsolais *et al.* 1993). Genetic variation of wild populations has been determined in wild populations of plants (Brauner *et al.* 1992, Chalmers *et al.* 1992a, Chalmers *et al.* 1992b, Dawson *et al.* 1995, Harada *et al.* 1994, Van Coppenolle *et al.* 1993), animals (Haig *et al.* 1994, Kim 1993,), and fungi (Hamelin *et al.* 1994). RAPDS also has been used to detect variation within populations of very closely related individuals and of endangered species (Baird *et al.* 1992, Gibbs *et al.* 1994, Haig *et al.* 1994, Luque *et al.* 1995, Martin *et al.* 1991, Van Buren *et al.* 1994, Waycott and Fort 1994, Yang and Quiros 1993).

The Hawaiian Campanulaceae includes examples of rare species and extremely reduced populations. It is important to know if the seedling populations produced from extremely bottlenecked species have variability. This knowledge could be used to determine conservation strategies where only one or a few populations can be saved. Because RAPDs may detect hidden variation, and can be used on plants of any population size (Andersen and Fairbanks 1990, Caetano-Anollés *et al.* 1991a, Kim 1993), an experiment was conducted using RAPDs to assess the genomic variability of seedling populations of two extremely bottlenecked Campanulaceae species. Knowing which populations contain the greatest genetic variation is also important. As mentioned above, somaclonal variation may result when some *in vitro* techniques are used in conservation programs of rare species. With a base line established using RAPDs clonal populations could be analyzed for presence of possible mutations. RAPDs offer a means to look for these, at least among dominant markers, and is much more sensitive than allozymes (Dawson *et al.* 1995, Puterka *et al.* 1993).

Methods and results for the *in vitro* propagation and RAPDs phases will be presented separately, for clarity. Chapter 2 will discuss the *in vitro* propagation of Hawaiian Campanulaceae and Chapter 3 will discuss the use of RAPDs in determining the genetic variability of seedling populations of the two species of Hawaiian Campanulaceae. Large data tables are in the appendices.

Chapter 2. *In vitro* propagation of Hawaiian Campanulaceae

Introduction

Hawai'i has been called the endangered species capital of the world. With 1,233 species of ferns and flowering plants native to the islands (Eldredge and Miller 1995) and 585 species either listed as endangered, proposed for listing, or candidates for listing under the Endangered Species Act (ESA), nearly 48% are in trouble in their native environment. The situation is equally dire for species in the Campanulaceae. Since their discovery by Western science nearly 25% of the species of Hawaiian Campanulaceae have gone extinct (Wagner *et al.* 1990). Of the 114 species remaining, 97 species are either listed as endangered, proposed for listing, or candidates for listing under the ESA. That is 85.1% of the remaining species.

The perilous conditions of Hawaiian Campanulaceae indicates a need to save the remaining Hawaiian plants. This need is immediate, since many of the remaining species have reduced populations, or are not reproducing in the wild; some have not flowered or produced seed in recent history. This study was conducted to see if *in vitro* techniques could be applied to species in the Hawaiian Campanulaceae. *In vitro* seed germination, organogenesis, and micropropagation (bud proliferation) techniques were studied to

ascertain if they could be used in a program for the *ex situ* conservation of these extremely rare species.

Materials and Methods

Plant Material

Plant material was collected over a four year period from August 2, 1991 to September 21, 1995 by various collectors and delivered to the micropropagation laboratory at Lyon Arboretum. Material was collected from existing populations of species on the islands of Kaua'i, O'ahu, Moloka'i, Lana'i, Maui, and Hawai'i. Non-wild-collected material was either obtained from plants growing in botanical gardens around the state or from the greenhouses at Lyon Arboretum. Material received included green fruit, ripe fruit, cleaned seeds, stem segments (cuttings), and leaves (see Table A.2, page 85). These plant parts gave rise to explants such as floral parts, embryos, immature seeds, mature seeds, buds, and leaf segments. Table A.1 on page 69 shows *in vitro* treatments for these explants. If seedlings or plantlets were produced then media tests were conducted to determine the appropriate medium for each species.

Forty-three of the species attempted (Table 2.1) are covered by the Endangered Species Act (ESA). Eighteen are listed as endangered, 12 are being proposed for immediate listing and 13 more are candidates for listing. The rarity and remoteness of many of these

species and populations limited the replication of most experiments. State of Hawai'i rules and regulations require permits for collection and possession of all species covered by the ESA. The Lyon Arboretum's permit only covers possession of the plant material so collections were performed by those possessing the appropriate permits, further limiting control over what was collected and replications of experiments. Fifteen taxa studied are not covered by the ESA (Table 2.2) and were collected by a number of local botanists.

In vitro treatments attempted on each species is shown in Table A.1. In most cases micropropagation (bud proliferation) treatments are not listed in Table A.1. Only if the species did not readily produce side shoots for propagation were experiments conducted to determine the best protocol for shoot production. Otherwise the naturally occurring *in vitro* side shoots were used as explants for multiplication of the seedlings. When seedlings or wild-collected plants were successfully cloned the resulting clonal populations were designated as seed lines.

Media

A variety of media was used for *in vitro* propagation. Murashige and Skoog medium (MS) (1962) was used at 1.0X, 0.5X, 0.25X or 0.125X strength macronutrients (while the micronutrients, iron, EDTA and vitamins were at full strength). Edamin, glycine, and the recommended plant growth regulators were not used and sucrose was added at 20 g/L. If plant growth regulators were used then 2,4-dichlorophenoxyacetic acid (2,4-D) (Sigma

Chemical), ∝-napthaleneacetic acid (NAA) (Sigma Chemical), and/or 6-benzylaminopurine (BA) (Sigma Chemical) were used at various concentrations as listed in Table A.1. Activated charcoal (AC) (Sigma Chemical) was added in some cases (but not in conjunction with plant growth regulators) at a rate of 2.0 g/L. The pH was adjusted to between 5.7 and 5.8. Linsmaier and Skoog (LS) medium (1965) was used at 1.0X and 0.5X macronutrient strengths, pH 5.55-5.65 and Woody Plant Medium (WPM) (Lloyd and McCown 1980) was used at full strength with or without activated charcoal at 2.0 g/L, pH 5.15-5.25. Vacin and Went medium (VW) (1949) was used full strength with or without 20 g/L sucrose and with 150 ml/L coconut water (CW), pH of 4.8-5.0. Semi-solid media were either solidified with purified agar (Sigma Chemical) or Phytagel™ (Sigma Chemical) at 8.0 g/L and 2.0 g/L respectively. In all cases the pH was adjusted using hydrochloric acid (HCl) or sodium hydroxide (NaOH) after all ingredients except the solidifying agent were added and the medium brought to volume. Solidifying agent was added and the medium microwaved to dissolve the solidifying agent before dispensing into the culture vessels before autoclaving. All media were autoclaved in a portable autoclave (Hirayama Model HA-300M) at 120°C and 1.2 kg/cm^2 for 20 minutes.

Growth Conditions

Culture vessels were either test tubes (Kimax, 25 x 150 mm) with Magenta™ closures (Sigma Chemical) (10 ml medium per tube), Erlenmeyer flasks (125 or 250 ml with 50 ml or 100 ml medium respectively) with aluminum foil closures, Phytacon™ culture tubs

(Sigma Chemical) (100 ml medium), or disposable petri dishes (100 x 15 mm, Fisher Scientific). Petri dishes were only used for tissue culture/organogenesis experiments and were sealed with Parafilm™. Culture conditions were the same for all cultures with commercial florescent lights running 24 hours a day (14.2 μmol/m^2/s) and a mean temperature of 22°C.

Disinfestation

Commercial bleach (White Magic™ Safeway, Inc. [5.27% by weight sodium hypochlorite]) was used for disinfestation at 10% (by volume) followed by 5% (by volume). No sterile water rinses were performed since this increased the amount of contamination. In general, the following procedures were used:

Disinfestation of green and ripe intact fruit: Fruit were washed in running tap water for up to two hours, trimmed of stems and any remaining floral parts and placed in a beaker with 10% bleach solution and one drop Tween 20 per 100 ml solution and spun using a magnetic stir bar and a stir plate (Corning PC-320) for 10-15 minutes. In the case of those fruits with seeds embedded in pulp (*Clermontia*, *Cyanea* and some *Delissea*) the outer rind of the fruit was then removed and the pulp containing the seeds was placed in fresh 10% (by volume) bleach solution and spun until the pulp dissolved away and the seeds were freed. After the bleach solution was decanted and the seeds repeatedly rinsed with

Table 2.1. Hawaiian Campanulaceae evaluated for *in vitro* propagation and covered by the Endangered Species Act (ESA). Authority names are from Wagner *et al.* (1990) unless otherwise indicated.

Species	ESA Status
Brighamia insignis A. Gray	Listed endangered
Clermontia drepanomorpha Rock	Proposed for listing
Clermontia lindseyana Rock	Listed endangered
Clermontia peleana Rock	Listed endangered
Clermontia pyrularia Hillebr.	Listed endangered
Clermontia tuberculata C. Forbes	Candidate level 2
Cyanea acuminata (Gaud.) Hillebr.	Proposed for listing
Cyanea asarifolia St. John	Listed endangered
Cyanea asplenifolia (H. Mann) Hillebr.	Candidate level 2
Cyanea copelandii ssp. *haleakalaensis* (St. John) Lammers	Candidate level 1
Cyanea crispa (Gaudichaud) Lammers, Givnish & Sytsma*	Listed endangered
Cyanea dunbarii Rock	Proposed for listing
Cyanea glabra (F. Wimmer) St. John	Candidate level 1
Cyanea grimesiana ssp. *grimesiana* Gaud.	Proposed for listing
Cyanea grimesiana ssp. *obatae* (St. John) Lammers	Listed endangered
Cyanea hamatiflora ssp. *carlsonii* (Rock) Lammers	Listed endangered
Cyanea hamatiflora ssp. *hamatiflora* Rock	Candidate level 1
Cyanea kolekoleensis (H. St. John) Lammers†	Candidate level 1
Cyanea kunthiana Hillebr.	Candidate level 1
Cyanea lanceolata (Gaudichaud) Lammers, Givnish & Sytsma*	Candidate level 1
Cyanea leptostegia A. Gray	Candidate level 3C
Cyanea linearifolia Rock	Candidate level 2
Cyanea longiflora (Wawra) Lammers, Givnish & Sytsma*	Proposed for listing
Cyanea manii (Brigham) Hillebr.	Listed endangered
Cyanea mceldowneyi Rock	Listed endangered
Cyanea membranacea Rock	Candidate level 2
Cyanea pinnatifida (Cham.) F. Wimmer	Listed endangered
Cyanea platyphylla (A. Gray) Hillebr.	Proposed for listing
Cyanea remyi Rock‡	Proposed for listing
Cyanea shipmanii Rock	Listed endangered
Cyanea st.johnii (Hosaka) Lammers, Givnish & Sytsma*	Proposed for listing
Cyanea superba ssp. *superba* (Cham.) A. Gray	Listed endangered

Table 2.1. (Continued) Hawaiian Campanulaceae evaluated for *in vitro* propagation and covered by the Endangered Species Act (ESA). Authority names are from Wagner *et al.* (1990) unless otherwise indicated.

Species	ESA Status
Cyanea truncata (Rock) Rock	Listed endangered
Delissea rhytidosperma H. Mann	Listed endangered
Delissea rivularis (Rock) F. Wimmer	Proposed for listing
Delissea subcordata Gaud.	Proposed for listing
Delissea undulata ssp. *undulata* Gaud.	Listed endangered
Lobelia gaudichaudii ssp. *koolauensis* (Hosaka & Fosb.) Lammers*	Proposed for listing
Lobelia hypoleuca Hillebr.	Candidate level 2
Lobelia monostachya (Rock) Lammers	Proposed for listing
Lobelia niihauensis St. John	Listed endangered
Lobelia oahuensis Rock	Listed endangered
Lobelia yuccoides Hillebr.	Candidate level 2

* (Lammers *et al.* 1993)
† (Lammers 1992)
‡ (Lammers and Lorence 1993)

Table 2.2. Hawaiian Campanulaceae evaluated for *in vitro* propagation but not covered by the Endangered Species Act (ESA). Authority names are from Wagner *et al.* (1990) unless otherwise indicated.

Species	Status
Clermontia fauriei H. Lév.	Stable
Clermontia grandiflora Gaud.	Stable
Clermontia kakeana Meyen	Stable
Clermontia kakeana Meyen X *C. arborescens* (H. Mann) Hillebr.	Possible hybrid*
Clermontia montis-loa Rock	Stable
Clermontia tuberculata C. Forbes	Stable
Cyanea aculeatiflora Rock	Stable
Cyanea angustifolia (Cham.) Hillebr.	Stable
Cyanea degeneriana F. Wimmer	Stable
Cyanea kuhihewa Lammers, *in ed.*†	New species, Rare
Cyanea oahuensis (sic)	Unknown, incorrectly named
Cyanea recta (Wawra) Hillebr.	Rediscovered, rare
Lobelia hillebrandii Rock	Stable
Trematolobelia kauaiensis (Rock) Skottsb.	Stable
Trematolobelia macrostachys (Hook. & Arnott) A. Zahlbr.	Stable

* (Rick Palmer, personal communication)
† (Dave Lorence, personal communication)

5% (by volume) bleach solution, the seeds were spun for another 10-15 minutes in 5% (by volume) bleach solution. Most of the bleach solution was removed, leaving some solution to cover the seeds for transport to the transfer case. A Pasteur pipet was sterilized and used in the sterile transfer case, to remove the remaining bleach solution with the beaker at an angle allowing the seeds to congregate in a clump to one side. A forceps was used to transfer the seeds to sterile tubes (25-50 seeds per tube) containing medium solidified with agar or Phytagel™.

If the seeds were not embedded in pulp (*Brighamia*, *Trematolobelia*, and some *Delissea*) the rind of the fruit was cut open and the seeds placed directly in a 5% (by volume bleach) solution and spun for 15 minutes. The seeds were then processed as above (from the Pasteur pipet stage).

Disinfestation of cleaned seeds: Seeds already removed from the fruit and cleaned by the collector were first soaked in tap water for up to two hours, placed in a 10% (by volume) bleach solution with one drop of Tween 20 per 100 ml and spun for 10-15 minutes. They were then treated as above (from the Pasteur pipet stage).

Disinfestation of leaf material: Leaf material was placed in running tap water (after making a fresh cut on the petiole) for two to 24 hours (the shorter time for freshly collected young material and the longer for either older leaf material or that collected

from one to several days before being delivered to the laboratory). The leaves were cut into segments approximately 2 cm^2, each containing either a segment of the main veins or part of the petiole, placed in 10% (by volume) bleach with Tween 20 at one drop per 100 ml and spun for 10-15 minutes, trimmed at all cut edges (which were bleached white by the bleach solution) and then placed in 5% (by volume) bleach solution and spun for 10-15 minutes. The segments were trimmed once again (resulting in size from 0.5 cm^2 to 1.0 cm^2) in the transfer case and placed on the medium. Attempts were made to propagate from all parts of the leaves, if available (petiole alone, petiole plus leaf blade and/or leaf blade segments). Disposable Petri dishes were used with medium solidified either by agar or Phytagel™ and sealed with Parafilm™ and placed in the growth room.

Disinfestation of stems (cuttings): Cuttings were placed in running tap water for two to 48 hours (the former for freshly collected, fairly clean material and the latter for material collected one day or more before being received by the laboratory, or for highly contaminated material). Leaves were removed and the stems cut into segments with at least one node or up to three nodes if the apical portion was used. These segments were placed in 10% (by volume) bleach solution with one drop per 100 ml of Tween 20 and spun for 10-20 minutes. The stem segments were trimmed at the cut surfaces and the first bud scale was removed, placed in 5% (by volume) bleach solution and spun for 10-15 minutes. With the aid of a dissecting scope, three more bud scales were removed (from well developed axillary buds and from apical buds; less for lesser developed axillary

buds). Apical buds were trimmed to 1-2 mm long and axillary buds were excised from the stem by cutting at an angle both above and below the bud and trimming away the stem tissue on either side of the bud, resulting in explants from 1-2 mm in length. These were placed in 1% (by volume) bleach solution for up to 15 minutes and placed directly on the medium in sterile culture vessels (either Petri dishes sealed with Parafilm™ or test tubes closed with Magenta™ closures).

If seeds germinated *in vitro* then seedlings were handled in several ways. If plants were extremely rare or had low germination, all seedlings were retained in the laboratory for cloning experiments. If many seeds germinated, 50% or more were sent directly to the greenhouse for further growth. The remainder were retained in the laboratory for cloning experiments. If leaves or buds produced plantlets, numbers were increased (using bud proliferation) until trials could be conducted in the greenhouse. Due to lack of greenhouse staff and bench space, not all species successfully propagated *in vitro* have yet been grown in the greenhouse.

Cloning experiments of *in vitro* seedlings or other plantlets were conducted in the same manner as *ex vitro* collected plant material, except the disinfestation stage was omitted.

Records

Records were kept for each collection using an IBM® computer and employing the Windows®-based relational database program Microsoft Access® version 2.0. Media were computer coded for date, amount, pH, and amount and type of solidifying agent (if used). All species attempted were given a species number (starting with the letter "S") in a table that had species-specific information (Latin name, authority, family, growth habit, nativity, Hawaiian status [endemic, indigenous, exotic], ecology [short description of natural habitat], and rating [Federal listing status, rarity comments]). A collection number (starting with the letter "P") was given to each accession. This number was attached to the collection information (species number, source's i.d. number, Lyon Arboretum's accession number, source code, date received, island, how received [green fruit, ripe fruit, seeds, cuttings, etc.], wild-collected or not, date collected, and anecdotal information [collector's information on collection location, plant appearance, elevation, etc.]).

For each treatment another four digits were added to the plant number creating a treatment number. This information was kept in a separate table and included the following fields: explant, explant notes (e.g. apical bud vs. axillary bud), disinfestation procedure, media code, date of treatment, comments, and purpose. The treatment number was also used as an identifier for separate seed lines (either *in vitro* seedlings or clones of wild-collected individuals). If cloning experiments other than bud proliferation were conducted then an additional letter was added after the treatment number alphabetically.

For example, if seed line/treatment P00580005 was the source of leaves used in an organogenesis experiment the first treatment was given the number P00580005A and the second given the number P00580005B and so on (see Table A.1).

Observation records were kept in a separate table. Dated entries were kept for general observations (germination of seeds, formation of organs, growth of callus, contamination, etc.) and also for each transflask operation, with a note of operator and medium used. Inventory was also kept and updated with each transflask operation, noting number and types of containers, explants per container and general condition of the explants.

Calculation of significant differences was done using the Student's t-test for species where enough germination replicates were available.

Results

Seed Germination

Table A.3, page 110 shows results of germination trials for the 52 species attempted. Total germination percentage ranged from 0.5% to 100% and within a taxon the widest germination percentage ranged from 0 to 100%. Seeds began to germinate in as little as 13 days and as long as 365 days with germination periods ranging from four days to as long as 508. Over 80% of the species attempted had a 1% or greater germination rate,

over 68% of them had a 25% or greater germination rate. Table 2.4 (page 25) shows average germination percentages for all species successfully germinated *in vitro* with their maintenance medium (Final Growth Medium).

It was possible only in a few cases to compare germination rates between immature and mature seeds within species (Table 2.3). Seeds were designated as immature if they came from unripe (green) fruit or had not fully developed their seed coat (white or soft seed coat). There was no significant difference (with a 95% confidence level) between germination percentages for immature or mature seeds for *Cyanea grimesiana* ssp. *obatae*, *Cyanea superba* ssp. *superba*, or *Delissea undulata* ssp. *undulata*. When germination percentages for all taxa germinated *in vitro* were analyzed there was also no significant difference. Immature germinated equally well as did mature seed. Germination rates were also not affected by maturity of seeds.

Cyanea pinnatifida seeds were available as a direct result of the conservation program to propagate this species. Seeds have not been produced in the wild for over 30 years. A plant growing in the greenhouse at Lyon Arboretum flowered for the first time in 1995 and produced immature fruit. While most fruit aborted, immature seed were collected from a couple of the fruits before they fell off. These seeds germinated in one case and the resulting seedlings were the first seed-produced progeny of this plant known in cultivation.

Table 2.3. Germination of Hawaiian Campanulaceae, immature (I) versus mature (M) seeds.

Species/Treatment I.D.	Maturity	%Germination	Germination Rate (Days)
Cyanea grimesiana ssp. *obatae*			
P00610001	M	86	170
P02950001	I	92	82
P02960001	M	90	103
P03390001	I	93	121
P05900001	I	97	61
Cyanea superba ssp. *superba*			
P02260001	I	0	n/a
P02270001	M	63	7
P02580001	M	0	n/a
P03450001	I	84	32
P03460001	I	63	15
P03470001	I	84	42
P05060001	M	8	18
P05070001	M	6	14
P05580001	M	56	16
P05590001	I	contaminated	n/a
Delissea undulata ssp. *undulata*			
P01630001	I	98	85
P01720001	I	92	147
P01770001	I	94	126
P01830001	I	91	85
P01830002	I	0	n/a
P02020001	M	81	49
P02140001	M	92	26

Table 2.4. Species successfully germinated *in vitro*.

Species	Immature (I) or Mature (M) Seeds	# of Successful Trials	Average Germination Percentage	Final Growth Medium
Brighamia insignis	I	2	92.5	0.5X MS
Clermontia drepanomorpha	I	2	100	0.5X MS with Activated Charcoal
Clermontia fauriei	M	1	96	0.5X MS
Clermontia kakeana	M	1	100	0.5X MS with Activated Charcoal
Clermontia kakeana x *C. arborescens*	I	2	50	0.5X MS with Activated Charcoal
Clermontia lindseyana	M	1	65	0.5X MS
Clermontia montis-loa	M	1	7	0.5X MS
Clermontia peleana	I	1	98	0.5X MS with Activated Charcoal
	M	1	96	0.5X MS with Activated Charcoal
Clermontia pyrularia	M	1	33	0.5X MS with Activated Charcoal
	I	1	15	0.5X MS with Activated Charcoal
Clermontia tuberculata	I	2	14.5	0.5X MS with Activated Charcoal

Table 2.4. (Continued) Species successfully germinated *in vitro*.

Species	Immature (I) or Mature (M) Seeds	# of Successful Trials	Average Germination Percentage	Final Growth Medium
Cyanea aculeatiflora	I	1	78	0.5X MS with Activated Charcoal
	M	1	63	0.5X MS with Activated Charcoal
Cyanea acuminata	M	3	13	0.5X MS with Activated Charcoal
Cyanea angustifolia	M	1	85	0.5X MS with Activated Charcoal
Cyanea asarifolia	I	1	95	0.5X MS
Cyanea copelandii ssp. *haleakalaensis*	I	1	89	0.5X MS
Cyanea crispa	M	2	89	0.5X MS
Cyanea dunbarii	M	1	12	0.5X MS
Cyanea glabra	I	1	97	0.5X MS with Activated Charcoal
Cyanea grimesiana ssp. *grimesiana*	M	2	51	0.5X MS
Cyanea grimesiana ssp. *obatae*	I	3	94	0.5X MS
	M	2	88	0.5X MS
Cyanea hamatiflora ssp. *hamatiflora*	M	1	80	0.5X MS with Activated Charcoal
Cyanea lanceolata	I	2	33.5	0.5X MS
Cyanea leptostegia	M	1	87	0.5X MS with Activated Charcoal
Cyanea longiflora	M	1	48	0.5X MS

Table 2.4. (Continued) Species successfully germinated *in vitro*.

Species	Immature (I) or Mature (M) Seeds	# of Successful Trials	Average Germination Percentage	Final Growth Medium
Cyanea mceldowneyi	I	2	33.75	0.5X MS with Activated Charcoal
Cyanea membranacea	M	1	29	0.5X MS
Cyanea oahuensis	I	1	29	0.5X MS with Activated Charcoal
Cyanea pinnatifida	I	1	40	0.5X MS with Activated Charcoal
Cyanea platyphylla	I	2	70.5	0.5X MS with Activated Charcoal
Cyanea recta	I	1	79	0.5X MS with Activated Charcoal
Cyanea remyi	I	3	7.33	0.5X MS
Cyanea shipmanii	M	1	92	0.5X MS with Activated Charcoal
Cyanea superba ssp. *superba*	I	3	77	0.5X MS
	M	4	33.25	0.5X MS
Delissea rhytidosperma	I	1	100	0.5X MS with Activated Charcoal
	M	1	84	0.5X MS with Activated Charcoal
Delissea rivularis	M	4	61	0.5X MS with Activated Charcoal
Delissea subcordata	M	1	56	0.5X MS with Activated Charcoal

Table 2.4. (Continued) Species successfully germinated *in vitro*.

Species	Immature (I) or Mature (M) Seeds	# of Successful Trials	Average Germination Percentage	Final Growth Medium
Delissea undulata ssp. *undulata*	I	4	93.75	0.5X MS with Activated Charcoal
	M	2	86.5	0.5X MS with Activated Charcoal
Lobelia hypoleuca	I	1	78	0.5X MS with Activated Charcoal
Lobelia gaudichaudii ssp. *koolauensis*	M	1	52	0.5X MS
Lobelia monostachya	I	1	55	0.5X MS with Activated Charcoal
Lobelia niihauensis	M	1	31	0.5X MS with Activated Charcoal
Lobelia oahuensis	I	1	64	0.5X MS with Activated Charcoal
	M	1	91	0.5X MS with Activated Charcoal
Trematolobelia kauaiensis	M	1	27	0.5X MS
Trematolobelia macrostachys	I	1	12	0.5X MS with Activated Charcoal
	M	1	28	0.5X MS

Seedling growth varied with different media. Some species did better on 0.5X MS and others on 0.5X MS with 2.0 g/L of activated charcoal added. Other media attempted did not improve growth of the seedling, and in some cases (VW, LS) hindered growth (vitrification, chlorosis, or necrosis). If VW with coconut water was used then growth was extremely distorted. Vitrification was a problem for those species that prefer charcoal in the medium when grown on charcoal-free medium though species that grew well on 0.5X MS grew equally well on 0.5X MS with activated charcoal.

Organogenesis

Table A.4 (page 120) shows that explants from leaves produced shoots organogenically in nearly 43% of the 29 species tried. Table 2.5 (page 32) lists all species successfully propagated using organogenesis techniques, with the medium that produced the best results. Media comparisons are shown in Table 2.6 and 2.7. Shoots were never produced without cytokinin or auxin (no PGRs). Auxin, either 2,4-D or NAA, was effectively unproductive without the addition of BA. Cytokinin (BA) alone was effective in producing shoots at 0.5 mg/L. The combination of 0.5 mg/L BA with either 0.05 mg 2,4-D or 0.5 mg/L NAA was the most effective in producing shoots among the species studied. The former produced the highest number of shoots in two out of the nine species that produced shoots from leaf explants. All other treatments were most effective for one species only.

Clermontia fauriei leaf explants produced an average of 47 plantlets from leaf blade explants in 0.5X MS with 0.5 mg/L BA and 0.05 mg/L of 2,4-D. *Clermontia kakeana* produced on average 67 shoots per explant in 0.5X MS with 1.0 mg/L BA and 0.1 mg/L 2,4-D. When 2,4-D was increased to 0.5 mg/L the number of shoots produced was considerably less. When BA was reduced to 0.5 mg/L and 2,4-D was added at 0.5 mg/L or 1.0 mg/L plantlets were also produced, with number of shoots averaging 26 and 43, respectively. Some of the plantlets produced organogenically were accidentally used in an outplanting experiment at Lyon Arboretum. Some of these survived the transplanting process but their leaves are different from any *Clermontia kakeana* seen before. The leaves are shortened by about ½ and slightly crinkled at their tips (Alvin Yoshinaga, personal communication).

Clermontia peleana produced an average of 125 shoots per explant in a treatment with 0.5 mg/L BA and 0.05 mg/L 2,4-D and when the 2,4-D was increased to 0.1 mg/L the number of shoots decreased (to 12). When NAA was used at 1.0 mg/L the number of shoots was similar (100). When BA was increased to 1.0 mg/L and 2,4-D was kept at 0.05 mg/L 90 shoots were produced. With this higher amount of BA the 2,4-D could be increased to 0.1 mg/L with no deleterious effects but at 0.5 mg/L or higher shoot production dropped off completely. *Cyanea asarifolia* produced an average of two shoots per explant in one treatment (0.5 mg/L BA and 0.05 mg/L 2,4-D). Using BA with NAA 15 shoots per explant were the maximum number of shoots produced (0.5 mg/L BA with

0.05 mg/L NAA). When either BA or NAA were increased shoot production dropped off but the combination of 1.0 mg/L BA with 0.5 mg/L NAA had six shoots on average.

Cyanea grimesiana ssp. *obatae* produced one shoot when BA was used at 0.5 mg/L and 2,4-D at either 0.05 or 0.1 mg/L. An average of 3 shoots per explant was produced when BA was used at 1.0 mg/L in conjunction with NAA at 1.0 mg/L. *Cyanea pinnatifida* produced shoots from wild-collected leaf material when treated with 0.5 mg/L BA and 0.5 mg/L 2,4-D. Four shoots were produced on average. *Delissea rhytidosperma* leaf explants produced most consistently when treated with BA in conjunction with NAA. When BA was used at 0.5 mg/L and NAA was used at 0.5 or 1.0 mg/L five and six shoots were produced, respectively. When BA was increased shoot production fell off (two shoots for 1.0 mg/L BA and 1.0 mg/L NAA).

Delissea subcordata produced its largest number of shoots (19) in treatments with 1.0 mg/L BA and 0.05 mg/L 2,4-D and both roots and shoots were produced when BA was used at 0.5 mg/L with 0.05 mg/L 2,4-D. Shoot production dropped off to only one shoot when BA was used at 0.5 mg/L with 2,4-D at 0.5 mg/L or when BA was used at 1.0 mg/L and 2,4-D at 0.5 or 1.0 mg/L. BA alone at 0.5 mg/L or 2,4-D alone at 0.05 mg/L were capable of producing shoots from leaf explants (9 and 1 respectively). *Delissea undulata* ssp. *undulata* produced one shoot per explant when BA was used at 1.0 mg/L with 2,4-D at 0.1 mg/L or with BA at 0.5 mg/L and 2,4-D at 1.0 mg/L. A better treatment for

producing shoots was BA at 0.5 mg/L in conjunction with 0.5 mg/L NAA (4 shoots per explant).

Table 2.5. Species successfully propagated from leaf explants.

Species	Highest number of shoot produced (average)	Plant growth regulator combination (mg/L)
Clermontia fauriei	47	0.5 mg BA; 0.05 mg 2,4-D
Clermontia kakeana	67	1.0 mg BA; 0.1 mg 2,4-D
Clermontia peleana	125	0.5 mg BA; 0.05 mg 2,4-D
Cyanea asarifolia	15	0.5 mg BA; 0.05 mg NAA
Cyanea grimesiana ssp. *obatae*	3	1.0 mg BA; 1.0 mg NAA
Cyanea pinnatifida	4	0.5 mg BA; 0.5 mg 2,4-D
Delissea rhytidosperma	8	0.5 mg BA; 0.1 mg 2,4-D
Delissea subcordata	19	1.0 mg BA; 0.05 mg 2,4-D
Delissea undulata ssp. *undulata*	4	0.5 mg BA; 0.5 mg NAA

Of the leaf explants that were wild-collected *Cyanea pinnatifida* and *Delissea rhytidosperma* produced shoots organogenically. All callus produced, either from wild-collected or *in vitro*-produced leaves, remained as callus no matter what treatment was tried (data not shown). All shoots that were produced using organogenesis techniques performed as well as seedlings of the same species. Roots were formed and shoots elongated when the shoots were placed on medium free of plant growth regulators and the plantlets were able to be propagated using micropropagation techniques. As noted above,

in the case of *Clermontia kakeana*, stable phenotypic mutations were formed. Variations were observed (leaf shape, pubescence, variegation) in shoots produced organogenically from leaf explants of *Clermontia peleana* and *Clermontia fauriei* but these variations disappeared over time *in vitro* and never expressed themselves in greenhouse-grown plants.

Table 2.6. BA and 2,4-D combinations tested for shoot production from leaf explants. The first number is the percentage of the species which produced at least one shoot from leaf explants. The second number is the number of species studied.

	0.0 mg 2,4-D	0.05 mg 2,4-D	0.1 mg 2,4-D	0.5 mg 2,4-D	1.0 mg 2,4-D
0.0 mg BA	0% (10)	100% (1)	0% (2)	0% (4)	0% (3)
0.5 mg BA	16.6% (6)	31.3% (16)	16.7% (18)	16.7% (18)	16.7 (12)
1.0 mg BA	0% (3)	12.5% (16)	17.6% (17)	11.1% (18)	5.6% (18)

Table 2.7. BA and NAA combinations tested for shoot production from leaf explants. The first number is the percentage of the species which produced at least one shoot from leaf explants. The second number is the number of species studied.

	0.05 mg NAA	0.5 mg NAA	1.0 mg NAA
0.0 mg BA	0% (1)	0% (1)	0% (2)
0.5 mg BA	6.25% (16)	21.4% (14)	12.5% (16)
1.0 mg BA	0% (15)	11.8% (17)	14.3% (14)

Table 2.8. Micropropagation results of Hawaiian Campanulaceae showing number of shoots produced and production rate.

Species			Species		
Shoots per Explant	**Shoot Production Rate (in months)**	**Medium***	**Shoots per Explant**	**Shoot Production Rate (in months)**	**Medium**
Brighamia insignis			*Cyanea mceldowneyi*		
3	2.5	1.0 BA	2	5	AC
Clermontia drepanomorpha			*Cyanea oahuensis*		
8	3.5	AC	6	3	AC
Clermontia fauriei			*Cyanea pinnatifida*		
8	3	0.5X MS	8	2.5	0.5X MS
Clermontia kakeana			*Cyanea recta*		
6	3	AC	3	6	AC
Clermontia peleana			*Cyanea remyi*		
10	3.5	AC	3	7	0.5X MS
Clermontia pyrularia			*Cyanea shipmanii*		
3	2.5	AC	4	3.5	AC
Cyanea aculeatiflora			*Cyanea superba* ssp. *superba*		
3	6	0.5X MS	2	4	AC
Cyanea acuminata			*Delissea rhytidosperma*		
3	5	AC	3	2.5	1.0 BA
Cyanea asarifolia			*Delissea rivularis*		
6	3.5	0.5X MS	3	2.5	AC
Cyanea crispa			*Delissea subcordata*		
4	3.5	AC	3	4.5	AC

Table 2.8. (Continued) Micropropagation results of Hawaiian Campanulaceae showing number of shoots produced and production rate.

Species			Species		
Shoots per Explant	**Shoot Production rate (in months)**	**Medium***	**Shoots per Explant**	**Shoot Production Rate (in months)**	**Medium***
Cyanea glabra			*Delissea undulata* ssp. *undulata*		
8	3	AC	3	4.5	AC
Cyanea grimesiana ssp. *grimesiana*			*Lobelia hypoleuca*		
4	4	0.5X MS	4	3	AC
Cyanea grimesiana ssp. *obatae*			*Lobelia niihauensis*		
4	4	0.5X MS	3	2.5	AC
Cyanea hamatiflora ssp. *hamatiflora*			*Lobelia oahuensis*		
3	3	AC	4	3	AC
Cyanea lanceolata			*Lobelia yuccoides*		
3	4	AC	6	3	AC
Cyanea leptostegia			*Trematolobelia macrostachys*		
3	6	AC	6	2.5	AC

* Media 0.5X MS basal salts. When noted otherwise, the main different component is listed where BA is given in mg/L and AC = activated charcoal at 2.0 g/L.

Micropropagation

Seedlings germinated *in vitro* were used for micropropagation experiments. In nearly every case bud proliferation occurred naturally and no plant growth regulators were used. When plant growth regulators were used the proliferation rate was often too fast (producing a mass of small shoots) and the resulting plants either vitrified or were quite distorted (data not shown). Table 2.8 shows the number of excisable shoots per "mother" shoot and the time needed to produce removable side shoots.

Wild-collected nodal explants or apical buds were more successful than wild-collected leaf explants in producing shoots (27.8% vs 12.5% respectively). Buds, either axillary or apical, did best when started on plant growth regulator-free medium. Plant growth regulator treatments usually resulted only in distorted growth and no shoots. On plant growth regulator-free medium, explants that survived the disinfestation stage and did not become contaminated elongated into healthy plantlets over a three to eight month period. Species successfully propagated in this way include *Cyanea kuhihewa*, *Cyanea pinnatifida*, *Delissea rhytidosperma*, *Delissea subcordata*, and *Lobelia yuccoides*.

All of the species successfully germinated *in vitro* produced side shoots for clonal propagation. Only two (*Brighamia insignis* and *Delissea rhytidosperma*) required the use of plant growth regulators to induce side shoots. The other species all produced side

shoots without manipulation. Most of these species are solitary-stemmed species in the wild or in cultivation, producing side shoots only when the apical portion is damaged.

Transfer to Greenhouse

Seedlings or clones of the following plants were successfully transferred to greenhouse conditions (success is being defined as surviving for at least two months after transfer): *Brighamia insignis, Clermontia fauriei, Clermontia kakeana, Clermontia peleana, Cyanea acuminata, Cyanea asarifolia, Cyanea crispa, Cyanea mceldowneyi, Cyanea pinnatifida, Cyanea shipmanii, Cyanea superba* ssp. *superba, Delissea rhytidosperma, Delissea subcordata, Delissea undulata* ssp. *undulata, Lobelia hypoleuca, Lobelia oahuensis*, and *Lobelia yuccoides*. Of these *Brighamia insignis, Clermontia kakeana, Cyanea asarifolia, Cyanea crispa, Cyanea superba* ssp. *superba, Delissea rhytidosperma, Delissea undulata* ssp. *undulata,* and *Lobelia hypoleuca* have been transferred to garden or forestry conditions. *Cyanea asarifolia, Delissea rhytidosperma, Delissea subcordata,* and *Lobelia hypoleuca* all reached flowering stages.

A recurring problem was encountered in trying to get *Cyanea grimesiana* ssp. *obatae* to transfer successfully to the greenhouse. The plants consistently died within a month of being removed from protective plastic bags. They died from a possible fungal contamination but tests conducted at the plant pathology laboratory at UH could not determine the causal agent. Fungicidal dips and media variations proved unsuccessful. An

experiment is now being conducted in conjunction with the Honolulu Botanical Garden system where a drier environment and a mycorrhizal treatment is being attempted. Early results indicate that the plants are successfully growing in pots, outside an initial protective bag (Kay Lynch, Joshlyn Sands, personal communication).

Discussion

When projects are undertaken to preserve whole groups of plants using *in vitro* techniques only one or two techniques are usually studied. For example, when Rublio *et al.* (1993) studied orchids and cacti they used seed germination and bud proliferation for the orchids and seed germination and organogenesis for the cacti. Bowes and Curtis (1991) reported on using organogenesis and bud proliferation techniques applied to the conservation of Begoniaceae. This study applied three separate *in vitro* techniques to the conservation of Hawaiian Campanulaceae — *in vitro* seed germination, organogenesis, and bud proliferation — making this study extremely valuable for attempts to save these rare species.

Since no significant difference was found between immature and mature seed germination collectors can now confidently collect green fruit in the field if that is all that is available. Before this, collectors had to take a chance that the seeds in green fruit were mature enough to grow under greenhouse conditions or leave the fruit on the plant. Often

the populations producing fruit are in hard-to-reach places, such as those that require a long hike, or even the use of helicopters, to reach, or are susceptible to damage by pests. With more than 80% of the species evaluated successfully germinated the chances are good that a species can be grown *in vitro*. Forty-four taxa were successfully germinated *in vitro*; some of these never before germinated. Even if no seeds are produced, though, a chance exists for growth *in vitro* if fresh bud material or leaf material is collected from healthy plants in the field.

The condition of the explants received by the propagation facility is very important. Though the viability of seeds before they were put into storage was not known, it was noted that seeds that had been in storage for as little as two months had little to no germination. Seeds cleaned by the collectors and put into storage became contaminated (as with *Cyanea hamatiflora* ssp. *carlsonii*) or never germinated. Seeds received in intact fruit were much easier to clean and were more likely to germinate without any contamination problems. The same can be said of vegetative material. If it were received quickly and in good condition by the lab, cleaning was easier and increased the likelihood of micropropagation or organogenesis being successfully performed.

As can be seen in Tables 2.6 and 2.7 on page 33, cytokinin or auxin alone does not effectively induce shoots from leaf explants. Combinations of cytokinin (BA) and auxins (2,4-D or NAA) were successful in producing shoots from leaf explants. No one ratio was

evidently superior for all the species tested. More studies need to be conducted to perfect this method of propagation of Hawaiian Campanulaceae. The results shown here show that BA alone or 2,4-D or NAA alone need not be further studied, and NAA should be used in concentrations higher than 0.05 mg/L. Other types of cytokinins (TDZ, kinetin, zeatin) may prove to be as successful or more successful in producing shoots from leaf explants of Hawaiian Campanulaceae.

The success of these *in vitro* germination and cloning experiments shows that these techniques are a valuable part of a conservation program of Hawaiian Campanulaceae in Hawai'i. Collection policies of the Center for Plant Conservation (CPC), a national organization dedicated to saving endangered plant species, assume on average a 10% success rate (surviving to maturity) in propagating wild native species (Center for Plant Conservation 1991, Guerrant 1992). Though this experiment did not follow individuals to maturity, having a copy of the collection represented *in vitro* will likely increase the percentage surviving since a duplicate can be tried again if the first fails to reach reproductive stage. The CPC policies also stress the need to avoid interfering with the reproductive ecology and demographics of rare plant populations. With the availability of an *in vitro* program, field collectors can collect less than they would normally (at least of seeds) since *in vitro* germination avoids the inherent problems of greenhouse-germinated collections (pests, diseases, etc.).

The ability of the seedlings "naturally" to produce side shoots *in vitro* is also advantageous for conservation. Little manipulation is required to increase propagules from the germinated seedlings, other than removing the side shoots and allowing them to grow and root as individual plants. If an increase in production is needed it could probably easily be induced by the addition of a very small amount of a plant growth regulator. Until the bottleneck that now exists between the production of clones and planting out in the field is broken, an increase in production is counterproductive, requiring more laboratory space and staff time for little return. The strict rule of the State of Hawai'i concerning the possession of endangered species also limits the usefulness of increasing production, since distributing any excess plant material produced is already legally difficult. The laboratory at Lyon Arboretum routinely throws away excess side shoots when maintaining the cultures, a distressing practice given the rarity of these species. A change in the administration of the state laws, or a change in the laws themselves, is needed to keep up to present propagation technologies and the ever-growing list of endangered plant species in Hawai'i. With an increase in interest among citizens of the state to grow Hawaiian plants, it would be good to be able to make some of these rare species available to collectors. Already more landscapers are using native plants in their projects. There is a fear that if demand for Hawaiian plants were to increase dramatically there would be collection pressures on the wild populations. Being able to produce large numbers of plants using *in vitro* techniques will relieve collecting pressures by supplying the market with the needed propagules.

Many species attempted are extremely rare and exist in very small populations. Knowing the genetic variability of the extant populations and of *ex situ* seedling populations would be helpful. Phenotypic variation was not observed among seedlings of any of the rare and endangered species attempted *in vitro*. They were uniform in growth pattern and ability to produce clonal propagules. The presence of leaf mutations among the organogenically-produced *Clermontia kakeana* raises the question of using techniques that produce adventitious buds for propagation of conservation-oriented collections (Cassells 1985, Geneve 1989). The introduced somatic mutations may or may not be sexually stable, and more research needs to be conducted on this phenomenon (Karp and Bright 1985, Klerk 1990, Scowcroft *et al.* 1984) . Meanwhile it would be advisable to restrict *in vitro* propagation to seed germination and shoot proliferation techniques if the propagules comprise a germplasm collection. A reliable technique to detect mutations that do not show up phenotypically is also needed to screen propagules for possible genomic mutation. The use of molecular techniques, such as RAPDs, may be useful in this regard. The work described in the following chapter discusses the use of RAPDs to detect variability among seedling populations of two of these bottlenecked populations.

Chapter 3. Seedling variability of *Cyanea asarifolia* St. John and *Delissea undulata* ssp. *undulata* Gaud. (Campanulaceae) as determined by RAPDs

Introduction

When populations are reduced to a low number of individuals genetic variability can be reduced or lost (Lacy 1992). If seeds are produced by such populations and used in an *ex situ* conservation program, it is desirable to know the innate variability of the seedling populations. *Cyanea asarifolia* St. John and *Delissea undulata* ssp. *undulata* Gaud. both existed in the wild as extremely reduced populations; the former with 15 individuals in the wild and the latter with only one. Morphology was identical among the seedlings so another character was needed to detect variation. The molecular technique of DNA amplification using RAPD primers has the potential to detect unseen differences between individuals of a population. This technique was used to screen seedling populations of these two extremely bottlenecked species. Results of the screening may be used as a comparison against clones produced using *in vitro* techniques, for detection of possible introduced mutations. RAPDs variation may be used as a baseline for future studies which might show either increased or decreased variability.

Materials and Methods

Plant Material

Seedlings of *Cyanea asarifolia* and *Delissea undulata* ssp. *undulata* were germinated *in vitro* (see previous chapter). In the case of the *Cyanea* there were 15 plants in the only known population with only some of those being mature. The pollination status (selfed or out-crossed) is unknown. Hurricane 'Iniki destroyed the majority (if not all) of those 15 plants since the collection of the immature fruit. There is only one mature plant of *D. undulata* ssp. *undulata* in the wild. Seeds collected were apparently produced from self-pollination.

Leaf material was collected aseptically and placed in sterile petri dishes and brought to the molecular genetics laboratory (St. John 510, University of Hawai'i at Mānoa). Young, but fully formed, leaves were used of each species from the original seedling, though clones had been produced from each of the seedlings used. Each individual seedling was given an identification number (as described above) and these numbers were used to identify the DNA extracted from the leaves.

DNA Extraction

DNA was extracted from leaf material collected from *in vitro* grown seedlings of both *Cyanea asarifolia* and *Delissea undulata* ssp. *undulata* using a miniprep extraction protocol. On average 0.03 grams of leaf material was used in a modified small scale extraction method (Doyle and Doyle 1987) as follows. A solution of 2X CTAB isolation buffer (100 mM tris-HCl at pH 8.0, 1.4M NaCl, 25 mM EDTA, 2% hexadecyltrimethyl-ammonium bromide [CTAB], and 1% sodium bisulfite) which was stored in the freezer, was heated in a water bath to 60°C. After the 2X CTAB isolation buffer thawed, 0.2% 2-mercaptoethanol was added. Two hundred microliters of this solution was added to a sterile 1.5 ml microfuge snap-top tube with a small amount of sand. The leaf material was added and ground by hand using a glass mortar until all leaf material had been crushed. Six hundred microliters of the 2X CTAB isolation buffer was added to each tube and the tubes inverted to mix and the tubes placed in the 60°C water bath for 10-15 minutes with occasional inverting. Tubes were removed from the water bath and 2/3 volume of SEVAG (chloroform:isoamylalcohol at 24:1) was added and the tubes inverted several times to form an emulsion. The tubes were then spun in an Eppendorf 5415C microcentrifuge at 14,000 rpm for 2 minutes. The aqueous layer was removed with a wide-bore pipet tip (1,000 μl pipet tip with the tip cut at an angle) and placed in a clean sterile 1.5 ml microfuge tube. This SEVAG step was repeated and the tubes spun again for 2 minutes and the aqueous layer removed and placed in another clean sterile 1.5 ml microfuge tube. Two-thirds volume of ice-cold isopropanol was added to each tube and

the tubes gently inverted to mix. The tubes were then stored in a -20°C freezer until needed (or at least overnight).

When the tubes were removed from the freezer they were spun in a microcentrifuge at 14,000 rpm for 10 minutes. The supernatant was removed (initially by pouring and finally by using a Pasteur pipet). Eight hundred milliliters of 76% EtOH/0.01 M NH_4OAc was added and the tubes allowed to sit for ten minutes. The supernatant was again removed as above. The DNA pellet was dried in a speedvac for 5-10 minutes. One hundred microliters of TE (0.05M tris-HCl at pH 8.0 and 0.001M EDTA) were added and the tubes placed in a 37°C water bath for 30 minutes to one hour to resuspend the DNA.

Large scale extraction was performed using leaves from *in vitro* seedlings of *Cyanea asarifolia* to compare with the miniprep extractions. This was performed by using 0.5 g of leaf material for each sample. Leaves were placed in a mortar heated to 60°C with 7.5 ml 2X CTAB with added 2-mercaptoethanol as described above and crushed with a pestle, also heated to 60°C, until a relatively homogeneous slurry resulted. This slurry was poured into a 30 ml centrifuge tube. The mortar and pestle were rinsed with 2.5 ml 2X CTAB/2-mercaptoethanol solution and the rinsate placed in the same tube. All samples were placed in a 60°C water bath and incubated for 60 minutes. An equal volume of SEVAG was added after the incubation period and mixed gently by rocking back and forth. The samples were spun in a centrifuge (IEC Centra MP4) for 5 minutes at

3,000 rpm. With a Pasteur pipet the aqueous layer was removed and placed in a fresh 30 ml centrifuge tube. Two-thirds (by volume) of ice-cold isopropanol was added to the tubes and the solution mixed gently to precipitate the DNA. This solution was then placed in a -20°C freezer overnight.

The samples were removed from the freezer and spun for three minutes at 3,000 rpm in the centrifuge to obtain a pellet of DNA. The liquid layer was poured off and the pellet allowed to dry. Three milliliters of resuspension buffer (10 mM ammonium acetate, 0.25 mM EDTA) were added to each sample and the solution placed in a 37°C water bath until the pellet was completely dissolved. The resuspended DNA was then placed in a -20°C freezer overnight.

The DNA was then purified using cesium chloride. Three grams of cesium chloride was added to the resuspended DNA (one gram for each milliliter of resuspension buffer added in the previous step). After the cesium chloride dissolved the solution was placed in an ultracentrifuge tube. The tubes were balanced by adding liquid cesium chloride (1.55 g/ml) to fill the tubes. Ethidium bromide was added for a final concentration of 200 μg/ml and a cap placed on each tube. The tubes were inverted to mix the ethidium bromide into the cesium chloride/DNA solution and the tubes placed in the ultracentrifuge rotor. They were spun at 55,000 rpm in a Beckman L-180 Ultracentrifuge for at least 5.5 hours, but usually overnight.

The tubes were removed from the ultracentrifuge and brought to a UV light source to observe the DNA band. The band was removed using a Pasteur pipet and placed in a plastic tube. The ethidium bromide was then washed out of the DNA solution by adding an equal volume of salt-saturated isopropanol to the DNA/ethidium solution. The tube was capped and inverted several times until the ethidium bromide was removed to the isopropanol layer. This layer was vacuumed off and the procedure repeated until no more ethidium bromide was visible and the DNA solution was clear.

The DNA solutions were then dialyzed by placing the solution into dialysis tubing (Spectra/Por 6.4 mm dia. MWCO 12-14,000, Fisher Scientific) and placing the tubing into dialysis buffer (10mM tris-HCl, pH 8.0, 1.0 mM EDTA pH 8.0) and gently stirred on a stir plate for one hour. The dialysis buffer was replaced with fresh buffer and stirred for another 24 hours. The dialysis buffer was replaced one more time and stirred for another 24 hours. The DNA solution was then removed from the dialysis tubing and placed in 1.5 ml centrifuge tubes with snap tops and stored at 4.0°C.

DNA solutions from either extraction method were evaluated by running 5μl of solution on a minigel (1.5% agarose, 0.5X TBE [0.225M tris-HCl, 0.225M boric acid, 0.05 M EDTA] at 200 volts for about 1 hour. A ladder (1 kb) was run with the DNA as a marker. The gels were stained in ethidium bromide (7×10^{-7} M) for 15 minutes, rinsed in distilled water for 15 minutes, and observed under UV light.

Concentration of DNA was calculated by using the absorption reading at 260 nm of a 1:100 dilution of DNA solution with sterile distilled H_2O. A working solution of each DNA was made up with a final concentration of 5 ng per μl.

RAPDs PCR

Amplification reactions were performed in volumes of 25 μl. Concentrations of dNTPs, $MgCl_2$, *Taq* and genomic DNA were all adjusted to optimize the amplification protocol and resulted in using 50 mM KCl; 10 mM tris-HCl; 0.1% Triton X-100 (all from Promega brand assay buffer); 2.04 mM $MgCl_2$; 0.1 mM each of dATP, dCTP, dGTP, and dTTP (Epicenter Technologies); 5.0 picomoles of primer (Operon Technologies); 5.0 ng of genomic DNA; and 0.65 units of *Taq* (Promega) topped with 30 μl of mineral oil (Sigma Chemical). Amplification was performed in a MJ Research PTC-100 programmable thermocycler set for 45 cycles of 1 minute at 94°C, 1 minute at 37°C, 2 minutes at 72°C; ending with 5 minutes at 72°C and held at 4°C until separated on a gel.

Amplification products were observed by running 20 μl of each product on a 1.5% agarose gel in 0.5X TBE buffer (0.45M tris-HCl, 0.45 M boric acid, and 0.1 M EDTA) for 2 hours at 200 volts. A 1 kb DNA ladder was run in a separate well using a gel loading dye buffer (solution of 0.25% bromphenol blue, 0.25% xylene cyanol ff, and 30% glycerol). The gel was stained with ethidium bromide ($7x10^{-7}$ M) for 15 minutes, rinsed

three times in distilled water and destained with distilled water for 15 minutes and observed under UV light and photographed with Polaroid 667 film.

When possible usable (polymorphic) amplification products were observed, the reactions were repeated at the same DNA concentration and with ± 25% concentrations of DNA to eliminate any possible false bands.

One hundred fifty-four primers were screened using genomic DNA of *Delissea undulata* ssp. *undulata*. Two hundred twenty primers were screened using genomic DNA of *Cyanea asarifolia*. Table B.1 on page 125 shows primers screened, their sequence, and DNA amplified.

Results

DNA was successfully extracted from *Delissea undulata* ssp. *undulata* using the small scale extraction protocol for use as template in the PCR reactions. Useable DNA was successfully extracted with either the large- or small-scale DNA extraction techniques for *Cyanea asarifolia*. When separated on a gel the cesium-cleaned DNA was of appreciably higher quality than the small-scale extracted DNA forming tighter bands (see Figure 3.1 page 56) but when used in PCR reactions they performed equally well. As a result only small-scale extraction DNA was used for PCR reactions. The largest band on the DNA

ladder is 12,216 bp and it can be seen that the native DNA is larger than that, as expected. In a few cases in the small-scale extractions RNA can be seen with one band at slightly larger than 2,036 bp and one band at slightly lower than 1,636 bp.

With *Cyanea asarifolia* 30 primers produced RAPD markers and were observed in 78 of the 154 primers screened with DNA from *Delissea undulata* ssp. *undulata*. In both cases most of the markers appeared uniform (monomorphic) among the individuals. Because possible polymorphisms were seen in the original screening DNA concentration tests were performed using *Cyanea* with primers OPA-5 (Figure 3.2), OPA-16, OPA-18, OPE-2, OPE-3, OPE-4, OPF-3, OPH-12, OPH-18, OPI-2, OPI-6, OPK-1, OPK-3, OPK-4, OPK-7, OPK-12 (Figure 3.3), and OPK-13. DNA concentration tests were performed using *Delissea* with primers OPA-3, OPA-12, OPB-1, OPB-2, OPB-5, OPB-6, OPB-7, OPB-8 (Figure 3.4), OPB-10, OPB-14, OPC-1, OPC-5, OPC-10, OPC-11 (Figure 3.5), OPB-17, and OPF-4. In no case was the polymorphism consistent but appeared to be due to variations in DNA concentrations.

Figure 3.2 shows the case of a possible marker (though not as bright) for OPA-5-800 but this band disappeared or appeared inconsistently when run at the bracketing DNA concentrations. In Figure 3.3 the possible polymorphic marker at OPK-12-3000 also appeared inconsistently at varying DNA concentrations. In Figure 3.4 a possible double band can be seen in samples 2, 5, and 6 at OPB-8-1200 (bp) when the DNA was run at

the original concentration. When the test was performed at 75% or 125% concentrations this possible double band appeared only as a single band. DNA concentrations did not produce a change in bright (but unvariable) bands in Figure 3.5 with OPC-11.

Figure 3.2 shows the case of a possible marker (though not as bright) for OPA-5-800 but this band disappeared or appeared inconsistently when run at the bracketing DNA concentrations. In Figure 3.3 the possible polymorphic marker at OPK-12-3000 also appeared inconsistently at varying DNA concentrations.

Discussion

Though it was possible to get RAPD markers with the DNA/primer combinations screened, no consistent polymorphic markers were found. The RAPDs technique failed to detect any genetic variability in either *Cyanea asarifolia* or *Delissea undulata* ssp. *undulata*. Since both species are extremely bottlenecked, this may not be too surprising. RAPDs is a random molecular sampling technique which has been shown to detect fine-scale variation in crops and wild plants (Baird *et al.* 1992, Gibbs *et al.* 1994, Haig *et al.* 1994, Luque *et al.* 1995, Martin *et al.* 1991, Van Buren *et al.* 1994, Waycott and Fort 1994, Yang and Quiros 1993). However, in these bottlenecked Hawaiian taxa such

variations were not detected even though a large number of primers were screened. Variability is low, at least for that part of the genome responsive to RAPD primers. Another technique may be required to detect the low levels of remaining variability.

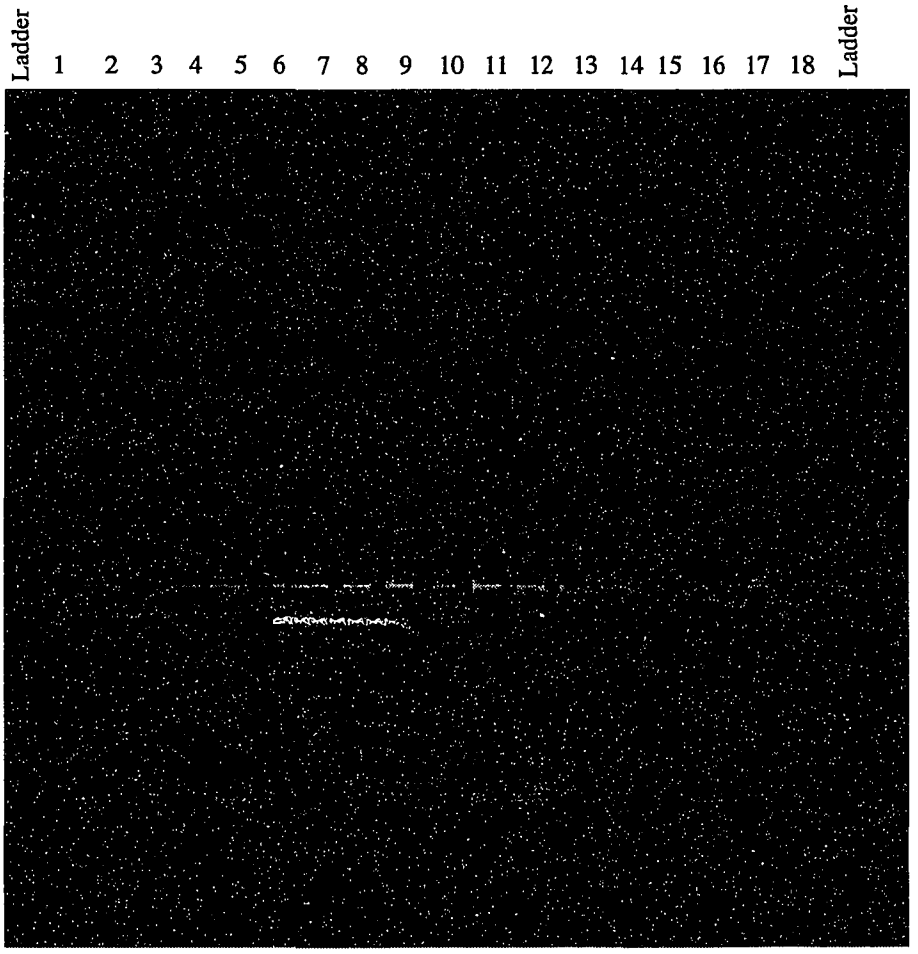
The lack of variation in Hawaiian Campanulaceae is similar to that found in another bottlenecked island endemic. Swensen *et al.* (1995) could find no polymorphisms using RAPDs in their study of *Malacohamnus fascuculatus* (Nutt.) Greene var. *nesioticus* (Rob.) Kearn (Malvaceae) on Santa Cruz Island, California. But RAPDs has been found to work with other Hawaiian endemics that are undergoing a bottleneck event. Friar *et al.* (unpublished manuscript) report detecting variation using RAPDs in *Argyroxiphium sandwicense* DC ssp. *sandwicense* (Asteraceae). Some primers have detected possible variations between individuals and populations of *Lipochaeta subcordata* A. Gray and *L. venosa* Sherff (Asteraceae) (Keeley, unpublished data). Even some species of Campanulaceae have had variation detected using RAPDs (Rick Palmer, personal communication). Variations among individuals may yet be detected in *Cyanea asarifolia* or *Delissea undulata* ssp. *undulata* using other molecular genetic techniques. It is possible, that as the populations increase using controlled breeding and propagation, variability will later be detected. This report can be used as a baseline for further studies.

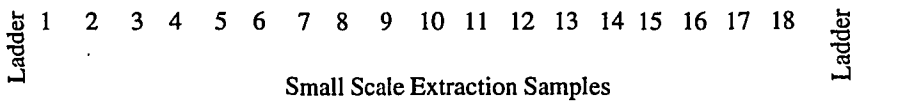
In the meantime, the lack of variability detected using RAPDs suggests that all seedlings resulting from propagation projects be conserved (easier to do using *in vitro* techniques).

Also, collectors in the field should be aware of the likelihood of extremely low genetic variation in small populations and collect accordingly. Propagules (clonal or sexual) should be collected from all remaining individuals if at all possible and without harming extant populations. With the use of *in vitro* techniques, clonal or sexual populations can be maintained and their genetic make-up compared with present and future individuals.

Figure 3.1. DNA cleaned with cesium chloride (top of gel) is notably cleaner than DNA extracted without a cesium-gradient step (bottom of gel).

Cesium-Cleaned Samples





Small Scale Extraction Samples

Figure 3.2. RAPDs markers resulting from using primer OPA-5 with DNA from *Cyanea asarifolia*. DNA at 100% concentration (right) and DNA at 75% and 125% (left).

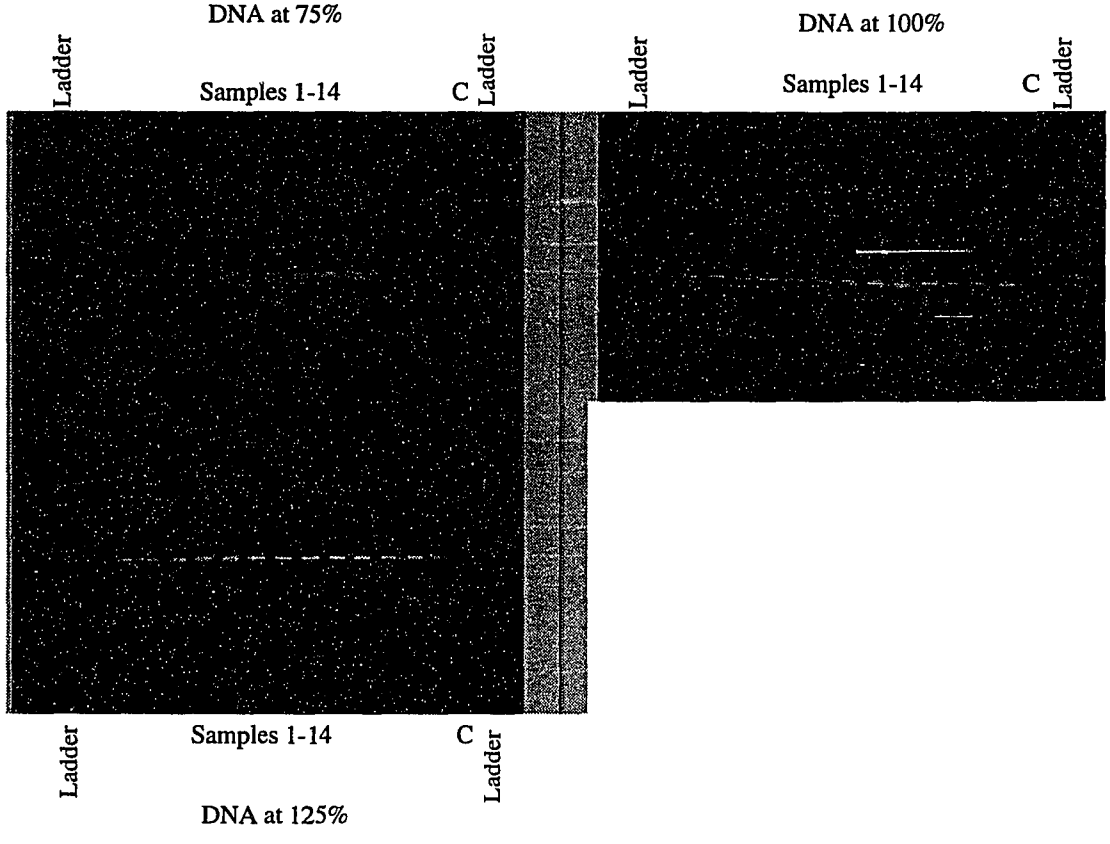

DNA at 75%
Ladder
Samples 1-14
C
Ladder
DNA at 100%
Ladder
Samples 1-14
C
Ladder
Ladder
Samples 1-14
C
Ladder
DNA at 125%

Figure 3.3. RAPDs markers resulting from using primer OPK-12 with DNA from *Cyanea asarifolia*. DNA at 100% concentration (right) and DNA at 75% and 125% (left).

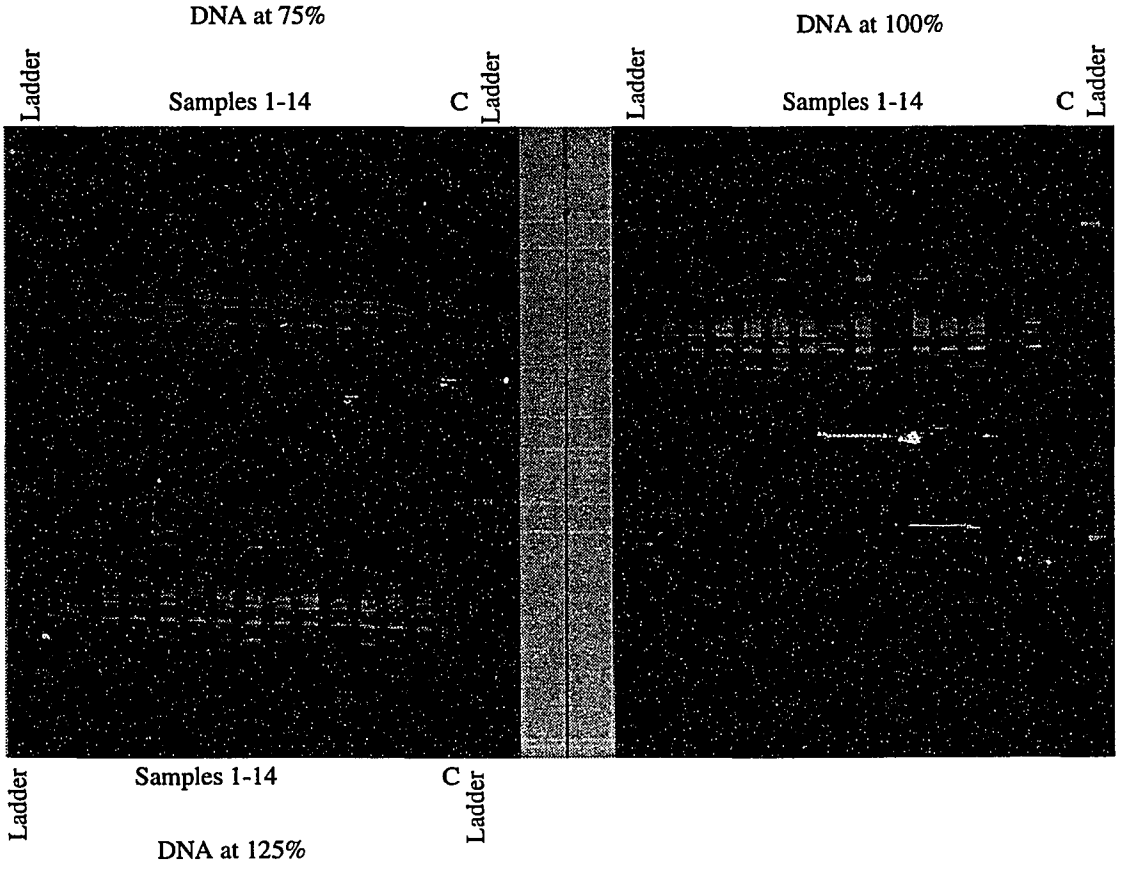

DNA at 75%
Ladder
Samples 1-14
C
Ladder
DNA at 100%
Ladder
Samples 1-14
C
Ladder
Ladder
Samples 1-14
C
Ladder
DNA at 125%

Figure 3.4. RAPDs markers resulting from using primer OPB-8 with DNA from *Delissea undulata* ssp. *undulata*. DNA at 100% concentration (top) and DNA at 75% (lower left) and 125% (lower right).

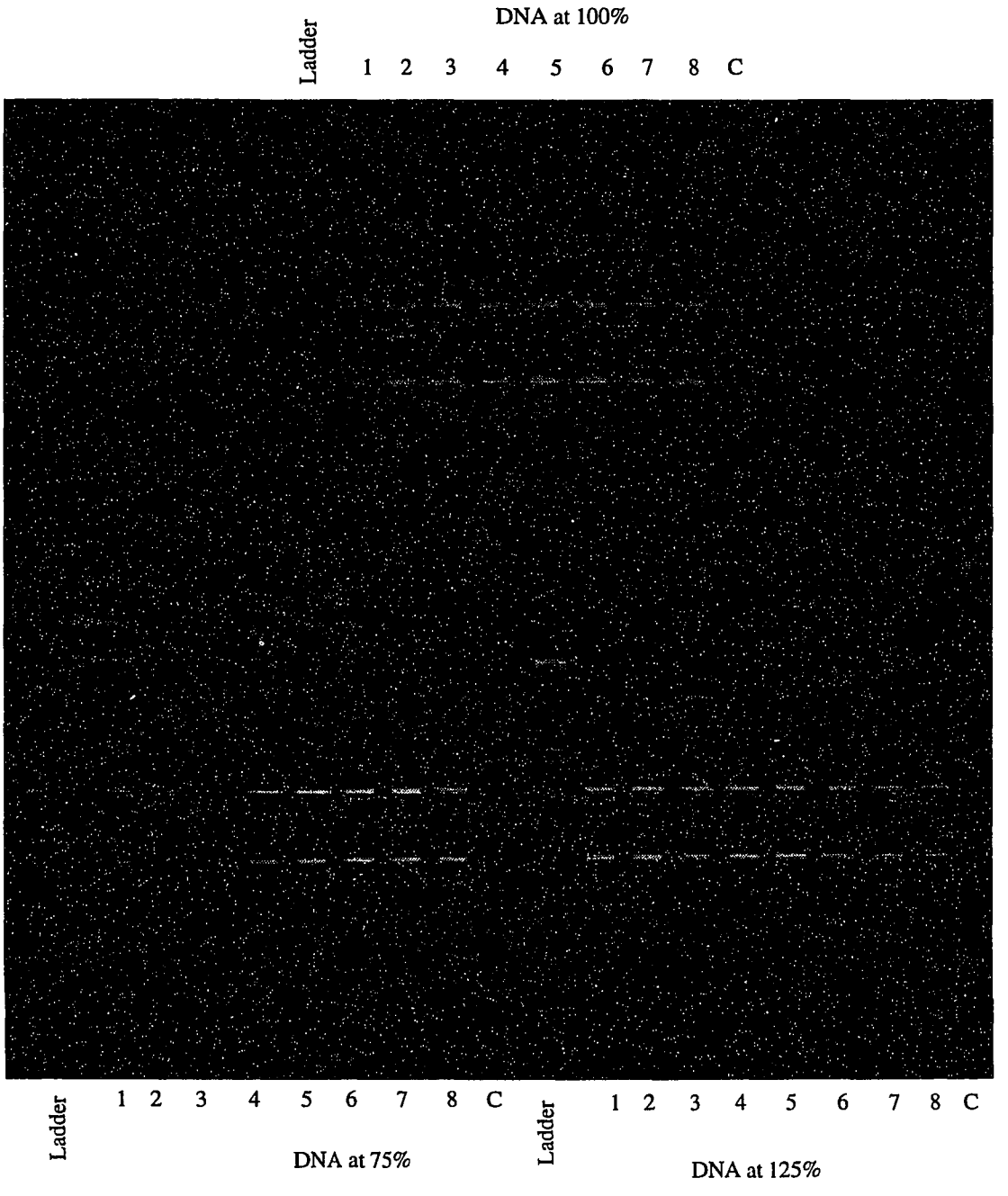
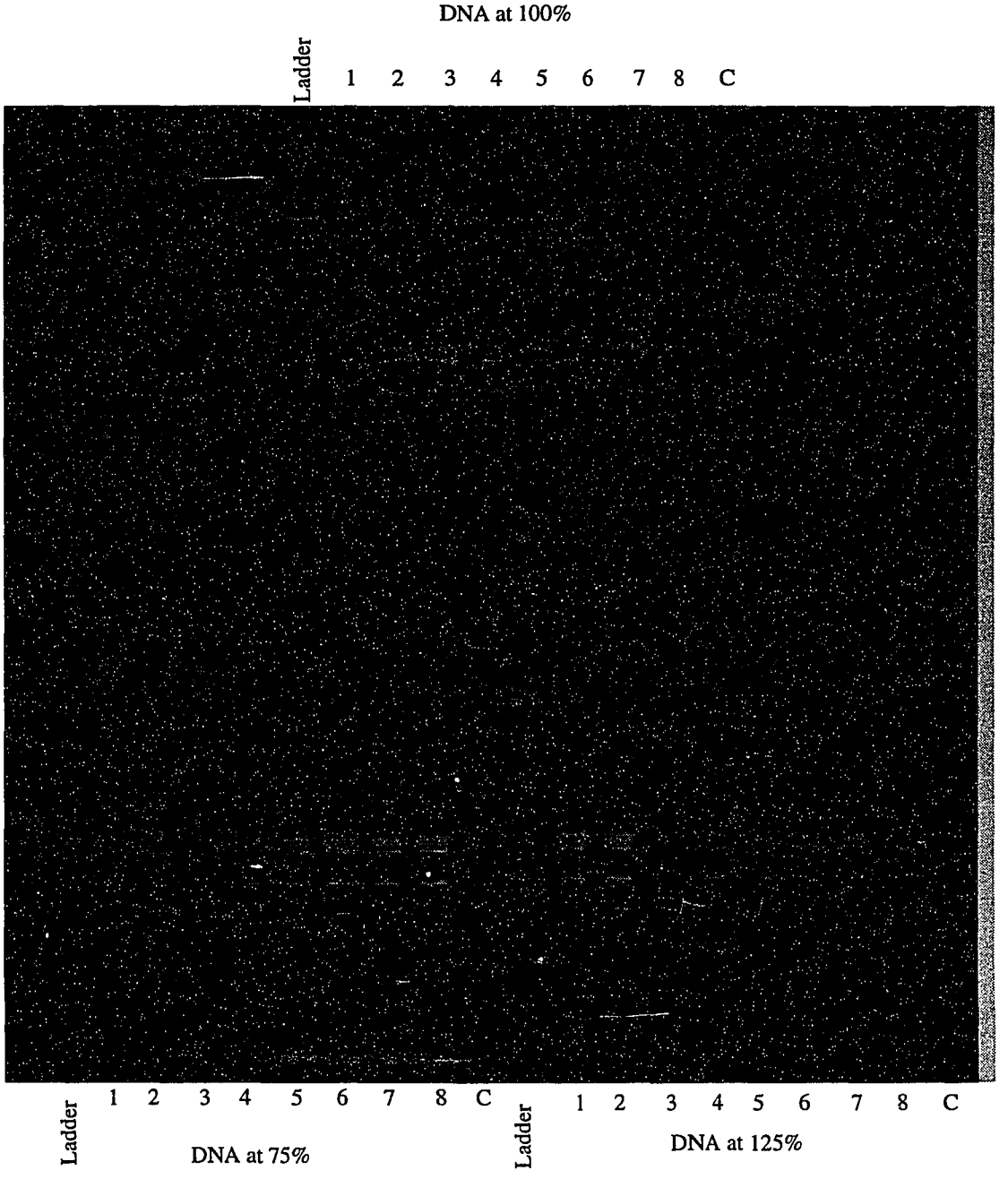

DNA at 100%
Ladder
1 2 3 4 5 6 7 8 C
Ladder
1 2 3 4 5 6 7 8 C
DNA at 75%
Ladder
1 2 3 4 5 6 7 8 C
DNA at 125%

Figure 3.5. RAPDs markers resulting from using primer OPC-11 with DNA from *Delissea undulata* ssp. *undulata*. DNA at 100% concentration (top) and DNA at 75% (lower left) and 125% (lower right).


DNA at 100%
Ladder 1 2 3 4 5 6 7 8 C
Ladder 1 2 3 4 5 6 7 8 C
DNA at 75%
Ladder 1 2 3 4 5 6 7 8 C
DNA at 125%

Chapter 4. General Discussion

In vitro propagation of Hawaiian Campanulaceae has proven useful for increasing the numbers of rare plants and requests for this type of propagation are increasing. For example, as state, federal and private land managers around the state have learned about the procedure they have requested work be done on species existing on their land. They are most concerned with (gross) preservation, that is, saving anything they can, as threats to populations are increasing and the plants are disappearing at an alarming rate. Gross preservation of species is important and sets the stage for evaluation of the variability inherent in these rare plants. But, reduced populations mean that only a limited amount of genetic information remains, and if the species are bottlenecked the variability may be fixed. Preservation of remaining variability is also subject to technical problems. For example, some mutations may occur when using some *in vitro* techniques (as with organogenesis of *Clermontia kakeana*) and the resulting populations should be closely monitored.

Variation occurs in all natural seedling populations but it is known that extremely bottlenecked species have lost much of their variability. Just how much is lost is not something that can be known in advance but must be discovered empirically for each species. For the two species analyzed here, a random sampling of the DNA for variable molecular markers resulted in no detectable variation in seedling populations. This lack

of variation apparently reflects the limited source. One population is derived from only one parent and the other from unknown parents or parent. Other molecular techniques could be used to look for variation in these species but would need to be extremely sensitive. DNA fingerprinting techniques such as the analysis of short tandemly repeated sequences known as microsatellites, short tandem repeats (STRs), or simple sequence repeats (SSRs) (Jarret and Bowen 1994) could be the next type of techniques used to determine variability in these populations. A technique known as SWAPP (DNA sequencing with arbitrary primer pairs) has also been developed to allow a closer look at markers found with the RAPDs technique (Burt *et al.* 1994) but has not been tried on any Hawaiian plants.

The future of *in vitro* propagation and molecular assays of genetic variation of Hawaiian plants looks good for Hawaiian Campanulaceae and other families. Much more work needs to be done, as this report shows, but it is possible both to preserve species and sample their genetic variation. Never before has such an extensive study been conducted on *in vitro* propagation of a group of Hawaiian plants. Time is running out for many species and more research needs to be conducted to learn the best protocol for cloning plants from the wild and keeping *in vitro* collections viable. With other propagation programs and a well thought-out conservation program, *in vitro* propagation has a large role to play in conserving Hawai'i's rare and endangered plants for future generations. Molecular marker detecting techniques are sensitive and new ones are appearing rapidly.

It seems likely that increasingly sensitive fingerprinting techniques will make it possible to monitor the evolution of outplanted individuals and give us a picture of the past (and present) mechanisms that have made Hawaiian Campanulaceae such exceptional examples of endemism and adaptive radiation.

This study provides a baseline for conservation of two rare and endangered Campanulaceae with extremely reduced populations. Little or no detectable variation is evident in the seed progeny of these species, indicating a genetic bottleneck. The effect of such a bottleneck is unclear. Hawaiian species have undergone bottlenecks in the distant past and have overcome these. Carson (1982) has postulated, for example, that while some *Drosophila* populations had low variation and small size (bottlenecks) these populations were able to overcome the bottleneck and subsequently become more variable. Studies to document this kind of spontaneous recovery of genetic variation are rare and the extent to which this applies to Hawaiian lobelioids is unknown. At the least, continued propagation of these rare plants means that the genes are still available and that new populations can be reestablished in nature or studied under conditions that will allow assessing inherent and spontaneous genetic changes. Cross-pollination experiments can also be conducted and the seeds grown and several generations established. After several generations have been crossed and propagated, another look at the variability of these populations can be done, either with the RAPDs technique or others. Naturally occurring asexual propagation of these species is unknown, and should also be investigated in the

future. Since both populations studied are being maintained clonally *in vitro* the "original" plant material can be used with another technique and compared with future populations.

Ideally, these species will recover some degree of variability and maintain themselves as viable wild populations in the future. This may never happen but that is no reason not to try to preserve what is left. Even if they only exist as "museum pieces," collections of what exist right now, they are still a valuable collection. Picasso and da Vinci are no longer producing works of art, but that is no reason to let the original *Sunflowers* or Mona Lisa turn to dust.

Appendix A. Additional *In Vitro* Propagation Data Tables

Table A.1 *In vitro* treatments used on Hawaiian Campanulaceae

Species	Treatment(s)	Explant	Explant Source/Notes	Media
Brighamia insignis				
	P00600001	Immature seed	Immature self-pollinated fruit	VW with sugar
	P00600051A through P00600051L	Leaves	*In vitro* seedlings.	0.5X MS, 150 ml/L CW or 0.5X MS with BA at 0.05 or 1.0 mg/L or 2,4-D at 0.1, 0.5, or 1.0 mg/L or combinations of BA and 2,4-D
	P01870001	Immature seeds	Immature fruit.	0.5X MS
Clermontia drepanomorpha				
	P03830001	Immature seed	Smaller than normal green fruit. Mature fruit is usually quite large (largest of the *Clermontia*).	0.5X MS
	P07170001	Immature seed	Green and slightly rotted fruit.	0.5X MS with charcoal
Clermontia fauriei				
	P01790001	Seed	Mature seeds already removed from fruit by the collector.	0.5X MS
	P01790002A through P01790014A	Leaf	*In vitro* seedling corresponding to plant number preceeding "A".	0.5X MS with BA and 2,4-D combinations or BA and NAA combinations. BA at 1.0 or 0.5 mg/L; 2,4-D at 0.05, 0.1, 0.5, or 1.0 mg/L and NAA at 0.05, 0.5 or 1.0 mg/L
Clermontia grandiflora				
	P01560001	Apical bud	Apical buds from mutli-branched branch of mature plant. There was one flower spike on the branch with an immature flower.	0.5X MS
	P01560002	Node	Nodal segments of green wood from branchlets from a mature, flowering branch.	0.5X MS
	P01560003	Leaf	Whole or partial leaf from mature, flowering branch.	MS 1/4

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
	P01560004	Petiole	Mature leaves from mature, flowering branch.	MS 1/4
	P01560005 through P01560008	Leaf segment	*In vitro* seedling.	0.5X MS with 150 ml/L CW or BA at 1.0 mg/L or a combination of BA and 2,4-D at 1.0 mg/L
	P07470001	Seeds	Ripe fruit (or nearly ripe).	0.5X MS
Clermontia kakeana				
	P00480001	Immature flower spike	Immature flower spike with two immature flower buds.	0.5X MS, 150 ml CW
	P00480002	Immature floral tube	Flower that had not yet opened.	0.5X MS, 150 ml CW
	P00480003	Peduncle	Immature (nearly mature) flower bud.	0.5X MS, 150 ml CW
	P00480004	Immature ovary	Whole immature flower buds.	0.5X MS, 150 ml CW
	P00480005	Node	Medial area of a side branch. The center of the explants is woody.	0.5X MS, 150 ml CW
	P00480006 through P00480017	Petal	P00480002	0.5X MS or 0.5X MS with BA at 0.5 or 1.0 mg/L or 2,4-D at 0.05, 0.5 or 1.0 mg/L or combinations of BA and 2,4-D
	P00480018	Style	P00480002	0.5X MS, 1.0 2,4-D
	P00480019 through P00480023	Fruit	P00480004	0.5X MS with 2,4-D at 0.5 mg/L alone or with combinations of BA at 0.5 and 1.0 mg/L and 2,4-D at 0.05 and 0.5 mg/L
	P00480024	Seed	Ripe fruit and stored at 4°C until use.	VW with sugar
	P00480031CA, P00480031HA, P00480021JA, P00480031LA through P00480031PA, P00480032A through P00480032L, P00480032JA and P00480032JB	Leaf	*In vitro* seedling and organogenically produced plantlets (P004800JA and JB).	0.5X MS with CW at 150 ml/L or 0.5X MS with BA and 2,4-D combinations or BA and NAA combinations. BA at 0.5 or 1.0 mg/L; 2,4-D at 0.05, 0.1, 0.5, or 1.0 mg/L and NAA at 0.05, 0.5 or 1.0 mg/L

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
Clermontia kakeana x Clermontia arborescens				
	P06410001	Immature seed	Green fruit.	0.5X MS
	P06870001	Immature seed	Green fruit.	0.5X MS
Clermontia lindseyana				
	P02490001	Immature seed	Green unopened fruit. Fruit had worms and only one seed was found.	0.5X MS
	P02880001	Seed	Nearly mature fruit.	0.5X MS
Clermontia montis-loa				
	P01990001	Seed	Mature seed.	0.5X MS
Clermontia peleana				
	P01690001	Seed	Unopened, ripe (orange) fruit.	0.5X MS
	P01690002	Seed	Unopened, immature (green) fruit.	0.5X MS
	P01690003A through P001690012A	Leaf	*In vitro* seedling.	0.5X MS with BA and 2,4-D combinations or BA and NAA combinations. BA at 0.5 or 1.0 mg/L; 2,4-D at 0.05, 0.1, 0.5, or 1.0 mg/L and NAA at 0.5 or 1.0 mg/L
	P01700001	Bud	Mature, flowering branch.	0.5X MS
Clermontia pyrularia				
	P02560001	Seed	Received mature, cleaned and dried.	0.5X MS
	P02570001	Seed	Received mature, clean and dried.	0.5X MS
	P03170001	Seed	Mature fruit.	0.5X MS
	P03180001	Seed	Mature fruit.	0.5X MS
	P03700001	Seed	Seed in storage.	0.5X MS
	P04740001	Immature seed	Green fruit.	0.5X MS
Clermontia tuberculata				
	P02820001	Immature seed	Immature (green) fruit.	0.5X MS
	P06880001	Immature seed	Green fruit.	0.5X MS
Cyanea aculeatiflora				
	P03870001	Immature seed	Young, yellowish fruit.	0.5X MS
	P05860001	Seed	Mature fruit.	0.5X MS
Cyanea acuminata				
	P00660001	Seed	Ripe fruit.	VW with sugar
	P00660002	Seed	Seeds left in carpel tissue.	VW with sugar

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
	P00660003BA through P00660003BM	Leaf	*In vitro* grown seedling.	0.5X MS with BA and 2,4-D combinations or BA and NAA combinations. BA at 0.5 or 1.0 mg/L; 2,4-D at 0.05, 0.1, 0.5, or 1.0 mg/L and NAA at 0.05, 0.5 or 1.0 mg/L
	P02100001	Seed	Mature fruit.	0.5X MS
	P02110001 through P02110003	Petiole	Mature leaf.	0.5X MS with BA at 0.5 or 1.0 mg/L and 2,4-D at 0.1 or 0.5 mg/L
Cyanea angustifola				
	P05850001	Seed	Both mature and immature fruit.	0.5X MS
	P07310001	Immature seed	Green fruit.	0.5X MS
Cyanea asarifolia				
	P00580001	Immature seed	Immature seed pods.	VW with sugar
	P00580015GA through P00580015GE, P00580015HA through P00580015HD, P00580015JA through P00580015JD	Leaf	*In vitro* seedling.	0.5X MS with BA and 2,4-D combinations or BA and NAA combinations. BA at 0.5 or 1.0 mg/L; 2,4-D at 0.05, 0.1, 0.5, or 1.0 mg/L and NAA at 0.05, 0.5 or 1.0 mg/L
	P00580016 through P00580026	Plantlet	Organogenically produced from leaf or cotyledon.	MS 1/4
	P02060001through P02060004	Petiole	Mature leaves.	0.5X MS with BA at .05 or 1.0 mg/L in combination with 2,4-D at 0.1 or 0.5 mg/L
	P02060005	Callus	P02060004.	0.5X MS
Cyanea asplenifolia				
	P07490001	Bud	Young shoots (apical buds).	0.5X MS
Cyanea copelandii ssp. *haleakalaensis*				
	P03860001	Immature seed	Purportedly ripe fruit but seeds (obviously) immature.	0.5X MS
Cyanea crispa				
	P00240001	Apical bud	Branch broken off mother plant by feral pigs.	0.5X MS, 150 ml CW

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species	Treatment	Explant	Explant Source/Notes	Media
	P00910001	Leaf	Various leaf parts including some main veins and some with side veins only.	0.5X MS
	P00910002	Node	Full node sections of green stem about 0.75 cm in diameter.	0.5X MS
	P01020001	Apical bud	Apical bud from side shoot growing from a cutting in the greenhouse.	0.5X MS
	P01500001	Seed	Very ripe fruit.	0.5X MS
	P01520001	Seed	Ripe fruit.	0.5X MS
	P01530001	Seed	Mature fruit collected from the ground.	0.5X MS
	P02120001	Bud	Mature branch.	LS-1/2
	P02540001 through P02540003	Bud	Apical bud.	0.5X MS with BA at 0.5, 1.0 or 2.0 mg/L
	P02540004 through P02540011	Leaf	Immature leaf.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, or 0.5 mg/L or NAA at 0.5 mg/L
	P03030001	Immature seed	Green fruit.	0.5X MS
	P03930001 through P03930005	Node segment	Seedlings collected in wild.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.5 or 1.0 mg/L or NAA at 0.5 or 1.0 mg/L
Cyanea degeneriana				
	P02000001	Seed	Mature seed.	0.5X MS
Cyanea dunbarii				
	P03490001	Seed	Nearly-ripe fruit.	0.5X MS
	P06740001 through P06740005	Bud	Young shoots, apical buds only. Remainder of shoots sent to greenhouse.	0.5X MS with charcoal, or 0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.5, or 1.0 mg/L
Cyanea glabra				
	P05840001	Immature seed	Green fruit.	0.5X MS
Cyanea grimesiana ssp. *grimesiana*				
	P03800001	Seed	Mature fruit.	0.5X MS
	P05550001	Seed	Ripe fruit.	0.5X MS

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
Cyanea grimesiana ssp. *obatae*				
	P00610001	Seed	Mature fruit.	VW with sugar
	P00610005GA, P00610005GB, P00610006AA, P00610006AB, P00610006BA through P00610006BD, P00610006CA through P00610006CE, P00610006DA through P00600006DE, P00610006EA through P00610006EC and P00610007A	Leaf	*In vitro* seedling.	0.5X MS with BA and 2,4-D combinations or BA and NAA combinations. BA at 0.5 or 1.0 mg/L; 2,4-D at 0.05, 0.1, 0.5, or 1.0 mg/L and NAA at 0.05, 0.5 or 1.0 mg/L
	P00740001 and P07400002	Petiole	Petiole previously disinfested and cultured temporarily in 0.5X MS.	0.5X MS with 2,4-D at 0.5 mg/L in combination with BA at 0.5 or 1.0 mg/L
	P00870001	Leaf	Greenhouse-grown plant.	0.5X MS with CW at 150 ml/L
	P00880001	Leaf	1-1/2 year old seedling.	0.5X MS
	P02950001	Immature seed	Green fruit.	0.5X MS
	P02960001	Seed	Mature fruit.	0.5X MS
	P03390001	Immature seed	Green fruit.	0.5X MS
	P05900001	Immature seed	Green fruit.	0.5X MS
Cyanea hamatiflora ssp. *carlsonii*				
	P00690001	Seed	Off-white to tan colored seeds about 1 mm long and 0.5 mm wide. Received from HPCC already removed from fruit and previously in storage.	VW with sugar
	P00690002	Embryo	Mature seeds.	VW with sugar
	P00700001	Seed	Off-white to tan colored seeds about 1 mm long and 0.5 mm wide. Received from HPCC already removed from fruit and previously in storage.	VW with sugar

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species	Treatment	Explant	Explant Source/Notes	Media
	P00710001	Seed	Off-white to tan colored seeds about 1 mm long and 0.5 mm wide. Received from HPCC already removed from fruit and previously in storage.	VW with sugar
	P05290001	Seed	From mature dried messy fruit that were gathered from the ground from around a dead plant.	0.5X MS
Cyanea hamatiflora ssp. *hamatiflora*				
	P05950001	Seed	From mature, but rotted fruit.	0.5X MS
	P05950003A through P05950003C, P05950004A through P05950004C, P05950005A, P05950005B, P05950008A through P05950008C, P05950009A through P05950009C	Leaf	*In vitro* seedling.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
Cyanea kolekoleensis				
	P01840001	Immature seed	Green fruit.	0.5X MS
	P04120001	Bud	Small side shoots from immature plants.	0.5X MS
Cyanea kuhihewa				
	P04850001through P04850003	Bud	Vegetative shoot.	0.5X MS with BA at 0.5 mg/L in combination with 2,4-D at 0.05 or 0.1 mg/L or NAA at 0.05 mg/L
	P04850003A through P04850003Mm P04850004 through P04850017	Leaf segment	P04850003 or wild-collected leaf (4-17).	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
Cyanea kunthiana				
	P06910001	Immature seed	Green fruit.	0.5X MS
Cyanea lanceolata				
	P02810001	Immature seed	Green fruit.	0.5X MS
	P03000001	Immature seed	Green fruit.	0.5X MS

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species	Treatment	Explant	Explant Source/Notes	Media
Cyanea leptostegia				
	P01800001	Seed	Mature seeds already removed from fruit by the collector.	0.5X MS
Cyanea linearifolia				
	P01460001	Seed	Mature seeds (very small).	0.5X MS
	P01460002	Seed	Mature seeds (very small).	0.5X MS
Cyanea longiflora				
	P01550001	Apical bud	Mature branch.	0.5X MS
	P01550002	Node	Mature branch. Green wood.	MS 1/4
	P01550003	Leaf	Mature branch.	MS 1/4
	P01550004 through P01550009	Leaf segment	P01550003.	0.5X MS with CW at 150 ml/L or 0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.1, 0.5 or 1.0 mg/L
	P02640001 through P02640002	Bud	Immature plant.	0.5X MS with BA at 0.5 or 2.0 mg/L
	P02860001	Seed	Mature fruit.	0.5X MS
	P04810001	Seed	Rotted and macerated mush with maggots in it. The whole mess was malordorous.	0.5X MS
Cyanea manii				
	P02380001	Seed	Immature fruit.	0.5X MS
Cyanea mceldowneyi				
	P00890001	Immature seed	Intact green fruit.	VW with sugar
	P00900001	Immature seed	Intact green fruit.	VW with sugar
	P03640001	Immature seed	Very very immature fruit.	0.5X MS
Cyanea membranacea				
	P00620001	Node/Apical stem	Segments of the stem were cut from a several branches after the leaves were removed, the apical bud was also used.	0.5X MS
	P02970001	Seed	Mature and green fruit.	0.5X MS
Cyanea oahuensis				
	P04980001	Immature seed	Green fruit.	0.5X MS
Cyanea pinnatifida				
	P00550001	Shoot	Young plant. One has small roots forming.	0.5X MS
	P00550002	Leaf	Young leaves from shoots used in P00550001. The leaves were stored in water in the fridge for one week.	0.5X MS

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species	Treatment	Explant	Explant Source/Notes	Media
	P02040001	Bud	Young shoots.	0.5X MS
	P02040002 through P02040003	Leaf	Young shoot.	0.5X MS, .5 BA, .5 2,4-D
	P02040004	Leaf	Small bit of petiole attached.	0.5X MS, .5 BA, .5 2,4-D
	P02040005 through P02050009	Petiole	Young leaves.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.1 or 0.5 mg/L
	P02040010	Leaf	P02040001.	0.5X MS, .5 BA, .1 2,4-D
	P02510001 through P02510004	Bud		0.5X MS or 0.5X MS with BA at 0.5, 1.0 or 2.0 mg/L
	P02510005 through P02510017	Leaf segment		0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5, or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P05870001	Bud	Apical bud (remainder of cutting placed in GH).	0.5X MS
	P07120001	Immature seed	Green fruit. The very first one harvested from the only blooming plant.	0.5X MS with charcoal
	P07520001	Seed	Very immature fruit.	0.5X MS
Cyanea platyphylla				
	P02790001through P02790067	Leaf	Mature leaf.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P05980001	Immature seed	Very moldy unripe fruit.	0.5X MS
	P06090001	Immature seed	Green fruit	0.5X MS
Cyanea recta				
	P02090001	Immature seed	Dry but immature fruit that was slightly moldy.	0.5X MS
Cyanea remyi				
	P00590001	Seed	Seeds still embedded in fruit with pericarp removed.	VW with sugar
	P00590002	Seed	Seeds removed from mature fruit.	VW with sugar
	P04110001	Seed	Still attached to inner fruit.	0.5X MS
	P04110002	Immature seed	Unopened fruit. Very immature seeds.	0.5X MS

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
Cyanea shipmanii				
	P02890001	Seed	Mature fruit.	0.5X MS
	P02890002A, P02890004A, P02890026A, P02890059A, P02890063A, P02890065A, P02890065B, P02890075A, P02890076A, P02890077A, P02890079A, P02890080A, P02890087A, P02890091A, P02890091B, P02890093A	Leaf	P02890002.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P02890101	Seedlings	P02890001.	LS
	P04920001 through P04920013	Bud	Broken off mature branch.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
Cyanea sp. (either *acuminata* or *truncata*)				
	P00230001	Apical Bud	Branch broken from mother plant.	0.5X MS, 150 ml CW
	P00230002 through P00230004	Axilary bud	Side buds were dormant and there was no obvious bud scales.	0.5X MS, 150 ml CW
	P03200001	Bud		0.5X MS, .5 NAA
	P03200002	Leaf segment		0.5X MS, 1 BA, .5 NAA
Cyanea st.-johnii				
	P07320001	Bud	Young branch.	0.5X MS
Cyanea superba ssp. *superba*				
	P02260001	Seed	Actually look like aborted seeds.	0.5X MS
	P02270001	Seed	Mature seed already removed from fruit.	0.5X MS
	P02580001	Seed	Received mature, cleaned and dried.	0.5X MS
	P03450001	Immature seed	Unripe fruit to nearly ripe fruit.	0.5X MS
	P03460001	Immature seed	Green and nearly-ripe fruit.	0.5X MS

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
	P03470001	Immature seed	Green and nearly ripe fruit.	0.5X MS
	P05060001	Mature seed	From storage.	0.5X MS
	P05070001	Mature seed	From storage.	0.5X MS
	P05580001	Seeds	Ripe and mushy fruit that was collected off the ground and full of maggots and junk.	0.5X MS
	P05590001	Immature seed	Green, almost ripe fruit.	0.5X MS
Delissea rhytidosperma				
	P01780001	Seed	From mature fruit.	0.5X MS
	P01780001A through P01780011A	Leaf	*In vitro* seedling.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P06560001 through P06560013	Leaf segment	Relatively young leaf.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P07700001	Immature seeds	Mostly immature fruit.	0.5X MS
Delissea rivularis				
	P01000001	Immature seed	Unripe fruit.	VW with sugar
	P01000002	Apical bud	Field-collected shoot.	0.5X MS
	P02030001	Seed/Placenta	Immature seed.	0.5X MS
	P02070001 through P02070004	Petiole	Mature leaves.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination wihth 2,4-D at 0.1 or 0.5 mg/L
	P02240001	Immature and mature seed	Fruit of various sizes.	0.5X MS
	P03760001	Seed	Mature fruit.	0.5X MS
	P03760001A through P03760001H	Leaf segment	*In vitro* seedling.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L or in 0.5X MS with 1.0 mg/L NAA
	P03770001	Seed	Mature fruit.	0.5X MS
	P03780001	Seed	Mature fruit.	0.5X MS
Delissea subcordata				
	P00380001	Seed	Seeds very small.	VW with sugar

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
	P00380002, P00380006, P00380010, P00380014, P00380018, P00380022, P00380026, P00380030, P00380034, P00380038, P00380042, P00380046	Root	*In vitro* seedling.	0.5X MS or 0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P00380003, P0038007, P00380011, P00380015, P00380019, P00380023, P00380027, P00380031, P00380035, P00380039, P00380043, P00380047.	Cotyledon	*In vitro* seedling.	0.5X MS or 0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P00380004, P00380008, P00380012, P00380016, P00380020, P00380024, P00380028, P00380032, P00380036, P00380040, P00380044, P00380048	Leaf	*In vitro* seedlings.	0.5X MS or 0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P00380005, P00380009, P00380013, P00380017, P00380021, P00380025, P00380029, P00380033, P00380037, P00380041, P00380045, P00380049.	Hypocotyl	*In vitro* seedlings.	0.5X MS or 0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
	P00380050 through P00380063, P00380069 through P00380091, P00380093 through P00380096	Shoot	*In vitro* seedings.	0.125X MS; 0.25X MS; 0.5X MS; 0.5MS with CW at 150 ml/L; 0.5X MS with 2,4-D at 0.05, 0.5, or 1.0 mg/L; 0.5X MS with BA at 1.0 mg/L in combination with 2,4-D at 0.05, 0.5 or 1.0 mg/L; VW with sugar; or VW with no CW
	P00380055A, P00380064 through P00380068, P00380092	Callus	I*n vitro* seedling.	0.125X MS, 0.25X MS, or 0.5X MS
	P00380058A, P00380059A, P00380060A, P00380061B, P00380063A, P00380080A, P00380083A, P00380093A, P00380094A, P00380095A.	Leaf	*In vitro* seedling.	0.5X MS with either BA at 0.5 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L or a combination of BA at 0.5 or 1.0 mg/L with NAA at 0.05, 0.5, or 1.0 mg/L
	P01010001	Apical bud	Greenhouse-grown seedling.	MS
	P01010002	Node	Upper stem of a greenhouse-grown seedling.	MS
	P01400001 through P01400004	Petiole	Whole leaf.	0.5X MS with BA at 0.5 mg/L and 2,4-D at 1.0 mg/L
	P01400005 through P01400006	Leaf blade	Whole leaf, blade segment with main vein.	0.5X MS with BA at 0.5 mg/L and 2,4-D at 1.0 mg/L
Delissea undulata ssp. *undulata*				
	P01630001	Immature seed	Immature fruit (blue/purple on top and green on bottom that were received intact).	0.5X MS
	P01720001	Immature seed	Immature fruit.	0.5X MS

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
	P01720008A throeugh P01720008Q, P01770028A through P01770033A, P01830013A through P01830015B, P01830019A through P01830019D, P01830036A through P01830037C, P02020006A through P02020006E, P02140003A through P02140003C, P02140006A through 11B, P02140014A through P02140015B, P02140019A and B, P02140022A and B, P02140022 A and B, P02140025A through P02140025D	Leaf	*In vitro* seedling.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
	P01770001	Immature seed	Immature fruit, some were removed from the tree and some were picked up from the ground. These collections were not kept separate.	0.5X MS
	P01830001	Seed	Immature fruit.	0.5X MS
	P01830002	Seed	Immature fruit.	VW with sugar
	P02020001	Seed	Mature seed.	0.5X MS
	P02140001	Seed	Ripe fruit.	0.5X MS
Lobelia gaudichaudii ssp. *koolauensis*				
	P01480001	Leaf	Plant in LA greenhouse under mist. Plant is sometimes wilted.	0.5X MS

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species	Treatment	Explant	Explant Source/Notes	Media
	P01480002 though P01480006	Leaf segment	P01480001.	0.5X MS with CW at 150 ml/L, 0.5X MS with BA at 0.5 mg/L alone or with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.5 or 1.0 mg/L
	P01980001	Seed	Received dry and cleaned from fruit.	0.5X MS
Lobelia hillebrandii				
	P02010001	Seed	Received dry and cleaned from fruit.	0.5X MS
Lobelia hypoleuca				
	P00650001	Immature seed	Immature fruits.	VW with sugar
	P00650004AA through P00650004AC, P00650004BA through P00650004CB, P00650004FA through P00650004GA, P00650005BA through P00650005BC	Leaf	*In vitro* seedling.	0.5X MS with BA at 0.5 or 1.0 mg/L in combination with 2,4-D at 0.05, 0.1, 0.5 or 1.0 mg/L or NAA at 0.05, 0.5 or 1.0 mg/L
Lobelia monostachya				
	P04460007	Immature seed	Unopened green fruit with some flower parts still attached. Seeds are very very small and may not be pollinated (fertilized) at all.	0.5X MS
	P07270001	Immature seed	Green fruit. Only one of seven fruit had any seeds and it was only 1/4 full.	0.5X MS
Lobelia niihauensis				
	P05130001	Seed	Dried fruit.	0.5X MS
	P05140001 through P05140005	Bud	Apical bud of side branch.	0.5X MS with BA at 1.0 mg/L in combination with 2,4-D at 0.05, 0.5, or 1.0 mg/L or NAA at 0.05 or 0.5 mg/L
Lobelia oahuensis				
	P01710001	Seed	Mature (recently) fruit.	0.5X MS
	P04960001	Immature seed	Rotting fruit.	0.5X MS
Lobelia yuccoides				

Table A.1 (Continued) *In vitro* treatments used on Hawaiian Campanulaceae

Species				
	Treatment	**Explant**	**Explant Source/Notes**	**Media**
	P01120001	Apical Bud	Axillary branches. Has a couple of nodes attached.	0.5X MS
	P01120002 through P01120003	Node	Axilary branches.	0.5X MS or 0.5X MS made with 0.32 ml/L hydrogen peroxide
	P01120004	Leaf	P01120001.	0.5X MS with BA at 0.5 mg/L and 2,4-D at 0.1 mg/L
Trematolobelia kauaiensis				
	P02310001	Seed	Nearly mature fruit.	0.5X MS
Trematolobelia macrostachys				
	P02160001	Seed	Received dry and cleaned from fruit already.	0.5X MS
	P06620001	Immature seed	Green fruit.	0.5X MS

Table A.2. Hawaiian Campanulaceae Evaluated for *in vitro* Propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Brighamia insignis							
	P0060	11/5/91	Perlman	Yes	Kaua'i	Green fruit	SP #12351. Kaua'i, Mt. Hā'upu gap area between Kīpū Kai and Māhā'ulepū, southwest side of Hā'upu Peak. Two plants on steep slope in mesic area.
	P0187	10/14/92	Perlman	Yes	Kaua'i	Green fruit	Collected on Mt. Hā'upu, gap up from Māhā'ulepū. Immature fruit.
Clermontia arborescens ssp. *arborescens*							
	P0748	9/18/95	Palmer	Yes	Maui	Green fruit	Information not available.
Clermontia drepanomorpha							
	P0383	2/15/94	Corn	Yes	Hawai'i	Green fruit	Wild-collected upper Hāmākua Ditch Trail, Hawai'i. One plant observed at 3900 foot elevetation. Three-fourths way to upper intake.
	P0717	7/27/95	Perlman	Yes	Hawai'i	Green fruit	Kohala Mts, upper Hāmākua Ditch trail between Alakahi and Kawai Nui Gulches on north side of ditch, 20 feet off trail, about 3/4 way to Kawai Nui Gulch. One plant, 4 ft tall with immature and mature fruit, no flowers. Gentle slope, scattered closure wet area. Associated with *Metrosideros-Cheirodendron-Cibotium*, montane wet forest, *Ilex*, *Coprosma*, *Myrsine*, *Diplazium sandwichianum*, *Vaccinium*, *Rubus hawaiiensis*, *Astelia menziesii*, *Carex alligata*, *Dicranopteris linearis*. Various weeds. Threatened by weeds, pigs, rats and slugs.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
Clermontia fauriei							
	P0179	9/24/92	Palmer	Yes	Kaua'i	Cuttings/fruit	Wild-collected 2nd wet bog - Alaka'i Swamp Trail (AST) - 5-5-92 - three cuttings plus fruit (Kaua'i)
Clermontia grandiflora							
	P0156	7/15/92	Mehrhoff	Yes	Moloka'i	Cuttings	Collected on Moloka'i. No other information at this time.
	P0747	9/18/95	Palmer	Yes	Maui	Ripe fruit	Information not available.
Clermontia kakeana							
	P0048	9/29/91	Koob	Yes	O'ahu	Ripe fruit	Collected on Mānoa Cliffs Trail, O'ahu from side of main Roundtop Drive side.
Clermontia kakeana x *Clermontia arborescens*							
	P0641	4/3/95	Palmer	Yes	Maui	Green fruit	1000 meters elevation — lower Waikamoi Flume Road East Maui
	P0687	6/27/95	Palmer	Yes	Maui	Green fruit	Upstream from old pumphouse on lower flume road in Waikamoi
Clermontia lindseyana							
	P0249	3/23/93	Perlman	Yes	Kaua'i	Green fruit	Kaua'i, Wahiawa Mountains, north of Houla, gulch below Hanapēpē Valley rim. Three trees observed, one has fallen down, other 2 have immature fruit. At 701 meters on steep slope in scattered closure in wet area.
	P0288	8/19/93	Jeffrey	Yes	Hawai'i	Ripe fruit	Collected 8-18-93 at Hakalau Forest National Wildlife Refuge, Hawai'i, Hawai'i at 6000 ft. elev. as edge of unnamed gulch directly below Magnetic Hill (Pua 'Ākala Quad).

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
Clermontia montis-loa							
	P0199	10/22/92	Palmer	Yes	Hawai'i	Ripe fruit	Collected on Big Island.
Clermontia peleana							
	P0169	9/1/92	Mehrhoff	Yes	Hawai'i	Green/Ripe fruit	Collected on Big Island near Wailuku River. One ripe fruit and four immature fruit. Only one population is known to exist.
	P0170	9/1/92	Mehrhoff	Yes	Hawai'i	Cuttings	Collected on Big Island near Wailuku River. Four cuttings received but I only kept the apical portion of two (green wood) and gave the rest to Karen Shigematsu.
Clermontia pyrularia							
	P0256	4/12/93	Mehrhoff	Yes	Hawai'i	Seeds	Collected by Jack Jeffery and originally given to Amy B.H. Greenwell Ethnobotanical Garden (received by them 8/31/92). Fruit were received very fermented by ABHGEG but were cleaned and some planted and remainder stored at 4°C. Cleaned seeds were received from Loyal Mehrhoff after 8 months in storage.
	P0257	4/12/93	Mehrhoff	Yes	Hawai'i	Seeds	Received from NTBG/HPCC (accession number 915468) by ABHGEG on 8/24/92 from wild collected source.
	P0317	10/6/93	Jeffrey	Yes	Hawai'i	Ripe fruit	Wild-collected as mature fruit on 9/28/93. On Big Island, Pīhā. Just off Keanakolu Road.
	P0318	10/6/93	Jeffrey	Yes	Hawai'i	Ripe fruit	Wild-collected as mature fruit on 9/28/93 on Big Island, Pīhā. Just off Keanakolu road.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species						
Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
P0370	1/27/94	Van Dyke	Yes	Hawai'i	Ripe fruit	Same as P0317 and P0318 but in storage in refrigerator at ABHGEG since September 1993. Wild collected as mature fruit on 9/28/93. On Big Island,Pīhā. Just off Keanakolu Road.
P0474	8/30/94	Bergfeld	Yes	Hawai'i	Green fruit	Pīhā Forest Reserve at 6200 foot elevation.
Clermontia tuberculata						
P0282	8/4/93	Palmer	Yes	Maui	Green fruit	Collected 8/2/93 at 5400' elevation along Carother's Trail, The Nature Conservancy of Hawai'i Waikamoi Preserve, East Maui. Immature fruit.
P0688	6/27/95	Palmer	Yes	Maui	Green fruit	Near end of upper Waikamoi Flume Road.
Cyanea aculeatiflora						
P0387	2/26/94	Mehrhoff	Yes	Maui	Green fruit	Wild-collected from Maui.
P0586	1/25/95	Palmer	Yes	Maui	Ripe fruit	Flume trail
Cyanea acuminata						
P0066	11/13/91	Obata	Yes	O'ahu	Ripe fruit	O'ahu, Makaua Gulch (Hidden Valley) Gully floor 1550' under canopy of *Clidemia hirta*.
P0210	11/12/92	Mehrhoff	Yes	O'ahu	Ripe fruit	Collected by John Obata 11/11/92/ in Hidden Valley, O'ahu.
P0211	11/12/92	Mehrhoff	Yes	O'ahu	Ripe fruit	Hidden Valley, O'ahu.
Cyanea angustifolia						
P0049	9/29/91	General	Yes	O'ahu	Cuttings	Collected from Mānoa Cliffs Trail, O'ahu, from side of trail.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
	P0585	1/31/95	Hiraoka	Yes	O'ahu	Ripe fruit	From Wai'anae Kai Contour trail
	P0731	9/6/95	Obata	Yes	O'ahu	Green fruit	Ko'olau Range, HI. Hawai`i Loa Ridge Trail. Into the native forest and on the final steep climb crest, about 300 meters to trail summit. Off trail, on the east-facing slope, one of two plants known of this species in extant. About 600 meters (2000ft). Growing through uluhe (*Driconopteris*). Just beginning to fruit. Ron Fenstemacher also present.
Cyanea asarifolia							
	P0058	11/5/91	Perlman	Yes	Kaua'i	Green fruit	Kaua'i, Blue hole at base of Mt. Wai'ale'ale, head of north fork of Wailua River. About 15 plants, west-facing vertical slope, open conditions, very wet. About 15 plants, flowers and fruit present but mostly immature.
	P0206	11/2/92	Mehrhoff	Yes	Kaua'i	Leaves	From Blue Hole region of Kaua'i. Last remaining plants are being eaten and killed by slugs.
Cyanea asplenifolia							
	P0749	9/18/95	Palmer	Yes	Maui	Cuttings	Information not available

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
Cyanea copelandii ssp. *haleakalaensis*							
	P0386	2/26/94	Mehrhoff	Yes	Maui	Green fruit	Maui - Waikamoi Flume area on February 22, 1994 with Bob Hobdy. The only population Bob Hobdy knows about is along a steep gully below the Lower Flume. There is a population of about 25 mature plants. Plants are scandents and it is difficult to count numbers. There were some small juvenile plants. This area is heavily damaged by pig. Almost all plants are in areas difficult for pigs to access. Blackberry is invading parts of drainage. Some plants were damaged by slumping hillsides. Associated species include: *Cybotium* sp. *Cyanea maceldownei*, *Clermontia kakeana*, *Clermontia arborescens*, *Stenogyne kamehameha* and *Broussasia* sp. Three plants had flowers, immature fruits and mature fruits. Most others were either sterile or had immature fruits. Plants produce relatively few flowers but seem to have high fruit set. Several mature fruit were taken for propagation at Lyon. [Actually fruit were slightly immature and seeds only lightly colored.]
Cyanea crispa							
	P0024	8/2/91	Obata	Yes	O'ahu	Cutting	Plants rooted up by pigs in Hidden Valley, O'ahu. Majority of plants put in greenhouse mist bed but some parts kept for tissue culture experiments. Only two populations known with about 30 individuals. Immature plants, never seen flowering.
	P0091	2/14/92	Mehrhoff	Yes	O'ahu	Cuttings	Plants rooted up by pigs in Hidden Valley, O'ahu. Majority of plants put in greenhouse mist bed but some parts kept for tissue culture experiments. Only two populations known with about 30 individuals. Immature plants, never seen flowering.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
	P0102	4/4/92	General	No	n/a	Cuttings	From greenhouse-grown cutting. Side shoot that emerged while cutting was under the mist.
	P0150	7/15/92	Mehrhoff	Yes	O'ahu	Ripe fruit	Collected 6/24/92 in Pia Valley, O'ahu from one of two populations. Loyal has this collection numbered 3/3.
	P0152	7/15/92	Mehrhoff	Yes	O'ahu	Ripe fruit	Collected on O'ahu in Pia Valley on 6/24/92. Loyal has this collection numbered 1/3, open.
	P0153	7/15/92	Mehrhoff	Yes	O'ahu	Ripe fruit	Collected on 6/24/92 from Pālolo Valley. Ripe fruit picked up from the ground. Loyal didn't have this one numbered but had the word "ground" on the package.
	P0212	11/12/92	Mehrhoff	Yes	O'ahu	Cuttings	Information not available.
	P0254	4/10/93	General	No	n/a	Cuttings	From greenhouse-grown cutting from main Lyon Arboretum greenhouse but kept in laboratory for two weeks before explants were removed.
	P0303	9/14/93	Mehrhoff	Yes	O'ahu	Green fruit	Collected by Ron Fenstemacher on O'ahu at Hidden Valley on 9/11/93 as green fruit.
	P0393	3/15/94	Obata	Yes	O'ahu	Cuttings	Wild-collected Wailupe, Laulaupoe Gulch, off gully floor at 1400' level, under a canopy of *Aleurites*, *Pipturus* and *Psidium*. Collected 3/13/94.
Cyanea degeneriana							
	P0200	10/22/92	Palmer	Yes	Hawai'i	Seeds	Collected on Big Island.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Cyanea dunbarii							
	P0349	12/9/93	Lau	Yes	Moloka'i	Ripe fruit	Collected on Moloka'i, Mokomoko Gulch, 2200 ft elevation, about 10-12 plants w/ flowers and fruit. Collected by Steve Perlman with Ken Wood and Joel Lau on 12/6/93 in *Metrosideros* lowland wet forest.
	P0674	6/17/95	Perlman	Yes	Moloka'i	Cuttings	Mokomoko gulch, 2280 foot elevation in soil, sun, and shade in a population of about 25 + plants.
Cyanea glabra							
	P0584	1/21/95	Ragone	Yes	Maui	Green fruit	Twelve plants in total in three groups. With flowers and fruit. West Maui, Kaua'ula Valley, 2640 ft elevation. Soil and rock and streambanks. Bob Hobdy and Ken Wood also present. Associated with *Metrosideros polymorpha*, lowland wet forest, *Xylosma*, *Dodonaea*, *Psychotria*, *Pipturus*, *Touchardia*, *Boehmaria*, *Clermontia kakeana*, *Cyanea elliptica*, *Perrottetia*, *Coprosma*, *Cibotium*, *Dubautia plantaginea*, *Cheirodendron*, *Thelypteris cyathioides*, *Diplazium*, *Sadleria*. North-facing and south-facing and moderate slope; scattered closure. Weeds present include *Coffea arabica*, *Tibouchina herbacea*, *Rubus rosifolius*.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species						
Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Cyanea grimesiana ssp. *grimesiana*						
P0380	2/8/94	Perlman	Yes	Lana'i	Ripe fruit	Collected 2/3/94 on Lana'i, Puhielelu 2900 feet with Bill Garnett. Only two plants known on Lana'i. Wild-collected Lana'i, Lana'i Summit Area, Puhielelu, gulch to south of Puhielelu Ridge Trail; 2 plants with fruit. 884 m alt. North-facing steep slope in scattered closure in wet area. Associated with *Pouteria sandwicensis, Pisonia, Cyrtandra, Metrosideros, Dicranopteris, Diplazium sandwichianum, Freycinetia, Viola lanaiensis, Myrsine, Rubus rosifolius, Myrica faya.*
P0555	12/27/94	Perlman	Yes	O'ahu	Ripe fruit	Kulu'ī Gulch, 1400 feet. Randy Kenedy also present.
Cyanea grimesiana ssp. *obatae*						
P0061	11/5/91	Perlman	Yes	O'ahu	Ripe fruit	O'ahu. Wai'anae Mountains, S. Kaluhā Gulch, main west fork, left (South) wall, just above stream bed, above fire break trail. Mature (1) 4' high with fruit, seedlings (6), 1-2 feet high, with no flowers. Last mature plant known from population of 40 plants, 10 years ago.
P0074	12/9/91	General	No	n/a	Cuttings	Collected from seedling grown in greenhouse at Lyon Arboretum.
P0087	2/5/92	General	No	n/a	Leaf	Collected from seed grown plant in main Lyon Arboretum greenhouse.
P0088	2/10/92	General	No	n/a	Leaf	Collected from seedling in main Lyon Arboretum greenhouse.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
	P0295	9/2/93	Obata	Yes	O'ahu	Green fruit	Collect 9/1/93 on O'ahu,Wai'anae Range, HI, south fork of Kaluhā Gulch, at 580 m (1900 ft) level; growing along a steep rock embankment, shaded and moist associated with few low growing natives and cryptograms and overshaded by a lot of *Clidemia*. Other collectors with John were Bill Garnett, Pat Conant and Julie Ishiki.
	P0296	9/2/93	Obata	Yes	O'ahu	Ripe fruit	Collect 9/1/93 on O'ahu,Wai'anae Range, HI, south fork of Kaluhā Gulch, at 580 m (1900 ft) level; growing along a steep rock embankment, shaded and moist associated with few low growing natives and cryptograms and overshaded by a lot of *Clidemia*. Other collectors with John were Bill Garnett, Pat Conant and Julie Ishiki.
	P0339	11/24/93	Obata	Yes	O'ahu	Green fruit	Collected 11/23/93 on O'ahu,Wai'anae Range. 'Ēkahanui Gulch, off ridge trail ascending Pu'u Kaua, about 150 m below ridge line at about 790 m (2600 ft.); along steep embankment, shaded by *Metrosideros*, *Pisonia*, etc.; seven matured plants observed; two in bud and in young fruit, possibly the rare variety *obatae*. Other collectors were Steve Perlman and Bill Garnett.
	P0590	2/7/95	Perlman	Yes	O'ahu	Green fruit	Kaluhā Gulch

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species						
Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Cyanea hamatiflora ssp. *carlsonii*						
P0069	11/27/91	Ragone	Yes	Hawai'i	Seed	Perlman # 12395. Hualālai, Honua'ula Forest Reserve, new fenced exclosure. Three plants, 8 to 10 feet high with old fruit and flowers. 167 m alt. on west-facing moderate slope in scattered closure in mesic area. Associated with *Acacia koa* montane mesic forest. *Ilex*, *Coprosma*, *Myoporum*, *Clermontia*, *Pipturus*, *Rubus*, *Dryopteris*, *Carex*, *Thelypteris*, *Phyllostegia*, *Cibotium*, *Cheirodendron*, *Hedyotis*, *Passiflora mollissima*, *Buddlea*, *Ehrharta*, *Ageratum riparia.* Seed were in storage at NTBG/HPCC.
P0070	11/27/91	Ragone	Yes	Hawai'i	Seed	Perlman # 12397. Other collection information as above.
P0071	11/27/91	Ragone	Yes	Hawai'i	Seed	Perlman # 12398. Other collection information as above.
P0529	12/6/94	Bergfeld	Yes	Hawai'i	Seed	Enclosure 1 Honua'ula Forest Reserve Plant A at 5660 feet. Plant died and mature fruit were picked up off the ground.
Cyanea hamatiflora ssp. *hamatiflora*						
P0595	2/21/95	Perlman	Yes	Maui	Fruit	Haipua'ena Gulch, 4250 foot elevation, about 10 plants, most fruit opened by birds or rats and have insect damage as well. Received from NTBG moldy and liquified fruit with only a few seeds.
Cyanea kolekoleensis						
P0184	10/14/92	Perlman	Yes	Kaua'i	Green fruit	Wild collected from Wahiawa Mountains north of Hōlua Gulch below Hanapēpē Valley rim. Population of three plants with flowers and immature fruit. East facing, steep slope with scattered closure forest in wet area. New species and should be added to the State's rare list. Fruit collected 10-7-92

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
	P0412	4/16/94	Mehrhoff	Yes	Kaua'i	Cuttings	Wild collected from Wahiawa Mountains north of Hōlua Gulch below Hanapēpē Valley rim. Population of three plants with flowers and immature fruit. East facing, steep slope with scattered closure forest in wet area. New species and should be added to the State's rare list.
Cyanea kuhihewa							
	P0485	9/30/94	Perlman	Yes	Kaua'i	Cuttings	Collected in back of Limahuli Preserve with Chipper Wichman. Only 7 plants in only known population and they aren't doing very well. I got three shoots and took the necessary explants and put three cuttings in the mist bed to use as stock plants if I need them.
Cyanea kunthiana							
	P0691	6/27/95	Palmer	Yes	Maui	Green fruit	Waikamoi Flume - 5th bridge
Cyanea lanceolata							
	P0281	8/2/93	Mehrhoff	Yes	O'ahu	Green fruit	Collected 7/31/93 by L. Mehrhoff and Ron Fenstermacher, Makaua Gulch (Hidden Valley), west fork about 100 m beyond fork. Under a canopy of *Hibiscus* and *Aleurites*. A seedling of the same population was placed under the mist in the greenhouse and has the same accession number.
	P0300	9/9/93	Obata	Yes	O'ahu	Green fruit	Wild-collected along Poamoho Stream, upper portion at the 1900 ft level; along river bank on September 5, 1993. Other collectors were Ron Fenstemacher and Dan Palmer.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
Cyanea leptostegia							
	P0180	9/24/92	Palmer	Yes	Kaua'i	Seed	Wild-collected 0.8 miles down Mākaha Ridge Road - north side-upslope - plant #2, approx. 100 m from road on edge of gulch - 8/4/92 - many fruit (very infested with mealy bugs).
Cyanea linearifolia							
	P0146	7/10/92	Ragone	Yes	Kaua'i	Seeds	Information not available. (Was considered to be extinct before being rediscovered on Kaua'i.)
Cyanea longiflora							
	P0155	7/15/92	Mehrhoff	Yes	O'ahu	Cuttings	Collected on July 8, 1992 at Pahole NARS, Wai'anae Mountains, O'ahu.
	P0264	5/25/93	Mehrhoff	No	n/a	Cuttings	From plantlet growing in greenhouse from original collection (see collection info for P0155). Plant brought into laboratory for several weeks to decrease number of contaminating spores and increase likelihood of successfull culture.
	P0286	8/13/93	Obata	Yes	O'ahu	Ripe fruit	Information not available.
	P0481	9/22/94	Reyes	Yes	O'ahu	Seed	Seeds still in rotting flesh of fruit. No collection information in packet.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Cyanea mannii							
	P0238	3/2/93	Ragone	Yes	Moloka'i	Green fruit	Collected on Moloka'i 2/23/93, Kawela Gulch, Kamakou Preserve near tunnel. Three plants with fruit. For DOFAW, TNC. At 1052 meter on SE steep slope in closed forest. Associated with *Metrosideros polymorpha* montane mesic forest; *Pouteria, Cheirendendron, Cibotium, Hedyotis, Broussaisia, Styphelia, Coprosma.* Received at laboratory 3/2/93 in poor condition. Fruit crushed and moldy. Only few seeds were salvageable.
Cyanea mceldowneyi							
	P0089	2/12/92	Ragone	Yes	Maui	Green fruit	Wild collected by Perlman (12525) on Maui, lower Waikamoi Flume Road, west side of road. Plants with immature fruit present, no flowers. About 15 plants total. Two groups, about 50 feet apart. Altitude of 1303 m, moderate north-facing slope with scattered closure. Plants about 1-2.5 meters in wet area.
	P0090	2/12/92	Ragone	Yes	Maui	Green fruit	Wild collected by Perlman (12527) on Maui, lower Waikamoi Flume Road, Flume Trail in gulch about 7.5 miles from old house at trail head, in stream bed near delapidated flume. Five plants observed. Immature fruit present. Altitude of 1071 meters on a north-facing moderate slope with scattered closure in wet soil.
	P0364	1/11/94	Palmer	Yes	Maui	Green fruit	Maui, Lower Waikamoi Flume Road about 3900 feet, 0.3 mi below 2nd bridge. Collected 1/8/94

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species						
Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Cyanea membranacea						
P0062	11/5/91	Perlman	Yes	O'ahu	Cuttings	Wild-collected on Wai'anae Mountains, central Kaluhā Gulch near *Joinvillea* subgulch. Scattered about 20-30 plants with fruit, shrubs 5-12 ft. high. In an natural community of alien vegetation in *Aleurites moluccana*-lowland mesic forest.
P0297	9/2/93	Obata	Yes	O'ahu	Green and Ripe Fruit	Collected 9/1/93 on O'ahu, Wainae Range, HI, south fork of Kaluhā Gulch, at 580 m (1900 ft) level; growing along a steep rock embankment, shaded and moist associated with few low growing natives and cryptograms and overshaded by a lot of *Clidemia*. Other collectors with John were Bill Garnett, Pat Conant and Julie Ishiki.
Cyanea oahuensis (sic)						
P0498	10/21/94	Kennedy	Yes	O'ahu	Green fruit	Waimānalo. Received via Julie Reyes. There is no listing of *Cyanea oahuensis* in the *Manual of Flowering Plants of Hawai`i.* Probably misidentified.
Cyanea pinnatifida						
P0055	11/5/91	Perlman	Yes	O'ahu	Cuttings	Collected on The Nature Conservancy land. Last remaining plant in the wild. Never has flowered and is threatened by a rock. O'ahu, North Kaluhā Gulch.
P0204	11/2/92	Bornhorst	Yes	O'ahu	Cuttings	From last remaining plant on Nature Conservancy Land, Kunia area, O'ahu.
P0251	3/25/93	General	No	n/a	Cuttings	From cutting now growing in main Lyon Arboretum main greenhouse.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
	P0587	2/7/95	Perlman	Yes	O'ahu	Cuttings	Kaluhā Gulch
	P0712	7/20/95	General	No	n/a	Green fruit	From plant growing in main Lyon Arboretum greenhouse
	P0722	8/19/95	General	No	n/a	Green fruit	From plant growing in Lyon Arboretum greenhouse. First known seeding in recent times. No seeds found in the fruit.
	P0752	9/19/95	General	No	n/a	Green fruit	From plant growing in main Lyon Arboretum greenhouse.
Cyanea platyphylla							
	P0279	7/17/93	Bergfeld	Yes	Hawai'i	Leaf	Collected 7/16/93 on Big Island and shipped next-day by air to DOFAW. Hand delivered by Caroline Corn to Lyon Arboretum.
	P0598	2/28/95	Brodie	Yes	Hawai'i	Moldy fruit	Laupāhoehoe at 2440 feet.
	P0609	3/10/95	Perlman	Yes	Hawai'i	Ripe fruit	Laupāhoehoe NAR between Blair Road and Kīlau Stream, about 3/4 mile from Blair Road, in fenced exclosure. 2740 feet, soil substrate, seven plant, two juvenile and one seedling, few fruits, no flowers, north facing moderate slope, wet area. Dying to feeble population.
Cyanea recta							
	P0209	11/9/92	Mehrhoff	Yes	Kaua'i	Green fruit	Fruit collected 11/4/92 by Steve Perlman on Kaua'i.
Cyanea remyi							
	P0059	11/5/91	Perlman	Yes	Kaua'i	Green fruit	Kaua'i, Blue Hole at base of Mt. Wai'ale'ale, head of north fork of Wailua River. Several hundred plants on east-facing moderate slope in wet conditions.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species						
Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
P0411	4/16/94	Mehrhoff	Yes	Kaua'i	Green fruit	Information not available.
Cyanea shipmanii						
P0289	8/19/93	Jeffrey	Yes	Hawai'i	Ripe fruit	Collected 8-18-93 at Hakalau Forest National Wildlife Refuge, Hawai'i, Hawai'i at 6000 ft. elev. at edge of unnamed gulch directly below Magnetic Hill (Pua 'Ākala Quad).
P0492	10/13/94	Harada	Yes	Hawai'i	Cuttings	Eastern slope of Mauna Loa. 5860 feet about 2.3 miles south of Power Line Road. Tree growing in small ravine. Shaded situation protected from wind. Multibranched plant >2 meters tall. Plant in area easily reached by ungulates and the branches received were probably broken off by mouflon.
Cyanea sp. (either *acuminata* or *truncata*)						
P0023	8/2/91	Obata	No	n/a	Cutting	Information not available.
P0320	9/26/93	Obata	Yes	O'ahu	Ripe fruit	Wild-collected on O'ahu, Ko'olau Range. Along summit trail, about 200 m (600 ft) west of Poamoho trail summit, along upper side of trail; growing mostly under low growing natives and most commonly under *Clidemia*; along a wet, heavily wind-blown slope; about 25 plants noted; one typical type of *Cyanea acuminata* note; young buds noted on mature plants. 800 m (2600 ft). Other collectors were Ron Fenstemacher and Kawehi Ryder.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
Cyanea st.-johnii							
	P0732	9/6/95	Obata	Yes	O'ahu	Cutting	Ko'olau Range. About 200 meters west of Hawai'i Loa Ridge Trail summit, slightly below crest on windward face. Growing around a canopy of *Clidemia*; habitat wet and under heavy wind constantly. Ron Fenstemacher, Amy Tsuneyoshi, Malikarjuna Aradhya and Bert Lum also present on hike.
Cyanea superba ssp. *superba*							
	P0226	1/14/93	Mehrhoff	Yes	O'ahu	Ripe fruit	Collected 12/30/92 at Pahole NARS, Makua Valley, O'ahu. Plant #2. Received three mature fruit but when opened only aborted seeds were found. Had been in refrigerator storage until received.
	P0227	1/14/93	Mehrhoff	Yes	O'ahu	Seeds	Collected 12/30/92 at Pahole NARS, Makua Valley, O'ahu. Plant # 1. Received eight seeds. Have been in storage in refrigerator from time of collection until received.
	P0258	4/12/93	Mehrhoff	Yes	O'ahu	Seeds	Plant number 3.
	P0345	12/6/93	Kennedy	Yes	O'ahu	Green fruit	Collected on O'ahu from Mākua exclosure plants.
	P0346	12/9/93	Obata	Yes	O'ahu	Green fruit	Mākua population, O'ahu. Only five plants in population. O'ahu, Mākua exclosure. Mākua Valley, Kahana Iki, fenced 5 plants in exclosure with fruits and one plant in exclosure. Six plants total, fruit present. 579 m alt. West-facing steep slope in scattered closure, mesic forest. Associated with *Pisonia-Charpentiera* lowland mesic forest with *Freycinetia*, *Canthium*, *Xylosma*, *Psychotria*, *Cyrtandra*, *Hedyotis terminalis*, *Delissea subcordata*, *Psidium cattleianum*. Possibly threatened by weeds or old age?

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species						
Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
P0347	12/9/93	Obata	Yes	O'ahu	Green fruit	From Pahole population, O'ahu. Only two plants in population. Fenced exclosure, only 2 plants left, one plant flowered and fruited. On 8/21/91 there were 6 plants living. 579 m alt. North-facing steep slope in closed forest mesic. Associated with *Pisonia-Charpentiera* lowland mesic forest with *Freycinetia, Canthium, Xylosma, Cyrtandra, Hedyotis terminalis, Delissea subcordata, Psidium cattleianum*. Threats may be weeds or old age?
P0506	11/5/94	Ragone	Yes	O'ahu	Seed	From Pahole population, O'ahu. Only two plants in population. Fenced exclosure, only 2 plants left, one plant flowered and fruited. On 8/21/91 there were 6 plants living. 579 m alt. North-facing steep slope in closed forest mesic. Associated with *Pisonia-Charpentiera* lowland mesic forest with *Freycinetia, Canthium, Xylosma, Cyrtandra, Hedyotis terminalis, Delissea subcordata, Psidium cattleianum*. Threats may be weeds or old age? These were in storage at HPCC/NTBG since 1/1/94.
P0507	11/5/94	Ragone	Yes	O'ahu	Seed	Mākua population, O'ahu. Only five plants in population. O'ahu, Mākua exclosure. Mākua Valley, Kahana Iki, fenced 5 plants in exclosure with fruits and one plant in exclosure. Six plants total, fruit present. 579 m alt. West-facing steep slope in scattered closure, mesic forest. Associated with *Pisonia-Charpentiera* lowland mesic forest with *Freycinetia, Canthium, Xylosma, Psychotria, Cyrtandra, Hedyotis terminalis, Delissea subcordata, Psidium cattleianum*. Possibly threatened by weeds or old age? These seeds were in storage at HPCC/NTBG since 1/1/94.
P0558	12/29/94	Reyes	Yes	O'ahu	Ripe fruit	From DOFAW land collected from the ground around the plant.
P0559	12/29/94	Reyes	Yes	O'ahu	Ripe fruit	From DOFAW land collected from the ground around the plant.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species	Plant #	Date Coll.	Collector	Wild?	Island	Plant Part(s)	Collection Information
Delissea rhytidosperma							
	P0129	3/20/92	Palmer	No	n/a	Ripe fruit	Fruit from cultivated plant. Has been in cold storage for some time.
	P0178	9/25/92	General	No	n/a	Ripe fruit	From mature fruiting plant growing at Lyon Arboretum main greenhouse holding area. Rick Palmer's plant (label says it's the fourth generation plant from a Waimea arboretum collection). Originates from Waimea Arboretum collection # 77s646
	P0656	5/9/95	Anon.	Yes	Kaua'i	Leaves	From possibly last plant in wild on Kaua'i.
	P0770	9/21/95	Anon.	No	Lihue	Green fruit	Līhu'e DLNR baseyard. Labeled as *Delissea rhytidosperma* and probably correctly labelled according to anonymous donor.
Delissea rivularis							
	P0100	4/3/92	Mehrhoff	Yes	Kaua'i	Green fruit	Collected on Kaua'i. The species was thought to be extinct and was last collected in 1916. Fruit are opened and stem is short (about 2 cm long)
	P0203	12/2/92	Mehrhoff	Yes	Kaua'i	Green fruit	Information not available.
	P0207	11/2/92	Mehrhoff	Yes	Kaua'i	Cutting	Information not available.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
	P0224	1/7/93	Perlman	Yes	Kaua'i	Green and ripe fruit	Wild-collected on Kaua'i — Upper Hanakoa Valley stream area, Honoonã pali NAR. About 20 plants, total population; alt of 1190 m, Steep-verticle slope, closed-scattered forest cover. *Metrosideros polymorpha-Cheirodendron trigynum* montane wet forest with *Sadleria cyathoides, Broussaisia, Carex alligata, Athyrium sandwichianum, Coprosma, Perottetia, Thelypteris sandwicensis, Machaerina angustifolia, Clermontia faureii, Dubautia knudsenii, Hedyotis terminalis, Cibotium, Urera glabra, Boehmaria, Pipturus, Dicranopteria* and *Astelia.*
	P0376	2/4/94	Mehrhoff	Yes	Kaua'i	Ripe fruit	Wild-collected on Kaua'i, Upper Hanakoa, 3540 feet, one plant on 1/31/94
	P0377	2/4/94	Mehrhoff	Yes	Kaua'i	Ripe fruit	Wild-collected Kaua'i, Upper Hanakoa Valley at 3460 feet. One plant. Collected 1/31/94. Hāmākua Valley stream area, Honoonāpali NAR. One shrub with flowers and fruit. Alt 1190 m closed scattered closure. Wet. Associated with *Metrosideros polymorpha-Cheirodendron trigynum* montane wet forest with *Sadleria cyathoides, Broussaisia, Carex alligata, Athyrium sandwichianum, Coprosma, Perrottetia, Thelypteris sandwicensis, Machaerina angustifolia, Clermontia faureii, Dubautia knudsenii, Hedyotis terminalis, Cibotium, Urera* and *Astelia.*

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
	P0378	2/4/94	Mehrhoff	Yes	Kaua'i	Ripe fruit	Wild-collected on Kaua'i at upper Hanako Valley at 3460 feet, 3 plants, on 1/31/94. Wild-collected Kaua'i, Upper Hanakoa Valley at 3460 feet. One plant. Collected 1/31/94. HanakoaValley stream area, Honoonāpali NAR. One shrub with flowers and fruit. Alt. 1190 m closed scattered closure. Wet. Associated with *Metrosideros polymorpha-Cheirodendron trigynum* montane wet forest with *Sadleria cyathoides, Broussaisia, Carex alligata, Athyrium sandwichianum, Coprosma, Perrottetia, Thelypteris sandwicensis, Machaerina angustifolia, Clermontia faureii, Dubautia knudsenii, Hedyotis terminalis, Cibotium, Urera* and *Astelia.*
Delissea subcordata							
	P0038	8/30/91	Ragone	Yes	O'ahu	Seeds	Collected by Steve Perlman on O'ahu: Pahole Gulch, near *Cyanea superba* fenced enclosure. Ten plants observed, 3 to 8 feet high with fruit on one. Seeds received in good condition in dry envelope.
	P0101	4/4/92	General	No	n/a	Cutting	From greenhouse grown seedling that was germinated *in vitro.*
	P0140	5/30/92	General	No	n/a	Leaf	Collected from mature (recently flowering) plant growing outside of the main Lyon Arboretum greenhouse.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species						
Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Delissea undulata ssp. *undulata*						
P0163	8/14/92	Giffin	Yes	Hawai'i	Green fruit	Collected from only existing rediscovered plant that was growing on the wall of a collapsed lava tube in a mamane-ohia woodland on the northern slopes of Hualālai near Pu'uwa'awa'a Wildlife Sanctuary at 3,520 feet. The plant was severely damaged by wind or possibly grazing ungulates prior to its discovery. It was propped up and started to flower several months later.
P0172	9/1/92	Mehrhoff	Yes	Hawai'i	Green fruit	Collection data as above.
P0177	9/24/92	Giffin	Yes	Hawai'i	Green fruit	Collection data as above.
P0183	10/5/92	Giffin	Yes	Hawai'i	Green fruit	Six fruit collected from tree on 10/2/92 but only four had seed inside. Collection data as above.
P0202	11/2/92	Giffin	Yes	Hawai'i	Green fruit	Collection data as above
P0214	11/18/92	Giffin	Yes	Hawai'i	Ripe fruit	Harvested 11/10/92 from Collection data as above. Collected by J. Giffin and R. Covington.
Lobelia gaudichaudii ssp. *koolauensis*						
P0148	7/15/92	Mehrhoff	No	O'ahu	Leaf	From plant in Lyon Arboretum greenhouse that was collected after a pig uprooted it in the Ko'olau Mountains.
P0198	10/21/92	Mehrhoff	Yes	O'ahu	Green fruit	Fruit received dried but intact. Was collected slightly immature.
Lobelia hillebrandii						
P0201	10/22/92	Palmer	Yes	Maui	Ribe fruit	Collected at Līhau, Maui.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
Lobelia hypoleuca							
	P0065	11/13/91	Obata	Yes	O'ahu	Green fruit	O'ahu, Moanalua-Kāne'ohe crest ridge, in open, heavily windbown terrain, heavy moss cover.
Lobelia monostachya							
	P0446	7/25/94	Obata	Yes	O'ahu	Green fruit	Ko'olau Range. Wailupe ('Āina-Haina), along slope on the western side of the valley and about 1 km inland of the last homes (1994); in a subgulch, at the same lattitude of where the large ridge radiating from the summit ends. Along a vertical rock cliff face; growing on a rocky substrate, generally associated with *Artemesia*, *Schinus* in general; rocky face rather dry but facing the prevailing winds. 210 meters (700 feet) elevation. Ron Fenstemacher, Daniel Chung and Al Benedict also present.
	P0727	8/22/95	Obata	Yes	O'ahu	Green fruit	Ko'olau Range. Wailupe ('Āina-Haina), as trail makes a right turn head directly to the left and follow ridge up, off ridge top along a verticle rock face, associated with *Schinus*, koa haole, grasses. found growing out of solid rock. Altitude of 240 m (800 feet). Daniel Chung, Amy Tsuneyoshi also present on collecting trip.
Lobelia niihauensis							
	P0513	11/17/94	Reyes	Yes	O'ahu	Seed	Collected by Ken Wood on 'Ōhikilolo Ridge
	P0514	11/17/94	Reyes	Yes	O'ahu	Cuttings	Collected by Ken Wood on 'Ōhikilolo Ridge
Lobelia oahuensis							
	P0171	9/1/92	Mehrhoff	Yes	O'ahu	Ripe fruit	Collected from Ko'olau Mts. 8/28/92.

Table A.2. (Continued) Hawaiian Campanulaceae Evaluated for *in vitro* propagation at Harold L. Lyon Arboretum Micropropagation Facility

Species							
	Plant #	**Date Coll.**	**Collector**	**Wild?**	**Island**	**Plant Part(s)**	**Collection Information**
	P0496	10/21/94	Kennedy	Yes	O'ahu	Fruit	Waimānalo. Received via Julie Reyes.
Lobelia yuccoides							
	P0112	4/24/92	Mehrhoff	Yes	O'ahu	Cutting	Collected on O'ahu, Pilikea, Wai'anae Mountains. Branches knocked off by pigs.
Trematolobelia kauaiensis							
	P0231	1/27/93	Palmer	Yes	Kaua'i	Ripe fruit	Collected on 1-18-93 by Kanehiro Kitayama at 1200 m alt. Pihea Trail (Alaka'i Swamp).
Trematolobelia macrostachys							
	P0216	11/30/92	Palmer	Yes	Hawai'i	Seeds	Collected by P. Welton 10/19/92 near Dog-leg helipad, Kīpahulu.
	P0662	5/25/95	Obata	Yes	O'ahu	Green fruit	Wai'anae Range. On the Wai'anae end of the "bog" trail, off narrow ridge top, under a canopy of *Metrosideros*, ca 3800 feet.

Table A.3. *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Brighamia insignis						
P00600001	I	VW with sucrose	90	13	61	0.5X MS
P01870001	I	0.5X MS	95	30	82	0.5X MS
Clermontia drepanomorpha						
P03830001	I	0.5X MS	100	58	49	0.5X MS AC
P07170001	I	0.5X MS AC	Contaminated	n/a	n/a	n/a
Clermonatia fauriei						
P01790001	M	0.5X MS	96	30	30	0.5X MS
Clermontia grandiflora						
P07470001	M	0.5X MS	No germination yet	n/a	n/a	n/a
Clermontia kakeana						
P00480024	M	VW with sucrose	100	30	25	0.5X MS AC
Clermontia kakeana x Clermontia arborescens						
P06410001	I	0.5X MS	0	n/a	n/a	n/a
P06870001	I	0.5X MS	100	86	15	0.5X MS AC

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Clermontia lindseyana						
P02490001	I	0.5X MS	Contaminated	n/a	n/a	n/a
P02880001	M	0.5X MS	65	42	75	0.5X MS
Clermontia montis-loa						
P01990001	M	0.5X MA	7	365	20	0.5X MS
Clermontia peleana						
P01690001	M	0.5X MS	96	26	11	0.5X MS AC
P01690002	I	0.5X MS	98	26	120	0.5X MS AC
Clermontia pyrularia						
P02560001	M	0.5X MS	0	n/a	n/a	n/a
P02570001	M	0.5X MS	0	n/a	n/a	n/a
P03170001	M	0.5X MS	Contaminated	n/a	n/a	n/a
P03180001	M	0.5X MS	31	69	508	0.5X MS AC
P03700001	M	0.5X MS	Contaminated	n/a	n/a	n/a
P04740001	I	0.5X MS	15	26	180	0.5X MS AC

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Clermontia tuberculata						
P02820001	I	0.5X MS	12	266	25	0.5X MS
P06880001	I	0.5X MS	17	81	10	0.5X MS AC
Cyanea aculeatiflora						
P03870001	I	0.5X MS	5	78	69	0.5X MS AC
P05860001	M	0.5X MS	8	63	10	0.5X MS AC
Cyanea acuminata						
P00660001	M	VW with sucrose	13	42	71	0.5X MS AC
P00660002	M	VW with sucrose	18	75	120	0.5X MS AC
P02100001	M	0.5X MS	8	51	180	0.5X MS AC
Cyanea angustifolia						
P05850001	M/I	0.5X MS	85	38	28	0.5X MS AC
P07310001	I	0.5X MS	not yet germinated	n/a	n/a	n/a
Cyanea asarifolia						
P00580001	I	VW with sucrose	95	49	210	0.5X MS

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Cyanea copelandii ssp. *haleakalaensis*						
P03860001	I	0.5X MS	89	41	12	0.5X MS
Cyanea crispa						
P01500001	M	0.5X MS	96	28	29	0.5X MS
P01520001	M	0.5X MS	82	60	31	0.5X MS
P01530001	M	0.5X MS	Contaminated	n/a	n/a	n/a
P03030001	I	0.5X MS	Contaminated	n/a	n/a	n/a
Cyanea degeneriana						
P02000001	M	0.5X MS	0	n/a	n/a	n/a
Cyanea dunbarii						
P03490001	M	0.5X MS	12	66	8	0.5X MS
Cyanea glabra						
P05840001	I	0.5X MS	97	39	55	0.5X MS AC
Cyanea grimesiana ssp. *grimesiana*						
P03800001	M	0.5X MS	82	55	20	0.5X MS
P05550001	M	0.5X MS	20	55	20	0.5X MS

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Cyanea grimesiana ssp. *obatae*						
P00610001	M	VW with sucrose	86	31	170	0.5X MS
P02950001	I	0.5X MS	92	49	82	0.5X MS
P02960001	M	0.5X MS	90	58	103	0.5X MS
P03390001	I	0.5X MS	93	46	121	0.5X MS
P05900001	I	0.5X MS	97	49	61	0.5X MS
Cyanea hamatiflora ssp. *carlsonii*						
P00690001	M	VW with sucrose	Contaminated	n/a	n/a	n/a
P00700001	M	VW with sucrose	Contaminated	n/a	n/a	n/a
P00710001	M	VW with sucrose	Contaminated	n/a	n/a	n/a
P05290001	M	0.5X MS	Contaminated	n/a	n/a	n/a
Cyanea hamatiflora ssp. *hamatiflora*						
P05950001	M	0.5X MS	80	57	8	0.5X MS AC
Cyanea kolekoleensis						
P01840001	I	0.5X MS	0	n/a	n/a	n/a

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Cyanea kunthiana						
P06910001	I	0.5X MS	No germination yet	n/a	n/a	n/a
Cyanea lanceolata						
P02810001	I	0.5X MS	2	223	5	0.5X MS
P03000001	I	0.5X MS	65	186	8	0.5X MS
Cyanea leptostegia						
P01800001	M	0.5X MS	87	31	243	0.5X MS AC
Cyanea linearifolia						
P01460001	M	0.5X MS	Contaminated	n/a	n/a	n/a
P01460002	M	0.5X MS	0	n/a	n/a	n/a
Cyanea longiflora						
P02860001	M	0.5X MS	48	149	15	0.5X MS
P04810001	M	0.5X MS	Contaminated	n/a	n/a	n/a

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Cyanea mannii						
P02380001	I	0.5X MS	0	n/a	n/a	n/a
Cyanea mceldowneyi						
P00890001	I	VW with sucrose	67	59	38	0.5X MS AC
P00900001	I	VW with sucrose	0	n/a	n/a	n/a
P03640001	I	0.5X MS	.5	160	1	0.5X MS AC
Cyanea membranacea						
P02970001	M/I	0.5X MS	29	49	61	0.5X MS
Cyanea oahuensis						
P04980001	I	0.5X MS	29	58	6	0.5X MS AC
Cyanea pinnatifida						
P07120001	I	0.5X MS AC	40	68	4	0.5X MS AC
P07520001	I	0.5X MS	0	n/a	n/a	n/a
Cyanea platyphylla						
P05980001	I	0.5X MS	63	74	6	0.5X MS AC
P06090001	I	0.5X MS	78	55	4	0.5X MS AC

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Cyanea recta						
P02090001	I	0.5X MS	79	72	35	0.5X MS AC
Cyanea remyi						
P00590001	I	VW with sucrose	8	114	33	0.5X MS
P00590002	M	VW with sucrose	8	33	90	0.5X MS
P04110001	I	0.5X MS	0	n/a	n/a	n/a
P04110002	I	0.5X MS	6	241	6	0.5X MS
Cyanea shipmanii						
P02890001	M	0.5X M	92	52	54	0.5X MS AC
Cyanea superba ssp. *suberba*						
P02260001	I	0.5X MS	0	n/a	n/a	n/a
P02270001	M	0.5X MS	63	83	7	0.5X MS
P02580001	M	0.5X MS	0	n/a	n/a	n/a
P03450001	I	0.5X MS	84	91	32	0.5X MS
P03460001	I	0.5X MS	63	80	15	0.5X MS
P03470001	I	0.5X MS	84	121	42	0.5X MS

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
P05060001	M	0.5X MS	8	41	18	0.5X MS
P05070001	M	0.5X MS	6	41	14	0.5X MS
P05580001	M	0.5X MS	56	53	16	0.5X MS
P05590001	I	0.5X MS	Contaminated	n/a	n/a	n/a
Delissea rhytidosperma						
P01780001	M	0.5X MS	84	31	7	0.5X MS AC
P07700001	I	0.5X MS	100	25	11	0.5X MS AC
Delissea rivularis						
P01000001	I	VW with sucrose	0	n/a	n/a	n/a
P02030001	I	0.5X MS	0	n/a	n/a	n/a
P02240001	M/I	0.5X MS	52	91	95	0.5X MS AC
P03760001	M	0.5X MS	68	31	42	0.5X MS AC
P03770001	M	0.5X MS	29	104	31	0.5X MS AC
P03780001	M	0.5X MS	95	106	46	0.5X MS AC
Delissea subcordata						
P00380001	M	VW with sucrose	56	48	27	0.5X MS AC

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Delissea undulata ssp. *undulata*						
P01630001	I	0.5X MS	98	32	85	0.5X MS AC
P01720001	I	0.5X MS	92	33	147	0.5X MS AC
P01770001	I	0.5X MS	94	31	126	0.5X MS AC
P01830001	I	0.5X MS	91	62	85	0.5X MS AC
P01830002	I	VW with sucrose	0	n/a	n/a	n/a
P02020001	M	0.5X MS	81	25	49	0.5X MS AC
P02140001	M	0.5X MS	92	26	55	0.5X MS AC
Lobelia hillebrandii						
P02010001	M	0.5X MS	0	n/a	n/a	n/a
Lobelia hypoleuca						
P00650001	I	VW with sucrose	78	42	105	0.5X MS AC
Lobelia gaudichaudii ssp. *koolauensis*						
P01980001	M	0.5X MS	52	48	21	0.5X MS

Table A.3. (Continued) *In vitro* seed germination trials and results

Species						
Treatment	**Immature (I) or Mature (M)**	**Initial Medium**	**% Germination**	**First Germination (Days)**	**Germination Period (Days)**	**Final Growth Medium**
Lobelia monostachya						
P04460007	I	0.5X MS	0	n/a	n/a	n/a
P07270001	I	0.5X MS	55	28	9	0.5X MS AC
Lobelia niihauensis						
P05130001	M	0.5X MS	31	39	10	0.5X MS AC
Lobelia oahuensis						
P01710001	M	0.5X MS	91	33	113	0.5X MS AC
P04960001	I	0.5X MS	64	49	15	0.5X MS AC
Trematolobelia kauaiensis						
P02310001	M	0.5X MS	27	66	90	0.5X MS
Trematolobelia macrostachys						
P02160001	M	0.5X MS	28	114	42	0.5X MS
P06620001	I	0.5X MS	12	96	36	0.5X MS AC

Table A.4. Leaf explants in 0.5X MS with plant growth regulator combinations (in mg/L)

Brighamia insignis								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	necrosis	necrosis	necrosis	xxx	xxx	xxx
0.5BA	necrosis	xxx	necrosis	necrosis	necrosis	xxx	xxx	xxx
1.0 BA	necrosis	xxx	necrosis	necrosis	necrosis	xxx	xxx	xxx
Clermontia fauriei								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	47 shoots	callus	cont.	xxx	necrosis	callus	callus
1.0 BA	xxx	callus	necrosis	necrosis	necrosis	necrosis	necrosis	callus
Clermontia grandiflora								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	necrosis	xxx	xxx	cont.	xxx	xxx	xxx	xxx
1.0 BA	xxx	xxx	xxx	xxx	necrosis	xxx	xxx	xxx
Clermontia kakeana								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	xxx	cont.	necrosis	necrosis	xxx	xxx	xxx
0.5BA	necrosis	callus	callus	embryoids and 26 shoots	embryoids and 43 shoots	callus	xxx	callus
1.0 BA	necrosis	callus	callus and 67 shoots	callus and 16 shoots	callus	callus	callus	callus

Table A.4. (Continued) Leaf explants in 0.5X MS with plant growth regulator combinations (in mg/L)

Clermontia peleana								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	125 shoots	12 shoots	necrosis	xxx	xxx	callus	100 shoots
1.0 BA	xxx	90 shoots	120 shoots	necrosis	necrosis	xxx	22 shoots	xxx
Cyanea acuminata								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	callus	necrosis	callus	xxx	necrosis	necrosis	necrosis
1.0 BA	xxx	necrosis	necrosis	necrosis	callus	necrosis	necrosis	necrosis
Cyanea asarifolia								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	callus and 2 shoots	callus	callus	xxx	15 shoots	callus and 3 shoots	necrosis
1.0 BA	xxx	callus	callus	callus	callus	necrosis	callus and 6 shoots	callus
Cyanea crispa								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	cont.	necrosis	xxx	xxx	cont.	xxx	xxx
1.0 BA	xxx	cont.	cont.	cont.	xxx	xxx	cont.	xxx

Table A.4. (Continued) Leaf explants in 0.5X MS with plant growth regulator combinations (in mg/L)

Cyanea grimesiana ssp. *obatae*								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	callus and 1 shoot	callus and 1 shoot	callus	callus	necrosis	necrosis	callus
1.0 BA	xxx	callus	necrosis	callus	callus	callus	cont.	3 shoots
Cyanea hamatiflora ssp. *hamatiflora*								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	callus	cont.	callus	callus	callus	cont.	callus
1.0 BA	xxx	cont.	callus	xxx	callus	callus	callus	callus
Cyanea kuhihewa								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	cont.	callus	cont.	callus	necrosis	cont.	necrosis
1.0 BA	xxx	callus	cont.	cont.	cont.	necrosis	necrosis	cont.
Cyanea longiflora								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	necrosis	xxx	cont.	xxx	necrosis	xxx	xxx	xxx
1.0 BA	xxx	xxx	cont.	cont.	xxx	xxx	xxx	xxx

Table A.4. (Continued) Leaf explants in 0.5X MS with plant growth regulator combinations (in mg/L)

Cyanea pinnatifida								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	cont.	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	cont.	necrosis	4 shoots	xxx	necrosis	cont.	necrosis
1.0 BA	xxx	cont.	cont.	necrosis	necrosis	cont.	necrosis	necrosis
Cyanea platyphylla								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	cont.	xxx	xxx	xxx	xxx
0.5BA	xxx	necrosis	necrosis	cont.	necrosis	necrosis	necrosis	necrosis
1.0 BA	xxx	necrosis	necrosis	necrosis	cont.	necrosis	necrosis	cont.
Cyanea shipmanii								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	necrosis	callus	necrosis	xxx	necrosis	necrosis	callus
1.0 BA	xxx	necrosis	xxx	necrosis	callus	necrosis	callus	necrosis
Delissea rhytidosperma								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	callus	8 shoots	necrosis	cont.	cont.	5 shoots	6 shoots
1.0 BA	xxx	callus	necrosis	callus	necrosis	cont.	cont.	2 shoots
Delissea rivularis								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	cont.
0.5BA	xxx	xxx	cont.	cont.	cont.	cont.	xxx	cont.
1.0 BA	xxx	xxx	cont.	cont.	xxx	cont.	necrosis	xxx

Table A.4. (Continued) Leaf explants in 0.5X MS with plant growth regulator combinations (in mg/L)

Delissea subcordata								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	necrosis	1 shoot	xxx	necrosis	necrosis	necrosis	necrosis	necrosis
0.5BA	9 shoots	roots and 5 shoots	xxx	1 shoot	callus	callus	callus	necrosis
1.0 BA	callus	callus and 19 shoots	xxx	callus and 1 shoot	callus and 1 shoot	callus	callus	necrosis
Delissea undulata ssp. *undulata*								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	callus	callus	necrosis	callus and one shoot	necrosis	callus and 4 shoots	callus
1.0 BA	xxx	callus	one shoot	callus	callus	necrosis	callus	callus
Lobelia gaudichaudii ssp. *koolauensis*								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	cont.	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	cont.	xxx	xxx	cont.	cont.	xxx	xxx	xxx
1.0 BA	xxx	xxx	xxx	xxx	cont.	xxx	xxx	xxx
Lobelia hypoleuca								
	0 auxin	**0.05 2,4-D**	**0.1 2,4-D**	**0.5 2,4-D**	**1.0 2,4-D**	**0.05 NAA**	**0.5 NAA**	**1.0 NAA**
0 BA	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
0.5BA	xxx	callus	callus	xxx	xxx	callus	callus	callus
1.0 BA	xxx	callus	callus	callus	callus	callus	callus	callus

Appendix B. RAPDs Data Tables

Table B.1. Operon™ primers screened and species

Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*	Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*
OPA-1	CAGGCCCTTC	Yes	Yes	OPA-2	TGCCGAGCTG	Yes	Yes
OPA-3	AGTCAGCCAC	Yes	Yes	OPA-4	AATCGGGCTG	Yes	Yes
OPA-5	AGGGGTCTTG	Yes	Yes	OPA-6	GGTCCCTGAC	Yes	Yes
OPA-7	GAAACGGGTG	Yes	Yes	OPA-8	GTGACGTAGG	Yes	Yes
OPA-9	GGGTAACGCC	Yes	Yes	OPA-10	GTGATCGCAG	Yes	Yes
OPA-11	CAATCGCCGT	Yes	Yes	OPA-12	TCGGCGATAG	Yes	Yes
OPA-13	CAGCACCCAC	Yes	Yes	OPA-14	TCTGTGCTGG	Yes	Yes
OPA-15	TTCCGAACCC	Yes	Yes	OPA-16	AGCCAGCGAA	Yes	Yes
OPA-17	GACCGCTTGT	Yes	Yes	OPA-18	AGGTGACCGT	Yes	Yes
OPA-19	CAAACGTCGG	Yes	Yes	OPA-20	GTTGCGATCC	Yes	Yes
OPB-1	GTTTCGCTCC	Yes	Yes	OPB-2	TGATCCCTGG	Yes	Yes
OPB-3	CATCCCCCTG	Yes	Yes	OPB-4	GGACTGGAGT	Yes	Yes
OPB-5	TGCGCCCTTC	Yes	Yes	OPB-6	TGCTCTGCCC	Yes	Yes
OPB-7	GGTGACGCAG	Yes	Yes	OPB-8	GTCCACACGG	Yes	Yes
OPB-9	TGGGGGACTC	Yes	Yes	OPB-10	CTGCTGGGAC	Yes	Yes
OPB-11	GTAGACCCGT	Yes	Yes	OPB-12	CCTTGACGCA	Yes	Yes
OPB-13	TTCCCCCGCT	Yes	Yes	OPB-14	TCCGCTCTGG	Yes	Yes

Table B.1. (Continued) Operon™ primers screened and species

Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*	Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*
OPB-15	GGAGGGTGTT	Yes	Yes	OPB-16	TTTGCCCGGA	Yes	Yes
OPB-17	AGGGAACGAG	Yes	Yes	OPB-18	CCACAGCAGT	Yes	Yes
OPB-19	ACCCCCGAAG	Yes	Yes	OPB-20	GGACCCTTAC	Yes	Yes
OPC-1	TTCGAGCCAG	Yes	Yes	OPC-2	GTGAGGCGTC	Yes	Yes
OPC-3	GGGGGTCTTT	Yes	Yes	OPC-4	CCGCATCTAC	Yes	Yes
OPC-5	GATGACCGCC	Yes	Yes	OPC-6	GAACGGACTC	Yes	Yes
OPC-7	GTCCCGACGA	Yes	Yes	OPC-8	TGGACCGGTG	Yes	Yes
OPC-9	CTCACCGTCC	Yes	Yes	OPC-10	TGTCTGGGTG	Yes	Yes
OPC-11	AAAGCTGCGG	Yes	Yes	OPC-12	TGTCATCCCC	Yes	Yes
OPC-13	AAGCCTCGTC	Yes	Yes	OPC-14	TGCGTGCTTG	Yes	Yes
OPC-15	GACGGATCAG	Yes	Yes	OPC-16	CACACTCCAG	Yes	Yes
OPC-17	TTCCCCCCAG	Yes	Yes	OPC-18	TGAGTGGGTG	Yes	Yes
OPC-19	GTTGCCAGCC	Yes	Yes	OPC-20	ACTTCGCCAC	Yes	Yes
OPD-1	ACCGCGAAGG	Yes	Yes	OPD-2	GGACCCAACC	Yes	Yes
OPD-3	GTCGCCGTCA	Yes	Yes	OPD-4	TCTGGTGAGG	Yes	Yes
OPD-5	TGAGCGGACA	Yes	Yes	OPD-6	ACCTGAACGG	Yes	Yes
OPD-7	TTGGCACGGG	Yes	Yes	OPD-8	GTGTGCCCCA	Yes	Yes

Table B.1. (Continued) Operon™ primers screened and species

Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*	Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*
OPD-9	CTCTGGAGAC	Yes	Yes	OPD-10	GGTCTACACC	Yes	Yes
OPD-11	AGCGCCATTG	Yes	Yes	OPD-12	CACCGTATCC	Yes	Yes
OPD-13	GGGGTGACGA	Yes	Yes	OPD-14	CTTCCCCAAG	Yes	Yes
OPD-15	CATCCGTGCT	Yes	Yes	OPD-16	AGGGCGTAAG	Yes	Yes
OPD-17	TTTCCCACGG	Yes	Yes	OPD-18	GAGAGCCAAC	Yes	Yes
OPD-19	CTGGGGACTT	Yes	Yes	OPD-20	ACCCGGTCAC	Yes	Yes
OPE-1	CCCAAGGTCC	Yes	Yes	OPE-2	GGTGCGGGAA	Yes	Yes
OPE-3	CCAGATGCAC	Yes	Yes	OPE-4	GTGACATGCC	Yes	Yes
OPE-5	TCAGGGAGGT	Yes	Yes	OPE-6	AAGACCCCTC	Yes	Yes
OPE-7	AGATGCAGCC	Yes	Yes	OPE-8	TCACCACGGT	Yes	Yes
OPE-9	CTTCACCCGA	Yes	Yes	OPE-10	CACCAGGTGA	Yes	Yes
OPE-11	GAGTCTCAGG	Yes	Yes	OPE-12	TTATCGCCCC	Yes	Yes
OPE-13	CCCGATTCGG	Yes	Yes	OPE-14	TGCGGCTGAG	Yes	Yes
OPE-15	ACGCACAACC	Yes	Yes	OPE-16	GGTGACTGTG	Yes	Yes
OPE-17	CTACTGCCGT	Yes	Yes	OPE-18	GGACTGCAGA	Yes	Yes
OPE-19	ACGGCGTATG	Yes	Yes	OPE-20	AACGGTGACC	Yes	Yes
OPF-1	ACGGATCCTG	Yes	Yes	OPF-2	GAGGATCCCT	Yes	Yes

Table B.1. (Continued) Operon™ primers screened and species

Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*	Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*
OPF-3	CCTGATCACC	Yes	Yes	OPF-4	GGTGATCAGG	Yes	Yes
OPF-5	CCGAATTCCC	Yes	Yes	OPF-6	GGGAATTCGG	Yes	Yes
OPF-7	CCGATATCCC	Yes	Yes	OPF-8	GGGATATCGG	Yes	Yes
OPF-9	GGGATATCGG	Yes	Yes	OPF-10	GGAAGCTTGG	No	Yes
OPF-11	TTGGTACCCC	No	Yes	OPF-12	ACGGTACCAG	No	Yes
OPF-13	GGCTGCAGAA	No	Yes	OPF-14	TGCTGCAGGT	No	Yes
OPF-15	CCAGTACTCC	No	Yes	OPF-16	GGAGTACTGG	No	Yes
OPF-17	AACCCGGGAA	No	Yes	OPF-18	TTCCCGGGTT	No	Yes
OPF-19	CCTCTAGACC	No	Yes	OPF-20	GGTCTAGAGG	No	Yes
OPG-1	CTACGGAGGA	Yes	Yes	OPG-2	GGCACTGAGG	Yes	Yes
OPG-3	GAGCCCTCCA	Yes	Yes	OPG-4	AGCGTGTCTG	Yes	Yes
OPG-5	CTGAGACGGA	Yes	Yes	OPG-6	GTGCCTAACC	Yes	Yes
OPG-7	GAACCTGCGG	Yes	Yes	OPG-8	TCACGTCCAC	Yes	Yes
OPG-9	CTGACGTCAC	Yes	Yes	OPG-10	AGGGCCGTCT	Yes	Yes
OPG-11	TGCCCGTCGT	Yes	Yes	OPG-12	CAGCTCACGA	Yes	Yes
OPG-13	CTCTCCGCCA	Yes	Yes	OPG-14	GGATGAGACC	Yes	Yes
OPG-15	ACTGGGACTC	Yes	Yes	OPG-16	AGCGTCCTCC	Yes	Yes

Table B.1. (Continued) Operon™ primers screened and species

Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*	Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*
OPG-17	ACGACCGACA	Yes	Yes	OPG-18	GGCTCATGTG	Yes	Yes
OPG-19	GTCAGGGCAA	Yes	Yes	OPG-20	TCTCCCTCAG	Yes	Yes
OPH-1	GGTCGGAGAA	Yes	Yes	OPH-2	TCGGACGTGA	Yes	Yes
OPH-3	AGACGTCCAC	Yes	Yes	OPH-4	GGAAGTCGCC	Yes	Yes
OPH-5	AGTCGTCCCC	Yes	Yes	OPH-6	ACGCATCGCA	Yes	Yes
OPH-7	CTGCATCGTG	Yes	Yes	OPH-8	GAAACACCCC	Yes	Yes
OPH-9	TGTAGCTGGG	Yes	Yes	OPH-10	CCTACGTCAG	Yes	Yes
OPH-11	CTTCCGCAGT	Yes	Yes	OPH-12	ACGCGCATGT	Yes	Yes
OPH-13	GACGCCACAC	Yes	Yes	OPH-14	ACCAGGTTGG	Yes	Yes
OPH-15	AATGGCGCAG	Yes	Yes	OPH-16	TCTCAGCTGG	Yes	Yes
OPH-17	CACTCTCCTC	Yes	Yes	OPH-18	GAATCGGCCA	Yes	Yes
OPH-19	CTGACCAGCC	Yes	Yes	OPH-20	GGGAGACATC	Yes	Yes
OPI-1	ACCTGGACAC	Yes	Yes	OPI-2	GGAGGAGAGG	Yes	Yes
OPI-3	CAGAAGCCCA	Yes	Yes	OPI-4	CCGCCTAGTC	Yes	Yes
OPI-5	TGTTCCACGG	No	Yes	OPI-6	AAGGCGGCAG	No	Yes
OPI-7	CAGCGACAAG	No	Yes	OPI-8	TTTGCCCGGT	No	Yes
OPI-9	TGGAGAGCAG	No	Yes	OPI-10	ACAACGCGAG	No	Yes

Table B.1. (Continued) Operon™ primers screened and species

Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*	Operon™ Code	Sequence 5' to 3'	With *D. undulata* ssp. *undulata*	With *C. asarifolia*
OPI-11	ACATGCCGTG	No	Yes	OPI-12	AGAGGGCACA	No	Yes
OPI-13	CTGGGGCTGA	No	Yes	OPI-14	TGACGGCGGT	No	Yes
OPI-15	TCATCCGAGG	No	Yes	OPI-16	TCTCCGCCCT	No	Yes
OPI-17	GGTGGTGATG	No	Yes	OPI-18	TGCCCAGCCT	No	Yes
OPI-19	AATGCGGGAG	No	Yes	OPI-20	AAAGTGCGGG	No	Yes
OPJ-1	CCCGGCATAA	No	Yes	OPJ-2	CCCGTTGGGA	No	Yes
OPJ-3	TCTCCGCTTG	No	Yes	OPJ-4	CCGAACACGG	No	Yes
OPJ-5	CTCCATGGGG	No	Yes	OPJ-6	TCGTTCCGTA	No	Yes
OPJ-7	CCTCTCGACA	No	Yes	OPJ-8	CATACCGTGG	No	Yes
OPJ-9	TGAGCCTCAC	No	Yes	OPJ-10	AAGCCCGAGG	No	Yes
OPJ-11	ACTCCTGCGA	No	Yes	OPJ-12	GTCCCGTGGT	No	Yes
OPJ-13	CCACACTACC	No	Yes	OPJ-14	CACCCGGATG	No	Yes
OPJ-15	TGTAGCAGGG	No	Yes	OPJ-16	CTGCTTAGGG	No	Yes
OPJ-17	ACGCCAGTTC	No	Yes	OPJ-18	TGGTCGCAGA	No	Yes
OPJ-19	GGACACCACT	No	Yes	OPJ-20	AAGCGGCCTC	No	Yes
OPK-1	CARRCGAGCC	No	Yes	OPK-2	GTCTCCGCAA	No	Yes
OPK-3	CCAGCTTAGG	No	Yes	OPK-4	CCGCCCAAAC	No	Yes

Table B.1. (Continued) Operon™ primers screened and species

Operon™ Code	**Sequence 5' to 3'**	**With *D. undulata* ssp. *undulata***	**With *C. asarifolia***	**Operon™ Code**	**Sequence 5' to 3'**	**With *D. undulata* ssp. *undulata***	**With *C. asarifolia***
OPK-5	TCTGTCGAGG	No	Yes	OPK-6	CACCTTTCCC	No	Yes
OPK-7	AGCGAGCAAG	No	Yes	OPK-8	GAACACTGGG	No	Yes
OPK-9	CCCTACCGAC	No	Yes	OPK-10	GTGCAACGTG	No	Yes
OPK-11	AATGCCCCAG	No	Yes	OPK-12	TGGCCCTCAC	No	Yes
OPK-13	GGTTGTACCC	No	Yes	OPK-14	CCCGCTACAC	No	Yes
OPK-15	CTCCTGCCAA	No	Yes	OPK-16	GAGCGTCGAA	No	Yes
OPK-17	CCCAGCTGTG	No	Yes	OPK-18	CCTAGTCGAG	No	Yes
OPK-19	CACAGGCGGA	No	Yes	OPK-20	GTGTCGCGAG	No	Yes

Table B.2. PCR results using RAPDs with *Cyanea asarifolia*. Headings are size of bands (in base pairs) and numbers in columns are the number of bands in that range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPA-1	0	2	1	3	2	1	OPA-2	0	0	0	0	2	3
OPA-3	0	1	0	1	0	3	OPA-4	0	0	1	0	0	3
OPA-5	0	0	0	1	0	0	OPA-6	0	0	0	0	0	0
OPA-7	0	0	2	0	1	1	OPA-8	0	0	2	1	1	2
OPA-9	0	0	0	2	0	1	OPA-10	0	1	2	0	2	0
OPA-11	0	0	0	0	0	0	OPA-12	0	0	0	0	0	0
OPA-13	0	0	0	0	0	0	OPA-14	0	0	0	0	0	0
OPA-15	0	0	0	0	0	0	OPA-16	1	1	1	2	0	0
OPA-17	0	0	0	0	0	0	OPA-18	0	0	3	0	3	1
OPA-19	0	0	0	0	0	0	OPA-20	0	0	0	0	0	0
OPB-1	0	0	0	3	0	0	OPB-2	0	0	0	0	0	0
OPB-3	0	0	0	0	0	0	OPB-4	0	0	0	0	0	0
OPB-5	0	0	0	0	0	0	OPB-6	0	0	0	0	0	0
OPB-7	0	1	0	3	1	1	OPB-8	0	0	0	0	0	0
OPB-9	0	0	0	0	0	0	OPB-10	0	0	0	0	0	0
OPB-11	0	0	0	0	0	0	OPB-12	0	0	0	0	0	0
OPB-13	0	0	0	0	0	0	OPB-14	0	0	0	0	0	0

Table B.2. (Continued) PCR results using RAPDs with *Cyanea asarifolia*. Headings are size of bands (in base pairs) and numbers in columns are the number of bands in that range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPB-15	0	0	0	0	0	0	OPB-16	0	0	0	0	0	0
OPB-17	0	0	0	0	0	0	OPB-18	0	0	0	0	0	0
OPB-19	0	0	0	0	0	0	OPB-20	0	0	0	0	0	0
OPC-1	0	0	0	0	0	0	OPC-2	0	0	0	0	0	0
OPC-3	0	0	0	0	0	0	OPC-4	0	0	0	0	0	0
OPC-5	0	0	0	0	0	0	OPC-6	0	0	0	0	0	0
OPC-7	0	0	0	0	0	0	OPC-8	0	0	0	1	1	1
OPC-9	0	1	0	1	1	1	OPC-10	0	0	0	0	0	0
OPC-11	0	0	0	0	0	0	OPC-12	0	0	0	0	0	0
OPC-13	0	0	0	0	0	0	OPC-14	0	0	0	0	0	0
OPC-15	0	0	0	0	0	0	OPC-16	0	0	0	0	0	0
OPC-17	0	0	0	0	0	0	OPC-18	0	0	0	0	0	0
OPC-19	0	0	0	0	0	0	OPC-20	0	0	1	2	1	0
OPD-1	0	0	0	0	0	0	OPD-2	0	0	0	0	0	0
OPD-3	0	0	0	0	0	0	OPD-4	0	0	0	0	0	0
OPD-5	0	0	0	0	0	0	OPD-6	0	0	0	0	0	0
OPD-7	0	0	0	0	0	0	OPD-8	0	0	0	0	0	0

Table B.2. (Continued) PCR results using RAPDs with *Cyanea asarifolia*. Headings are size of bands (in base pairs) and numbers in columns are the number of bands in that range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPD-9	0	0	0	0	0	0	OPD-10	0	0	0	2	0	0
OPD-11	0	0	2	2	0	2	OPD-12	0	0	0	0	0	0
OPD-13	0	0	0	0	0	0	OPD-14	0	0	0	0	0	0
OPD-15	0	0	0	0	0	0	OPD-16	0	0	0	0	0	0
OPD-17	0	0	0	0	0	0	OPD-18	0	0	0	0	0	0
OPD-19	0	0	0	0	0	0	OPD-20	0	0	0	0	0	0
OPE-1	0	0	0	0	0	0	OPE-2	0	1	1	0	1	1
OPE-3	0	0	0	2	1	2	OPE-4	0	0	1	0	0	2
OPE-5	0	0	0	0	0	0	OPE-6	0	0	0	0	0	0
OPE-7	0	0	0	0	0	0	OPE-8	0	0	0	0	0	0
OPE-9	0	0	0	0	0	0	OPE-10	0	0	0	0	0	0
OPE-11	0	0	0	0	0	0	OPE-12	0	0	0	0	0	0
OPE-13	0	0	0	0	0	0	OPE-14	0	0	0	0	0	0
OPE-15	0	0	0	0	0	0	OPE-16	0	0	0	0	0	0
OPE-17	0	0	0	0	0	0	OPE-18	0	0	0	0	0	0
OPE-19	0	0	0	0	0	0	OPE-20	0	0	0	0	0	0
OPF-1	0	0	0	0	0	0	OPF-2	0	0	0	0	0	0

Table B.2. (Continued) PCR results using RAPDs with *Cyanea asarifolia*. Headings are size of bands (in base pairs) and numbers in columns are the number of bands in that range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPF-3	0	0	0	0	2	0	OPF-4	0	0	0	0	0	0
OPF-5	0	0	0	0	0	0	OPF-6	0	0	0	0	0	0
OPF-7	0	0	0	0	0	0	OPF-8	0	0	0	0	0	0
OPF-9	0	0	0	0	0	0	OPF-10	0	0	0	0	0	0
OPF-11	0	0	0	0	0	0	OPF-12	0	0	0	0	0	0
OPF-13	0	0	0	0	0	0	OPF-14	0	0	0	0	0	0
OPF-15	0	0	0	0	0	0	GPF-16	0	0	0	0	0	0
OPF-17	0	0	0	0	0	0	OPF-18	0	0	0	0	0	0
OPF-19	0	0	0	0	0	0	OPF-20	0	0	0	0	0	0
OPG-1	0	0	0	0	0	0	OPG-2	0	0	0	0	0	0
OPG-3	0	0	0	0	0	0	OPG-4	0	0	0	0	0	0
OPG-5	0	0	0	0	0	0	OPG-6	0	0	0	0	0	0
OPG-7	0	0	0	0	0	0	OPG-8	0	0	0	0	0	0
OPG-9	0	0	0	0	0	0	OPG-10	0	0	0	0	0	0
OPG-11	0	0	0	0	0	0	OPG-12	0	0	0	0	0	0
OPG-13	0	0	0	0	0	0	OPG-14	0	0	0	0	0	0
OPG-15	0	0	0	0	0	0	OPG-16	0	0	0	0	0	0

Table B.2. (Continued) PCR results using RAPDs with *Cyanea asarifolia*. Headings are size of bands (in base pairs) and numbers in columns are the number of bands in that range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPG-17	0	0	0	0	0	0	OPG-18	0	0	0	0	0	0
OPG-19	0	0	0	0	0	0	OPG-20	0	0	0	0	0	0
OPH-1	0	0	0	0	0	0	OPH-2	0	0	0	0	0	0
OPH-3	0	0	0	0	0	0	OPH-4	0	0	0	0	0	0
OPH-5	0	0	0	0	0	0	OPH-6	0	0	0	0	0	0
OPH-7	0	0	0	0	0	0	OPH-8	0	0	0	0	0	0
OPH-9	0	0	0	0	0	0	OPH-10	0	0	0	0	0	0
OPH-11	0	0	0	0	0	0	OPH-12	0	0	0	2	1	2
OPH-13	0	0	0	0	0	0	OPH-14	0	0	0	0	0	0
OPH-15	0	0	0	0	0	0	OPH-16	0	0	0	0	0	0
OPH-17	0	0	0	0	0	0	OPH-18	0	0	0	2	3	2
OPH-19	0	0	0	0	0	0	OPH-20	0	0	0	0	0	0
OPI-1	0	0	0	0	0	0	OPI-2	0	0	0	2	1	1
OPI-3	0	0	0	0	0	0	OPI-4	0	0	0	0	0	0
OPI-5	0	0	0	0	0	0	OPI-6	0	0	2	1	3	0
OPI-7	0	0	0	0	0	0	OPI-8	0	0	0	0	0	0
OPI-9	0	0	0	0	0	0	OPI-10	0	0	0	0	0	0

Table B.2. (Continued) PCR results using RAPDs with *Cyanea asarifolia*. Headings are size of bands (in base pairs) and numbers in columns are the number of bands in that range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPI-11	0	0	0	0	0	0	OPI-12	0	0	0	0	0	0
OPI-13	0	0	0	0	0	0	OPI-14	0	0	0	0	0	0
OPI-15	0	0	0	0	0	0	OPI-16	0	0	0	0	0	0
OPI-17	0	0	0	0	0	0	OPI-18	0	0	0	0	0	0
OPI-19	0	0	0	0	0	0	OPI-20	0	0	0	0	0	0
OPJ-1	0	0	0	0	0	0	OPJ-2	0	0	0	0	0	0
OPJ-3	0	0	0	0	0	0	OPJ-4	0	0	0	0	0	0
OPJ-5	0	0	0	0	0	0	OPJ-6	0	0	0	0	0	0
OPJ-7	0	0	0	0	0	0	OPJ-8	0	0	0	0	0	0
OPJ-9	0	0	0	0	0	0	OPJ-10	0	0	0	0	0	0
OPJ-11	0	0	0	0	0	0	OPJ-12	0	0	0	0	0	0
OPJ-13	0	0	0	0	0	0	OPJ-14	0	0	0	0	0	0
OPJ-15	0	0	0	0	0	0	OPJ-16	0	0	0	0	0	0
OPJ-17	0	0	0	0	0	0	OPJ-18	0	0	0	0	0	0
OPJ-19	0	0	0	0	0	0	OPJ-20	0	0	0	0	0	0
OPK-1	0	0	0	0	0	0	OPK-2	0	0	0	0	0	0
OPK-3	0	0	2	0	0	0	OPK-4	0	0	0	0	0	0

Table B.2. (Continued) PCR results using RAPDs with *Cyanea asarifolia*. Headings are size of bands (in base pairs) and numbers in columns are the number of bands in that range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPK-5	0	0	0	0	0	0	OPK-6	0	0	0	0	0	0
OPK-7	0	0	1	1	1	1	OPK-8	0	0	0	0	0	0
OPK-9	0	0	0	0	0	0	OPK-10	0	0	0	0	0	0
OPK-11	0	0	0	0	0	0	OPK-12	0	1	2	0	3	3
OPK-13	0	0	1	0	1	2	OPK-14	0	0	0	0	0	0
OPK-15	0	0	0	0	0	0	OPK-16	0	0	0	0	0	0
OPK-17	0	0	0	0	0	0	OPK-18	0	0	0	0	0	0
OPK-19	0	0	0	0	0	0	OPK-20	0	0	0	0	0	0

Table B.3. PCR results using RAPDs with *Delissea undulata* ssp. *undulata*. Headings indicate size of bands (as base pairs) and numbers in the columns indicate the number of bands in that size range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPA-1	1	0	2	0	3	2	OPA-2	0	1	0	1	1	0
OPA-3	0	0	1	1	2	2	OPA-4	0	2	2	2	1	3
OPA-5	1	0	1	1	1	2	OPA-6	0	0	1	2	0	0
OPA-7	0	0	0	2	1	1	OPA-8	0	0	0	1	1	2
OPA-9	0	0	0	2	0	1	OPA-10	0	0	0	0	0	0
OPA-11	0	1	1	1	0	0	OPA-12	0	0	1	1	1	2
OPA-13	0	0	0	0	0	0	OPA-14	0	0	0	0	0	3
OPA-15	0	0	0	1	1	2	OPA-16	0	1	1	2	1	0
OPA-17	0	0	1	0	2	0	OPA-18	0	1	1	1	1	0
OPA-19	0	0	1	1	0	0	OPA-20	0	0	0	0	0	0
OPB-1	0	0	1	2	0	1	OPB-2	0	0	1	0	0	1
OPB-3	1	0	1	0	0	0	OPB-4	0	0	1	0	0	2
OPB-5	0	1	1	0	1	2	OPB-6	1	1	2	1	0	0
OPB-7	0	1	0	1	1	1	OPB-8	0	0	0	3	1	1
OPB-09	0	0	0	1	0	0	OPB-10	0	0	2	1	1	1
OPB-11	0	0	0	1	1	2	OPB-12	0	0	1	1	1	2
OPB-13	0	1	1	0	0	0	OPB-14	1	2	0	0	1	1

Table B.3. (Continued) PCR results using RAPDs with *Delissea undulata* ssp. *undulata*. Headings indicate size of bands (as base pairs) and numbers in the columns indicate the number of bands in that size range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPB-15	0	0	1	1	1	1	OPB-16	0	0	0	0	0	0
OPB-17	0	0	1	1	2	6	OPB-18	0	0	0	0	0	0
OPB-19	0	0	0	0	0	0	OPB-20	0	0	0	0	0	0
OPC-1	1	0	0	2	0	1	OPC-2	0	0	1	1	1	2
OPC-3	0	0	0	0	0	0	OPC-4	0	0	0	0	0	0
OPC-5	0	0	1	2	0	3	OPC-6	0	0	0	2	1	2
OPC-7	0	1	0	0	3	0	OPC-8	0	0	2	0	2	0
OPC-9	0	0	0	0	0	0	OPC-10	0	0	1	0	2	0
OPC-11	0	1	0	2	1	2	OPC-12	0	0	0	2	0	1
OPC-13	0	0	0	1	1	1	OPC-14	0	0	0	0	0	0
OPC-15	0	0	0	2	0	1	OPC-16	0	0	0	1	2	0
OPC-17	0	0	2	2	1	0	OPC-18	0	0	0	0	1	0
OPC-19	0	0	1	1	2	1	OPC-20	0	0	1	1	2	1
OPD-1	0	0	0	0	1	0	OPD-2	0	0	2	2	0	2
OPD-3	0	0	0	1	3	2	OPD-4	0	0	0	2	1	0
OPD-5	0	0	0	0	0	0	OPD-6	0	0	0	1	0	0
OPD-7	0	0	0	0	0	0	OPD-8	0	0	0	0	0	0

Table B.3. (Continued) PCR results using RAPDs with *Delissea undulata* ssp. *undulata*. Headings indicate size of bands (as base pairs) and numbers in the columns indicate the number of bands in that size range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPD-9	0	0	0	1	1	2	OPD-10	0	0	0	0	0	0
OPD-11	0	0	0	1	0	3	OPD-12	0	0	0	0	0	0
OPD-13	0	0	0	0	0	0	OPD-14	0	0	0	0	0	0
OPD-15	0	0	0	0	1	0	OPD-16	0	0	0	0	0	0
OPD-17	0	0	0	0	0	0	OPD-18	0	0	0	0	1	2
OPD-19	0	0	0	0	0	0	OPD-20	0	0	0	0	0	0
OPE-1	0	0	0	0	0	0	OPE-2	0	0	0	0	1	0
OPE-3	0	0	0	0	0	0	OPE-4	0	0	0	0	0	0
OPE-5	0	0	0	0	0	0	OPE-6	0	0	0	0	0	0
OPE-7	0	0	0	0	0	0	OPE-8	1	1	1	1	1	1
OPE-9	0	0	0	0	0	0	OPE-10	0	0	0	0	0	0
OPE-11	0	0	0	0	0	0	OPE-12	0	0	0	0	0	0
OPE-13	0	0	0	0	0	0	OPE-14	0	0	0	0	0	0
OPE-15	0	0	0	0	0	0	OPE-16	0	0	0	0	0	0
OPE-17	0	0	0	0	0	0	OPE-18	0	0	0	0	0	0
OPE-19	0	0	0	0	0	0	OPE-20	0	0	0	0	0	0
OPF-1	0	0	0	0	1	0	OPF-2	0	0	0	1	0	1

Table B.3. (Continued) PCR results using RAPDs with *Delissea undulata* ssp. *undulata*. Headings indicate size of bands (as base pairs) and numbers in the columns indicate the number of bands in that size range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPF-3	0	0	1	1	0	1	OPF-4	0	0	0	1	1	1
OPF-5	0	0	0	0	0	0	OPF-6	0	0	1	0	1	1
OPF-7	0	0	0	0	0	0	OPF-8	0	0	0	0	0	0
OPF-9	0	0	0	0	0	0	OPF-10	0	0	0	0	0	0
OPF-11	0	0	0	0	0	0	OPF-12	0	0	0	0	0	0
OPF-13	0	0	0	0	0	0	OPF-14	0	0	0	0	0	0
OPF-15	0	0	0	0	0	0	OPF-16	0	0	0	0	0	0
OPF-17	0	0	0	0	0	0	OPF-18	0	0	0	0	0	0
OPF-19	0	0	0	0	0	0	OPF-20	0	1	0	0	0	1
OPG-1	0	0	0	0	0	0	OPG-2	0	0	0	0	0	0
OPG-3	0	0	0	0	0	0	OPG-4	0	0	0	0	0	0
OPG-5	0	0	0	0	0	0	OPG-6	0	0	0	0	0	0
OPG-7	0	0	0	0	0	0	OPG-8	0	0	0	0	0	0
OPG-9	0	0	0	0	0	0	OPG-10	0	0	0	0	0	0
OPG-11	0	0	0	0	0	0	OPG-12	0	0	0	0	0	0
OPG-13	0	0	0	0	0	0	OPG-14	0	0	0	0	0	0
OPG-15	0	0	1	1	2	1	OPG-16	0	0	0	0	0	0

Table B.3. (Continued) PCR results using RAPDs with *Delissea undulata* ssp. *undulata*. Headings indicate size of bands (as base pairs) and numbers in the columns indicate the number of bands in that size range.

Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0	Operon™ Code	4000-3000	2999-2000	1999-1500	1499-1000	999-750	749-0
OPG-17	0	0	0	0	0	0	OPG-18	0	0	0	0	0	0
OPG-19	0	0	0	0	0	0	OPG-20	0	0	0	0	0	0
OPH-1	0	0	0	3	3	3	OPH-2	0	0	1	2	2	2
OPH-3	0	0	0	1	2	5	OPH-4	0	0	2	0	3	5
OPH-5	0	0	2	2	1	1	OPH-6	0	0	1	3	3	0
OPH-7	0	0	0	2	2	0	OPH-8	0	0	1	1	2	2
OPH-9	0	0	0	0	0	0	OPH-10	0	0	0	0	0	0
OPH-11	0	0	2	1	2	0	OPH-12	0	0	0	0	0	0
OPH-13	0	0	0	1	1	1	OPH-14	0	0	0	0	0	0
OPH-15	0	0	0	0	0	0	OPH-16	0	1	0	0	2	2

Literature Cited

Akopyanz, N., N. O. Bukanov, T. U. Westblom, S. Kresovich, and D. E. Berg. 1992. DNA diversity among clinical isolates of *Helicobacter pylori* detected by PCR-based RAPD fingerprinting. **Nucleic Acids Research 20**:5137-5142.

Andersen, W. R., and D. J. Fairbanks. 1990. Molecular markers:important tools for plant genetic resource characterization. **Diversity 6**:51-53.

Arnheim, N., T. White, and W. Rainey. 1990. Application of PCR: organismal and population biology. **Bioscience 40**:174-182.

Baird, E., S. Cooper-Bland, R. Waugh, M. DeMaine, and W. Powell. 1992. Molecular characterisation of inter- and intra-specific somatic hybrids of potato using randomly amplified polymorphic DNA (RAPD) markers. **Molecular and General Genetics 233**:469-475.

Bayliss, M. W. 1980. Chromosomal variation in plant tissue cultures. Pages 113-144 *in* I. K. Vasil, editor. Perspectives in plant cell and tissue culture, International review of cytology Edition. Volume Supplement 11A. Academic Press, New York.

Black, W. C., IV, N. M. DuTeau, G. J. Puterka, J. R. Nechols, and J. M. Pettorini. 1992. Use of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) to detect DNA polymorphisms in aphids (Homoptera: Aphidadae). **Bulletin of Entomological Research 82**:151-159.

Bowes, B. G. and E. W. Curtis. 1991. Conservation of the British National *Begonia* collection by micropropagation. **New Phytologist 119:**169-181.

Brandt, K. 1992. Micropropagation of *Campanula isophylla* Moretti. **Plant Cell, Tissue and Organ Culture 29**:31-36.

Brauner, S., D. J. Crawford, and T. F. Stuessy. 1992. Ribosomal DNA and RAPD variation in the rare plant family Lactoridaceae. **American Journal of Botany 79**:1436-1439.

Burt, A., D. A. Carter, T. J. White, and J. W. Taylor. 1994. DNA sequencing with arbitrary primer pairs. **Molecular Ecology 3**:523-525.

Caetano-Anollés, G., B. J. Bassam, and P. M. Gresshoff. 1991a. DNA amplification fingerprinting: a strategy for genome analysis. **Plant Molecular Biology Reporter 9**:294-307.

---. 1991b. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. **Bio/Technology 9**:553-557.

Carson, H. L. 1982. Evolution of Drosophila on the newer Hawaiian volcanoes. **Heredity 48**:3-25.

Cassells, A. C. 1985. Genetic, epigenetic and non-genetic variation in tissue culture derived plants. Pages 111-120 *in* A. Schafer-Menuhr, editor. *In vitro* techniques - propagation and long term storage. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht, Germany, Proceedings of a seminar in the CEC Programme of Co-ordination of Research on Plant Productivity, held at Braunschweig, Federal Republic of Germany, 27-29 November, 1984.

Center for Plant Conservation. 1991. Genetic sampling guidelines for conservation collections of endangered plants. Pages 225-238 *in* D. A. Falk and K. E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University PRess, New York.

Chalmers, K. J., R. Waugh, J. I. Sprent, A. J. Simons, and W. Powel. 1992a. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. **Heredity 69**:465-472.

Chalmers, K. J., R. Waugh, J. I. Sprent, A. J. Simons, and W. Powell. 1992b. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. **Heredity 69**:465-472.

Crowhurst, R. N., B. T. Hawthorne, E. H. A. Rikkerink, and M. D. Templeton. 1991. Differentiation of *Fusarium solani* f. sp. *cucurbitae* races 1 and 2 by random amplification of polymorphic DNA. **Current Genetics 20**:391-396.

Dawson, I. K., A. J. Simons, R. Waugh, and W. Powell. 1995. Diversity and genetic differentiation among subpopulations of *Gliricidia sepium* revealed by PCR-based assays. **Heredity 74**:10-18.

Deragon, Jean-M., and B. S. Landyr. 1992. RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. **PCR Methods and Applications 1**:175-180.

Devos, K. M., and M. D. Gale. 1992. The use of random amplified polymorphic DNA markers in wheat. **Theoretical Applied Genetics 84**:567-572.

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. **Phytochemical Bulletin 19**:1-15.

Duvall, F. 1991. Cultivation of *Clermontia, Cyanea, Lobelia*, and *Trematolobelia* from seed. **Hawaii's Forests and Wildlife 6**:12-13.

Echt, C. S., L. A. Erdahl, and T. J. McCoy. 1992. Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. **Genome 35**:84-87.

Eldredge, L.G. and S.E. Miller. 1995. How many species are there in Hawaii? **Bishop Museum Occassional Papers: No. 41**:3-18.

Engelmann, F. 1991. *In vitro* conservation of tropical plant germplasm — a review. **Euphytica 57**:227-243.

Erlich, H. A., D. Gelfand, and J. J. Sninsky. 1991. Recent advances in the polymerase chain reaction. **Science 252**:1643-1651.

Fay, M. F. 1992. Conservation of rare and endangered plants using in vitro methods. **In Vitro Cellular Developmental Biology 28**:1-4.

Friar, E. A., R. H. Robichaux, and D. W. Mount. 1996. In Press. Molecular genetic variation following a population crash in the endangered Mauna Kea silversword, *Argyroxiphium sandwicense* ssp. *sandwicense* (Asteraceae). **Molecular Ecology**.

Fukuoka, S., K. Hosaka, and O. Kamijima. 1992. Use of random amplified polymorphic DNAs (RAPDs) for identification of rice accessions. **Japanese Journal of Genetics 67**:243-252.

Geneve, R. L. 1989. Variation within a clone during tissue culture propagation. **Proceedings of the International Plant Propagators' Society 39**:458-462.

Gibbs, H. L., K. A. Prior, and P. J. Weatherhead. 1994. Genetic analysis of populations of threatened snake species using RAPD markers. **Molecular Ecology 3**:329-337.

Gilbert, D. A., N. Lehman, S. J. O'Brien, and R. K. Wayne. 1990. Genetic fingerprinting reflects population differentiation in the California Channel Island fox. **Nature 344**:764-767.

Givnish, T. J., K. J. Sytsma, J. F. Smith, and W. J. Hahn. 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae), the largest plant genus endemic to Hawai`i. Pages 288-337 *in* W. J. Wagner and V. L. Funk, editors. Origin and Adaptive Radiation of the Hawaiian Biota. Smithsonian Institution Press, Washington DC.

Guerrant, E. O., Jr. 1992. Genetic and demographic considerations in the sampling and reintroduction of rare plants. Pages 321-344 *in* P. L. Fielder and S. K. Jain, editors. Conservation biology the theory and practice of nature conservation preservation and management. Chapman and Hall, New York.

Haberlandt, G. 1902. Kulturversuche mit isolierten Pflansenzellsn. **Sitzungsber. Akad. der Wiss. Wien, Math.-Naturwiss 111**:69-92.

Haig, S. M., J. M. Rhymer, and D. G. Heckel. 1994. Population differentiation in randomly amplified polymorphic DNA of red-cockaded woodpeckers *Picoides borealis*. **Molecular Ecology 3**:581-595.

Halward, T. M., H. T. Stalker, E. A. LaRue, and G. Kochert. 1991. Genetic variation detectable with molecular markers among unadapted germ-plasm resources of cultivated peanut and related wild species. **Genome 34**:1013-1020.

Hamelin, R. C., R. L. Doudrick, and W. L. Nance. 1994. Genetic diversity in *Cronartium quercuum* f. sp. *fusiforme* on loblolly pines in southern U.S. **Current Genetics 26**:359-363.

Harada, K., A. Kinoshita, N. A. A. Shukor, H. Tachida, and T. Yamazaki. 1994. Genetic variation estimated in three *Shorea* species by the RAPD analysis. **Japanese Journal of Genetics 69**:713-718.

Hartman, H. T., and D. E. Kester. 1983. Plant propagation, principles and practices, 4th Edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

He, S., H. Ohm, and S. Mackenzie. 1992. Detection of DNA sequence polymorphisms among wheat varieties. **Theoretical Applied Genetics 84**:573-578.

Hedrick, P. 1992. Shooting the RAPDs. **Nature 355**:679-680.

Horsch, R. B., J. E. Fry, N. L. Hoffmann, D. Eighholtz, S. G. Rogers, and R. T. Fraley. 1985. A simple and general method for transferring genes into plants. **Science 227**:1229-1231.

Hu, J., and C. F. Quiros. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. **Plant Cell Reports 10**:505-511.

Iqbal, M. J., D. W. Paden, and A. L. Rayburn. 1995. Clonal stability of RAPD markers in three *Rhododendron* species. **Journal of Environmental Horticulture 13**(1):43-46.

Jarret, R. L., and N. Bowen. 1994. Simple sequence repeats (SSRs) for sweetpotato germplasm characterization. **Plant genetic resources newsletter 100**:9-11.

Karp, A., and S. W. J. Bright. 1985. On the causes and origins of somaclonal variation. **Oxford Surveys of Plant Molecular & Cell Biology 2**:199-234.

Kidwell, K. K., and T. C. Osborn. 1993. Variation among alfalfa somaclones in copy number of repeated DNA sequences. **Genome 36**:906-912.

Kim, J. 1993. Improving the accuracy of phylogenetic estimation by combining different methods. **Systematic Biology 42**:331-340.

Klein-Lankhorst, R. M., A. Vermunt, R. Weide, T. Liharska, and P. Zabel. 1991. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). **Theoretical Applied Genetics 83**:108-114.

Klerk, G. J. 1990. How to measure somaclonal variation. **Acta Botanica Neerlandica 39**:129-144.

Kyte, L. 1987. Plants From Test Tubes, Revised Edition Edition. Timber Press, Portland, Oregon.

Lacy, R. C. 1992. The effects of inbreeding on isolated populations: Are mimimum viable populations sizes predictable? Pages 277-296 *in* P. L. Fiedler and S. K. Jain, editors. Conservation Biology - the theory and practice of natuyre conservation preservation and management. Chapman and Hall, New York.

Lamboy, W. F. 1994a. Computing genetic similarity coefficients from RAPD data: Correcting for the effects of PCR artifacts caused by variation in experimental conditions. **PCR Methods and Applications** 4:38-43.

---. 1994b. Computing genetic similarity coefficients from RAPD data: the effects of PCR artifacts. **PCR Methods and Applications** 4:31-37.

Lammers, T. G. 1989. Revision of *Brighamia* (Campanulaceae: Lobelioideae), a caudiciform succulent endemic to the Hawaiian Islands. **Systematic Botany 14**:133-138.

---. 1991. Systematics of *Clermontia* (Campanulaceae - Lobelioideae): Systematic Botany Monographs. Volume 32. The American Society of Plant Taxonomists, Ann Arbor, Michigan.

---. 1992. Two new combinations in the endemic Hawaiian genus *Cyanea* (Campanulaceae: Lobelioideae). **Novon 2**:129-131.

Lammers, T. G., and D. H. Lorence. 1993. A new species of *Cyanea* (Campanulaceae: Lobelioideae) from Kaua`i, and the resurrection of *Cyanea remyi*. **Novon 3**:431-436.

Lammers, T. G., T. J. Givnish, and K. J. Sytsma. 1993. Merger of the endemic Hawaiian genera *Cyanea* and *Rollandia* (Campanulaceae: Lobelioideae). **Novon 3**:437-441.

Larkin, P. J., and W. R. Scowcroft. 1981. Somaclonal variation — a novel source of variability from cell cultures for plant improvement. **Theoretical Applied Genetics 60**:197-214.

Linsmaier, E. M. and F. Skoog. 1965. Organic growth factor requirements of tobacco tissue cultures. **Physiologia Plantarum 18:** 100-127.

Lloyd, G., and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. **Proceedings of the International Plant Propagators' Society 30**:421-427.

Luque, T., C. Ruiz, J. Avalos, I. L. Calderon, and M. E. Figueroa. 1995. Detection and analysis of genetic variation in *Salicornieae (Chenopodiaceae)* using random amplified polymorphic DNA (RAPD) markers. **Taxon 44**:53-63.

Marsolais, J. V., J. S. Pringle, and B. N. White. 1993. Assessment of random amplified polymorphic DNA (RAPD) as genetic markers for determining the origin of interspecific lilac hybrids. **Taxon 42**:531-537.

Martin, G. B., J. G. K. Williams, and S. D. Tanksley. 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. **Proceedings of the National Academy of Sciences 88**:2336-2340.

Michelmore, R. W., I. Paran, and R. V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. **Proceedings of the National Academy of Sciences 88**:9828-9832.

Morel, G. M. 1960. Producing virus-free cymbidiums. **American Orchid Society Bulletin 29**:495-497.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. **Physiologia Plantarum 15**:473-497.

Nagy, G. 1995. Haberlandt Gottlieb. The establisher of the plant tissue culture. **Chronica Horticulturae 35**:15.

N'Goran, J. A. K., V. Laurent, A. M. Risterucci, and C. Lanaud. 1994. Comparative genetic diversity studies of *Theobroma cacao* L. using RFLP and RAPD markers. **Heredity 73**:589-597.

Pierik, R. L. M. 1987. In Vitro Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht.

Power, J. B., S. E. Cummins, and E. C. Cocking. 1970. Fusion of isolated protoplasts. **Nature 225**:1016-1018.

Puterka, G. J., W. C. Black IV, W. M. Steiner, and R. L. Burton. 1993. Genetic variation and phylogenetic relationships among worldwide collections of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), inferred from allozyme and RAPD-PCR markers. **Heredity 70**:604-618.

Rublio, A., V. Chávez, A. P. Martínez and O. Martínez-Vázquez. 1993. Strategies for the recovery of endangered orchids and cacti through in-vitro culture. **Biological Conservation 63:**163-169.

Rock, J. F. 1919. A monographic study of the Hawaiian species of the tribe Lobelioideae family Campanulaceae. **Memoirs of the Bernice Pauahi Bishop Museum VII**:1-394.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. **Science 239**:487-491.

Schieder, O., and I. K. Vasil. 1980. Protoplast fusion and somatic hybridization. Pages 21-46 *in* I. K. Vasil, editor. Perspectives in plant cell and tissue culture, International review of cytology Edition. Volume Supplement 11B. Academic Press, New York.

Scott, M. P., K. M. Haymes, and S. M. Williams. 1992. Parentage analysis using RAPD PCR. **Nucleic Acids Research 20**:5493.

Scowcroft, W. R., S. A. Ryan, R. I. S. Brettel, and P. J. Larkin. 1984. Somaclonal variation: a 'new' genetic resource. Pages 258-267 *in* J. H. W. Holden and J. T. Williams, editors. Crop genetic resources: conservation & evaluation. George Allen & Unwin, London.

Scragg, A. H., and M. W. Fowler. 1985. The mass culture of plant cells. Pages 103-128 *in* I. K. Vasil, editor. Cell Culture and Somatic Cell Genetics of Plants. Volume 2. Academic Press, New York.

Skoog, F., and C. O. Miller. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. **Symp. Soc. for Exp. Biol. 11**:118-131.

Swensen, S. M., G. J. Allan, M. Howe, W. J. Elisens, S. A. Junak, and L. H. Reiseberg. 1995. Genetic analysis of the endangered island endemic *Malacothamnus fasciculatus* (Nutt.) Greene var. *nesioticus* (Rob.) Kearn. (Malvaceae). **Conservation Biology 9**:404-415.

Towill, L. E., and E. E. Roos. 1989. Techniques for preserving of plant germplasm. Pages 379-403 *in* L. Knutson and A. K. Stoner, editors. Beltsville Symposia in Agricultural Research, 13: Biotic diversity and germplasm preservation, global imperatives. Kluwer Academic Publishers, The Netherlands.

Vacin, E. F., and F. W. Went. 1949. Some pH changes in nutrient solutions. **Botanical Gazette 110**:605-613.

Van Buren, R., K. T. Harper, W. R. Andersen, D. J. Stanton, S. Seyoum, and J. L. England. 1994. Evaluating the relationship of autumn buttercup (*Ranunculus acriformis* var. *aestivalis*) to some close congeners using random amplified polymorphic DNA. **American Journal of Botany 81**:514-519.

Van Coppenolle, B., I. Watanabe, C. Van Hove, and S. R. McCouch. 1993. Genetic diversity and phylogeny analysis of *Azolla* based in DNA amplification by arbitrary primers. **Genome 36**:686-693.

Vasil, I. K. 1980. Androgenetic haploids. Pages 195-223 *in* I. K. Vasil, editor. Perspectives in plant cell and tissue culture, International review of cytology Supplement 11A. Academic Press, New York.

Vasil, I. K., and V. Vasil. 1980. Isolation and culture of protoplasts. Pages 1-19 *in* I. K. Vasil, editor. Perspectives in plant cell and tissue cutlure, International Revies of Cytology Supplement 11B. Academic Press, New York.

Vierling, R. A., and H. T. Nguyen. 1992. Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. **Theoretical Applied Genetics 84**:835-838.

Virk, P. S., B. V. Ford-Lloyd, M. T. Jackson, and H. J. Newbury. 1995. Use of RAPD for the study of diversity within plant germplasm collections. **Heredity 74**:170-179.

Vosberg, Hans-P. 1989. The polymerase chain reaction: an improved method for the analysis of nucleic acids. **Human Genetics 83**:1-15.

Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1990. Manual of flowering plants of Hawai'i. Volume 1-2. Bishop Museum, Honolulu.

Waycott, W., and S. B. Fort. 1994. Differentiation of nearly identical germplasm accessions by a combination of molecular and morphologic analyses. **Genome 37**:577-583.

Welsh, J., and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. **Nucleic Acids Research 18**:7213-7218.

White, P. R. 1963. The cultivation of animal and plant cells, 2nd Edition. Ronald Press, New York.

Wilde, J., R. Waugh, and W. Powell. 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. **Theoretical Applied Genetics 83**:871-877.

Wilkins, C. P., and J. H. Dodds. 1983. The application of tissue culture techniques to plant genetic resources. **Science Progress, Oxford 68**:259-284.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbritary primers are useful as genetic markers. **Nucleic Acids Research 18**:6531-6535.

Withers, L. A. 1991a. In-vitro conservation. **Biological Journal of the Linnean Society 43**:31-42.

---. 1991b. Tissue culture in the conservation of plant genetic resources. Pages 1-18 *in* FRIM/MNCPGR, editor. Conservation of Plant Genetic Resources Through *In Vitro* Methods. FRIM/MNCPGR.

Withers, L. A., S. K. Wheelans, and J. T. Williams. 1990. *In vitro* conservation of crop germplasm and the IBPGR databases. **Euphytica 45**:9-22.

Yang, X., and C. Quiros. 1993. Identification and classification of celery cultivars with RAPD markers. **Theoretical Applied Genetics 86**:205-212.

Yoshimura, S., A. Yoshimura, and N. Iwata. 1992. Simple and rapid PCR method by using crude extracts from rice seedlings. **Japanese Journal of Breeding 42**:669-674.

Zenk, M. H., M. Ruffer, T. M. Kutchan, and E. Galneder. 1988. Biotechnological approaches to the production of isoquinoline alkaloids. Pages 213-227 *in* G. Bock and J. Marsh, editors. Application of plant and cell tissue culture. John Wiley & Sons, Chichester.