CHARACTERIZATION AND PROPAGATION OF *PA'UOHI'IAKA* (*JACQUEMONTIA SANDWICENSIS A. GRAY)* FOR POTENTIAL USE AS A HANGING BASKET PLANT

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ABSTRACT

The use of native Hawaiian plants as ornamentals has increased in the last 28 years. Despite active promotion, efforts to expand selections for horticultural use have been minimal. Pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) is a prostrate-growing, endemic vine commonly found in coastal areas. In the wild, morphological variation exists but efforts to collect and characterize these variations for hanging basket use have been limited. To develop the use of pa‘uohi‘iaka as a hanging basket plant, six accessions were collected, characterized and assessed for rooting response. Morphological characterization indicated that each accession has its own unique set of qualitative and quantitative characters. Principal component analysis identified leaf shape, leaf length, adaxial and abaxial stem color, length of internodes and length of lateral branches, flower color and number and flowers as important characters that contribute to the variation of the six accessions. Cluster analysis revealed three distinct groups. Lyon Arboretum, Puhala Bay and South Point were selected for further evaluation because of their shorter internodes and lateral branching. Rooting response was associated with high leaf retention and longer cutting length (i.e. four nodes). Leaf retention was negatively affected by leaf pubescence. Due to poor rooting and survival of stem cuttings after transplanting, the South Point accession was dropped for further evaluation.

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CHAPTER 1

INTRODUCTION

The use of native Hawaiian plants as ornamentals has increased in the last 28 years due to state laws that require its use in publicly funded landscaping projects (Acts 73 and 236). It has been encouraged to mitigate the spread of invasive species and to conserve the local biodiversity (Tamimi, 1999; Ricordi et al., 2014). Promotion of native plants in nurseries and garden centers can lessen demand and/or replace ornamentals that have escaped cultivation and pose threats to natural areas (Ruchala, 2002). Despite active promotion, limited plant availability and the lack of knowledge on the use of native Hawaiian plants continue to be key constraints (Tamimi, 1999 and Ricordi et al., 2014). Studies to develop feasible propagation and production methods are important to increase the availability of native plants in the nursery trade (Ruchala, 2002).

Pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) is a prostrate, endemic vine that grows in coastal habitats (Wagner et al., 1991). It was formerly classified as *J. ovalifolia* subsp. *sandwicensis*, but molecular and morphological data support it as being a distinct species (Shay and Drake, 2018; Namoff et al 2010). As an ornamental, pa‘uohi‘iaka is typically used as a groundcover in landscaping but will also do well in a large pot or hanging basket (Bornhorst and Rauch, 2003). In the wild, morphological variations of pa‘uohi‘iaka exist. Leaves and stems can be glabrous to densely tomentose and its flowers maybe pale blue or white (Wagner et al., 1991). Inflorescence branches and calyces can also vary greatly (Robertson, 1974). Leaf shape can range from elliptic to suborbicular (Wagner et al., 1991). These variations exist within islands, populations or even on some individual plants (Robertson, 1974).

Despite the existence of morphological variation, efforts to collect and characterize these variations for horticultural use have been limited. To increase the variety of pa‘uohi‘iaka in the nursery trade, collection and characterization of wild and cultivated plant material for ornamental use are key activities. Accurate documentation, characterization and evaluation of germplasm are essential for effective conservation and use (Biodiversity International, 2007). Characterization of accessions can identify the ornamental potential of a collection and can also provide information for future ornamental breeding programs (de Souza et al, 2012).

Due to poor consumer receptivity to native plants (Hooper et al., 2008), studies to evaluate new uses are needed. Urbanization in Hawaii provides an opportunity to introduce new uses for pa‘uohi‘iaka. Cultivating these plants in hanging baskets is ideal in an urban setting. Hanging baskets can fill the need for vertical gardening in small homes that lack landscape spaces (Starman and Eixmann, 2006).

To increase the availability of pa‘uohi‘iaka selections in the nursery trade, improved propagation protocols are necessary. Vegetative propagation is the preferred method for ornamental production. Vegetative propagation maintains uniformity and is a practical solution to assure a dependable supply of desired genotypes (Zohary, 2001). Although pa‘uohi‘iaka can be easily propagated from four node stem cuttings (Bornhorst, 1996), propagation using single node cuttings may be useful to increase limited planting material.

In this thesis, morphological characteristics and rooting response of six pa‘uohi‘iaka accessions from wild and cultivated sources were assessed. Morphological characterization was done to determine the identifiable and unique set of characters in each accession The information provided by morphological characterization was also used to identify and select accessions that are highly suitable as a hanging basket/container plant. Principal component analysis and cluster analysis were conducted to determine the important morphological characters that contribute to the variation and similarity of the accessions.

Aside from morphological characterization, the rooting response of the six accessions were evaluated using four node and single node stem cuttings propagated in two propagating dates (March and October 2018). The goal was to determine accessions that are most responsive to rooting and to test the feasibility of using single node stem cutting.

The information generated from the morphological characterization and rooting response evaluation of the six accession will aid development pa‘uohi‘iaka as a potential hanging basket plant.

CHAPTER 2

MORPHOLOGICAL CHARACTERIZATION AND IDENTIFICATION OF PA‘UOHI‘IAKA (*JACQUEMONTIA SANDWINCENSIS* A. GRAY) ACCESSIONS FOR HANGING BASKET USE

Introduction

The use of native plants in landscaping has been actively promoted in Hawaii for the past 28 years. Despite increased use of native plants in landscaping, a number of challenges still exist preventing their wide usage. Landscape professionals find it difficult to specify native plants instead of non-native plant species due to the lack of availability of desired plant species and sizes as well as the lack of consumer receptivity and customer unfamiliarity with native plants (Hooper et al., 2016, Ricordi et al., 2014). To increase availability of native Hawaiian plants, new species and selections must be identified and evaluated for various uses such as hanging basket plants.

Pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) (Convolvulaceae) is a perennial vine endemic to Hawai‘i. It is commonly found on all main islands at elevations ranging from sea level to 30.4 m (100 ft) (Wagner et al., 1999). It is a component of coastal vegetation that often grows with *Sida fallax* (Shay & Drake, 2018) and is highly salt and wind tolerant (Bezona et al., 2001). According to Wagner et al (1999), pa‘uohi‘iaka can be glabrous to densely tomentose, with flowers ranging from white to pale blue. Despite the existence of these variations within the species, there has been limited efforts to collect and identify selections for naming as cultivars.

As an ornamental plant, pa‘uohi‘iaka has been commonly used as a ground cover for landscaping. Although it can also be used as a hanging basket or potted plant (Bornhorst & Rauch, 2003), no selections have been identified for this purpose. In this study, six accessions, collected from Oahu, Maui and Hawaii Island, were grown in pots and characterized to identify selections for potential use as a hanging basket/container plant. A principal component analysis and cluster analysis was also done to determine the important morphological characters that contribute to the variation and similarity of the accessions

Materials and Methods

The study was conducted from October 10, 2017 to February 18, 2018 at Magoon Research Facility, University of Hawaii at Manoa, Honolulu, USA (Lat: 21.306163, Long: -157.809243, Elevation: ~48 m above sea level). Pa‘uohi‘iaka accessions were collected as stem cuttings *in situ* or from cultivated sources on Oahu, Maui and Hawaii islands (Table 1.1). Stock plants were established by rooting cuttings on a mist bench in 1:1 by volume mix of perlite and vermiculite. Rooted cuttings were planted in 15 cm (6 in) plastic pots filled with a 1:1 by volume mix of coir dust and 1.9 cm (3/4 inch) diameter cinder.

Table 1. 1. Provenance information of pa‘uohi‘iaka accessions used for the characterization study.

Accession Name/Provenance	Collection Site	Genetic Status
Ahihi-Kinau	Maui Nui Botanical Gardens, Maui	Wild
Lyon Arboretum*	Leeward Community College, Oahu	Cultivated, seed bank accession
McGregor	Maui Nui Botanical Gardens, Maui	Wild
Puhala Bay	Maui Nui Botanical Gardens, Maui	Wild
Shidler College*	Shidler College Business School, Oahu	Cultivated
South Point	South Point, Hawaii	Wild

* unknown provenance

Plants used for morphological characterization were propagated from stock plants on October 10, 2017. Four to six node cuttings of each accession were treated with Hormex 1 (1000ppm IBA) and inserted vertically in 15 cm (6 in) pots with equal parts of perlite and vermiculite. Cuttings were allowed to root in a mist bench inside a glasshouse for 30 days. Mist was programmed to turn on for 10 seconds every six minutes. Rooted cuttings were planted vertically in Deepot Cells D40H (volume: 656 ml; Stuewe and Sons) filled with equal parts of coconut coir and cinder. Controlled release fertilizer (Nutricote 13-4.8-9.1, Arysta LifeScience) was also incorporated into the growing media at a rate of 6,992 grams/cubic meter (198 grams/cubic foot). Deepot Cells were placed under full sun and watered twice daily for five minutes through sprinkler irrigation. Each pot received approximately 220 ml of water daily. After one month, the plants were potted in 15 cm (6 in) diameter pots using equal parts of coconut coir and cinder (Appendix Figure 1). Plants were held for another month under the same outdoor conditions. Since each pot developed only one main stem (Appendix Figure 1.1), plants were pruned 10 cm (4 inches) from the base to promote lateral branching.

Morphological characterization

Morphological characterization was conducted one month after pruning the plants. Six plants were used to record a total of 17 qualitative and quantitative traits. Qualitative traits recorded for each accession were flower color, stem color, leaf pubescence and stem pubescence. Flower color and stem color were determined using the Royal Horticultural Society 5th edition (2007) color swatches. Leaf pubescence of the six accessions was categorized either as dense, medium or absent, while stem pubescence of the six accessions was categorized either as dense, medium or sparse. Images of the plant and its leaves and flowers were recorded using both a digital camera (Canon EOS Rebel T7i) and a flatbed scanner (Epson Model EU 88).

Aside from qualitative characters, quantitative characters were also measured from each plant. Average leaf length, average leaf width, average leaf thickness and average petiole length were measured from 10 mature leaves of each plant in each accession. Average peduncle length and diameter, and floral diameter were recorded by randomly selecting 10 flowers in the mid portion of the lateral branches. The average length of internodes was obtained by individually measuring all the internodes of the longest stem. The average number of lateral branches and the average length of lateral branches for each plant in each accession were calculated. The average number of flowers and average number of pre-formed roots (i.e. nodes with pre-formed roots) for each plant in each accession were also calculated.

Statistical and Principal Component Analysis

Quantitative data were analyzed as a Randomized Complete Block Design with the six sample plants that served as replicates in Statistix 10 software (Analytical Software) using the ANOVA function. Tukey HSD was used to separate accession means.

To determine relationships and similarities among the six accessions and to determine the correlation of morphological characters, principal component analysis and a cluster dendrogram was generated using the 17 quantitative and qualitative characters. The qualitative data were transformed by assigning ordinal numbers for each character state. Data were scaled using prcomp function and were visualized using a combination of ‘FactoMiner’ (Husson et al., 2018) and ‘factoextra’ (Kassambara and Mundt, 2017: Kasambara, 2018) packages in R studio version 3.4.4 (RStudio, Inc.). Aside from conducting and plotting the Principal Component Analysis, bi-plot, factor map and individual factor maps were also generated. Contributions of the morphological characters in the components were also calculated. Principal Component loadings greater than 0.3 or less than -0.3 were accepted as significant (Peres-Neto et al., 2010; Richman, 1988). To generate the cluster dendrogram, packages ‘dyplyr’, ‘plyr’ and ‘ggplot2’ (Wickham et al., 2019) were also installed in R studio version 3.4.4.

Results

Qualitative Data

Qualitative morphological characters indicate that each accession has its own unique set and combination of characters. Leaf shape, flower color, stem color and the degree of stem and leaf pubescence can be used to identify an accession (Table 1.2).

Table 1.2.Qualitative morphological characters assessed in six accessions of pa‘uohi‘iaka.

Accession	Leaf Shape	Leaf Pubescence	Stem Pubescence	Stem Color*	Flower Color*
Ahihi-Kinau	Obovate	None	Sparse	Purple N77A	Violet-Blue 91C
Lyon Arboretum	Obovate	None	Medium	Purple N77A	White N155 A
McGregor	Ovate	Dense	Dense	Adaxial: Yellow-Green 144D Abaxial: Purple N77 C	White N155 A
Puhala Bay	Ovate with undulate or wavy leaf margin	Dense	Dense	Yellow-Green 144D	Violet-Blue 91B
Shidler College	Obovate	None	Sparse	Purple N79B	Violet-Blue 91B
South Point	Ovate	Medium	Medium	Yellow-Green 144D	White N155 A

* Stem and flower color were determined using the Royal Horticultural Society Colour Chart (2007).

Figure 1.1. Leaves, flowers and stems of six pa‘uohi‘iaka accessions; A) Ahihi-Kinau, B) Lyon Arboretum, C) McGregor, D) Puhala Bay, E) Shidler College and F) South Point.

Quantitative Data

At one month after pruning, significant differences were found among accessions for average leaf length, leaf thickness, petiole length, number of lateral branches, length of internodes, number of flowers and floral diameter (Table 1.3). No significant differences between accessions were detected for leaf width (*P=0.30*), peduncle length (*P=0.09*), peduncle diameter (*P=0.33*), length of lateral branches (*P=0.52*) and number of pre-formed roots (*P= 0.2850*).

Table 1.3. Quantitative data recorded from six accessions of pa‘uohi‘iaka one month after pruning. Means and standard errors presented were rounded off to the nearest tenths. Values with common letters are not significantly different using Tukey’s HSD pairwise comparison test at P < 0.05, n=6.

Accessions	**Leaf Length (cm)**	**Leaf Thickness (mm)**	**Petiole Length (cm)**	**Number of Lateral Branches**	**Length of Internodes (cm)**	**Flower Diameter (mm)**	**Number of Flowers**
Ahihi-Kinau	4.2±0.1ab	0.42±0.33 a	1.5±0.1a	10.0±0.8 ab	1.7±0.2 ab	11.3±0.4 b	2.8±0.8 ab
Lyon Arboretum	4.3±0.3 ab	0.33±0.33 b	1.4±0.1 a	13.3±1.8 a	1.4±0.2 b	10.8 b	0.16±0.2 c
McGregor	3.0±0.1c	0.31±0.33 b	1.0 b	9.7±1.2 ab	1.7±0.2 ab	13.0±0.4 b	4.5±0.8 a
Puhala Bay	3.4±0.2bc	0.45±0.33 a	1.4±0.1 ab	12.3±1.6 ab	1.1±0.1 b	11.4±0.4 b	0.7±0.5 bc
Shidler College	4.7±0.3a	0.32±0.33 ab	1.6±0.1 a	7.0±1.0 b	2.1±0.3 a	14.5±0.4 a	2.2±0.5 abc
South Point	2.9±0.2c	0.33±0.33 b	1.3±0.1 ab	8.2±0.4 ab	1.3±0.2 b	12.0 ±0.3 b	1.7±0.3 bc

Significant differences (*P<0.01*) in length of leaves were observed among the six different accessions of pa‘uohi‘iaka. The Shidler College accession exhibited the longest leaf length (4.7cm) while South Point exhibited the shortest leaf length. Significant differences (*P<0.01*) in leaf thickness were also observed between accessions. Puhala Bay possessed the thickest leaves (0.5mm) while South Point possessed the thinnest leaves (0.3 mm). Average petiole length among accessions were significantly different (*P<0.01*). The McGregor accession exhibited the shortest petiole length (1 cm) while the Shidler College accession exhibited the longest petiole length (1.6 cm). Ahihi-Kinau and Lyon Arboretum exhibited petiole lengths that were not significantly different from Shidler College. Puhala Bay and South Point accessions possessed intermediate petiole lengths.

Average number of lateral branches among accessions was significantly different ($P<0.01$). Lyon Arboretum exhibited the highest number of lateral branches (13.3) while Shidler College only produced an average of seven lateral branches. The rest of the accessions possessed intermediate lateral branch numbers. The average length of internodes of the main stem among the six accessions was significantly different ($P<0.01$). The Shidler College accession exhibited the longest internodes (2.1 cm) while Puhala Bay exhibited the shortest internodes (1.0 cm). Ahihi-Kinau and McGregor exhibited intermediate internode lengths while Lyon Arboretum and South Point accessions exhibited similar internode lengths as Puhala Bay.

Average number of flowers between accessions was significantly different ($P<0.01$). Lyon Arboretum exhibited the least number of flowers while McGregor exhibited the most number of flowers. The rest of the accessions exhibited intermediate flower numbers. Average floral diameter among accessions was significantly different ($P<0.01$). The Shidler College accession exhibited the widest floral diameter (14.5 mm) among all accessions.

Principal Component Analysis (PCA)

Table 1.4. Principal component analysis of the 17 morphological characters.

	PC1	**PC2**
Eigenvalue	0.90	0.33
Proportion of Variance	0.3961	0.2918
Morphological Characters		
Leaf Shape	0.014786	**-0.34765**
Leaf Length (cm)	**-0.30864**	-0.26303
Leaf Width (cm)	-0.29103	-0.28629
Leaf Thickness (mm)	-0.01444	-0.05841
Leaf Pubescence	0.284544	0.223899
Adaxial Stem Color	**-0.35203**	-0.1729
Abaxial Stem Color	-0.23169	**0.300933**
Density Stem Pubescence	0.226527	0.089506
Length of Internodes (cm)	**-0.37612**	-0.16403
Number of Lateral Branches	0.17827	-0.16403
Length of Lateral Branches (cm)	**-0.32344**	0.212551
Number preformed roots	-0.04846	0.251474
Peduncle Length (cm)	-0.0358	0.251474
Peduncle Diameter(mm)	0.249987	-0.29847
Flower Color	**0.317332**	0.067092
Number of Flowers	-0.08901	**0.323439**
Flower Diameter (mm)	-0.23901	0.158758

Table 1.4 shows that 90% of the variation in the morphological characters were explained by PC1 and only 33% explained by PC2. Since there is a decrease in the variation explained by PC3 in comparison to PC1 and PC2, only the first two components were reviewed for the variables (morphological characters) that were used in constructing the variable factor map (Figure 1.2). Among the 17 morphological characters, five make significant contributions in PC1 and three in PC2. The significant morphological characters in PC1 are length of internodes, stem color (adaxial), length of lateral branches, flower color and leaf length. In PC2, leaf shape, number of flowers and stem color (abaxial) are significant.

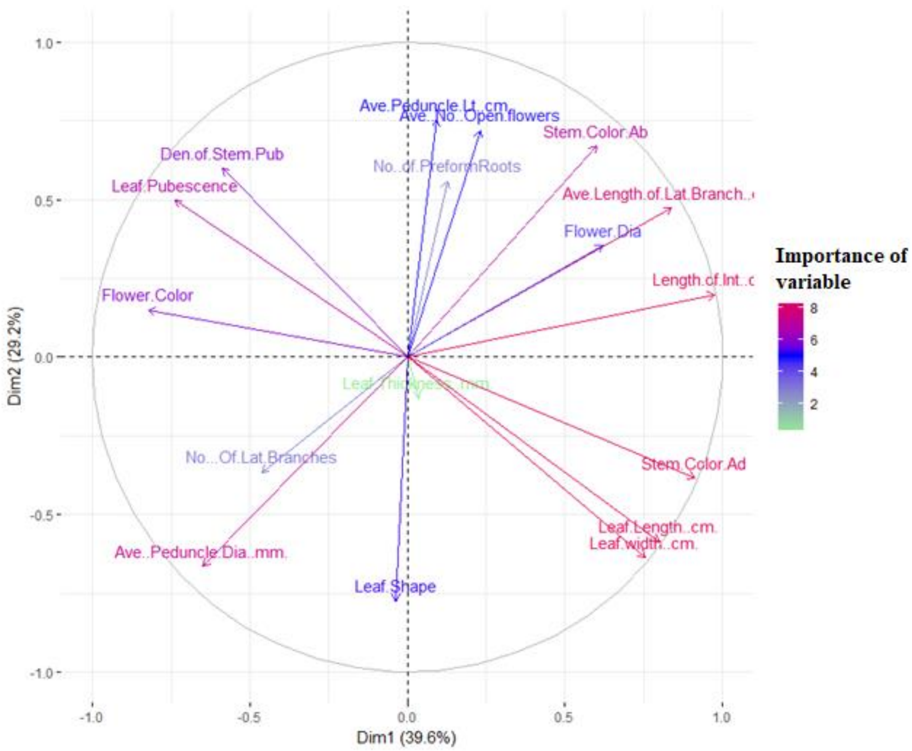


Figure 1.2. Variable factor map of 17 morphological characters that contributed to the construction of PC1 and PC2. Proximity of arrow points to the perimeter of the circle indicate strength of correlation between a morphological character and PC1 and PC2. Colors of morphological characters represent the strength of contribution and importance of each morphological character (Low: Light green, Medium: Blue and Strong: Red) in the construction of PC1 and PC2.

Figure 1.2 illustrates the morphological characters that are well represented or important (long red arrows) and those are not (shorter blue and light green arrows). It also illustrates morphological characters that are negatively correlated with each other (opposite quadrants). Among the morphological characters, leaf thickness is the least important in the construction of the components. Those characters with strong contributions in the components are shown in Figures 1.3 and 1.4.

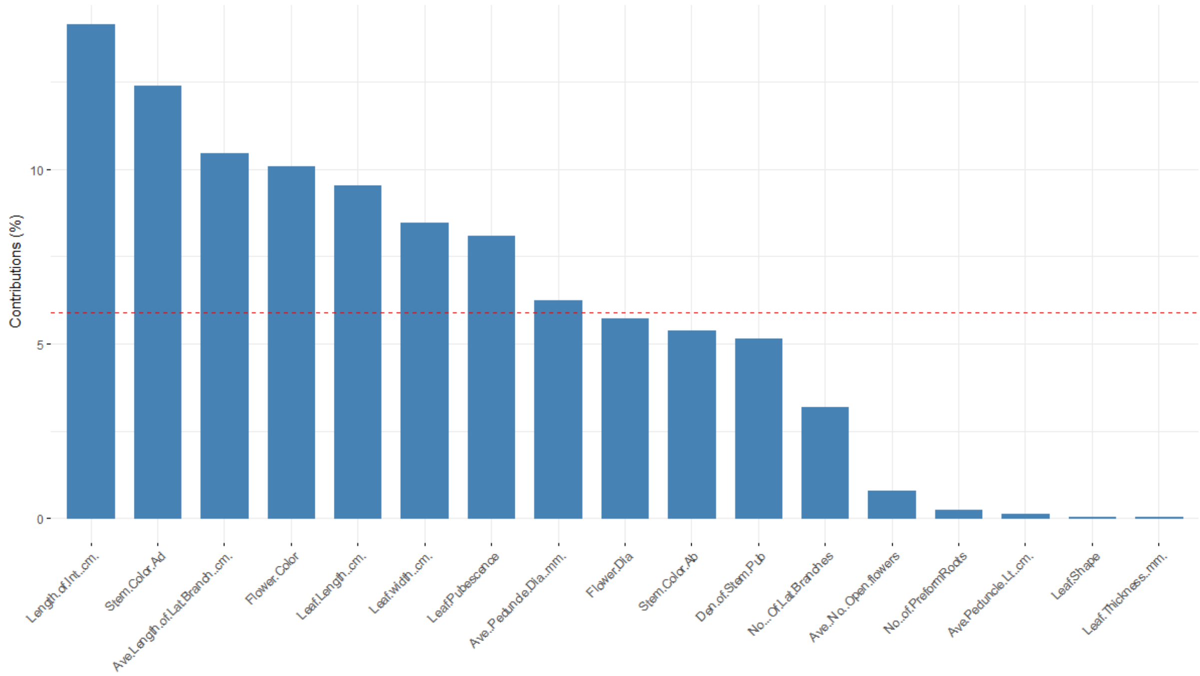


Figure 1.3. Top eight morphological characters of PCA that contributed to the construction of PC1. Red dashed line on the graph above indicates the expected average contributions of morphological characters to the construction of components.

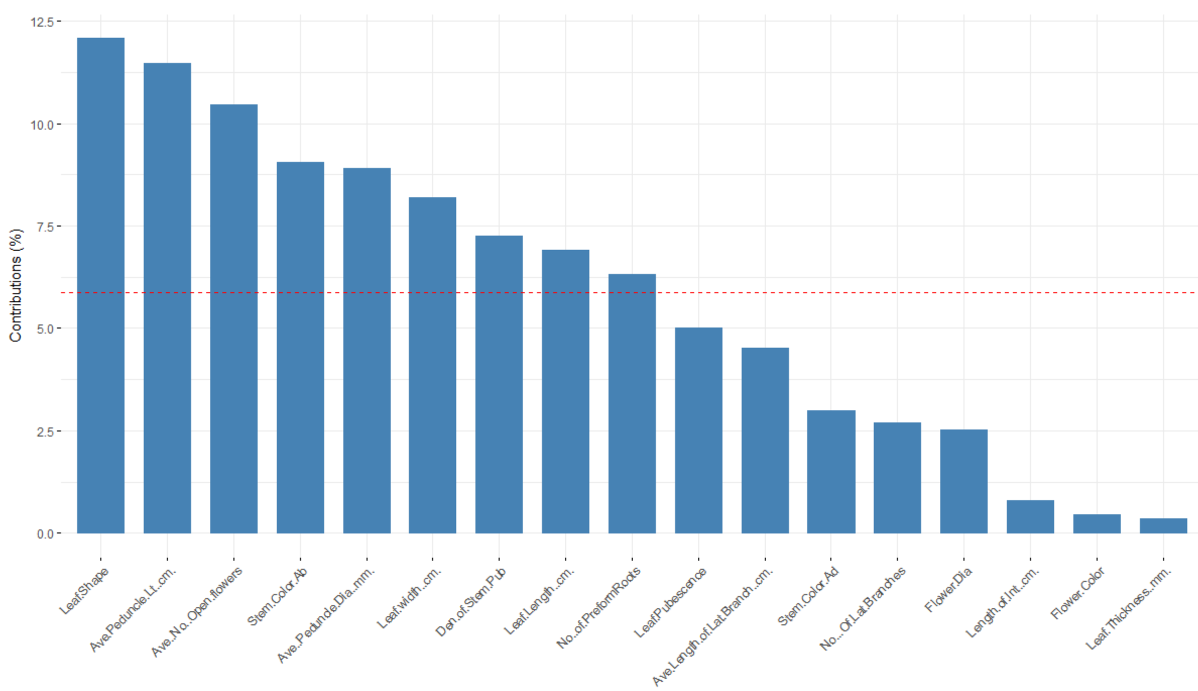


Figure 1. 4. Top nine morphological characters of PCA that contributed to the construction of PC2. Red dashed line on the graph above indicate the expected average contributions of morphological characters to the construction of components.

The morphological characters that had the most contribution to the construction of PC1 were length of internodes, stem color (adaxial), length of lateral branches, flower color, leaf length and width, leaf pubescence and peduncle diameter. The morphological characters that had the most contribution for PC2 were leaf shape, peduncle length, number of flowers, stem color (abaxial), peduncle diameter, leaf width, density of stem pubescence, leaf length and number of preformed roots. Characters like leaf thickness, number of lateral branches and flower diameter did not contribute for construction of these components.

Cluster Analysis

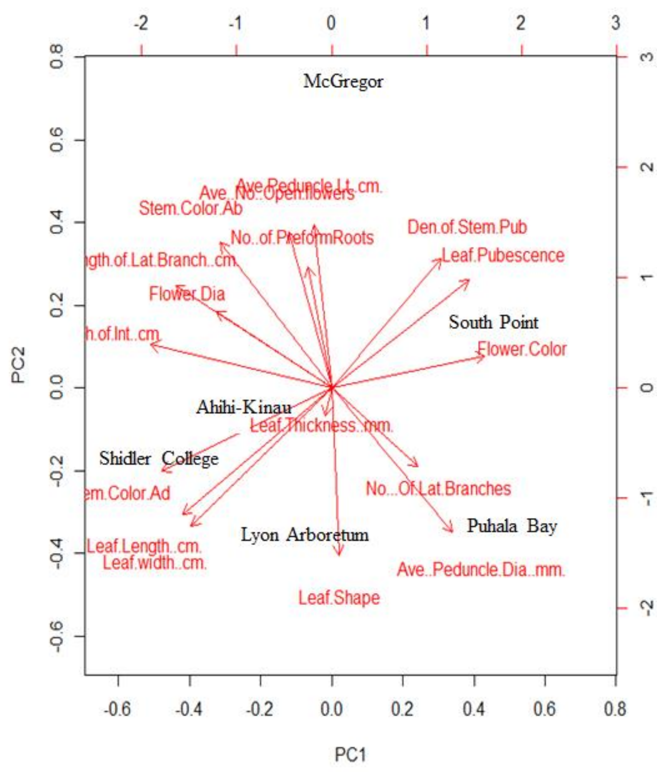


Figure 1.5. Bi-plot of six pa‘uohi‘iaka accessions generated by 17 qualitative and quantitative morphological characters.

Bi-plot of the six accessions (Figure 1.5) reveal that McGregor is associated with a set of characters that are not shared by other accessions. Leaf pubescence, density of stem pubescence, peduncle length, number of flowers and preformed roots, stem color (abaxial) makes the McGregor accession unique from the rest of the accessions and explains why it has its own cluster (Figure 1.8). South Point and Puhala Bay accessions are closer from one another and share characters such as number of lateral branches and average peduncle diameter. Ahihi-Kinau, Shidler College and Lyon Arboretum are also relatively closer from one another compared to the previous accessions. These accessions were grouped together because of likeness in morphological characters such as leaf shape, leaf length and width, and adaxial stem color. Because of these shared characteristics, they belong to the same cluster (Figure 1.6 and Figure 1.7).

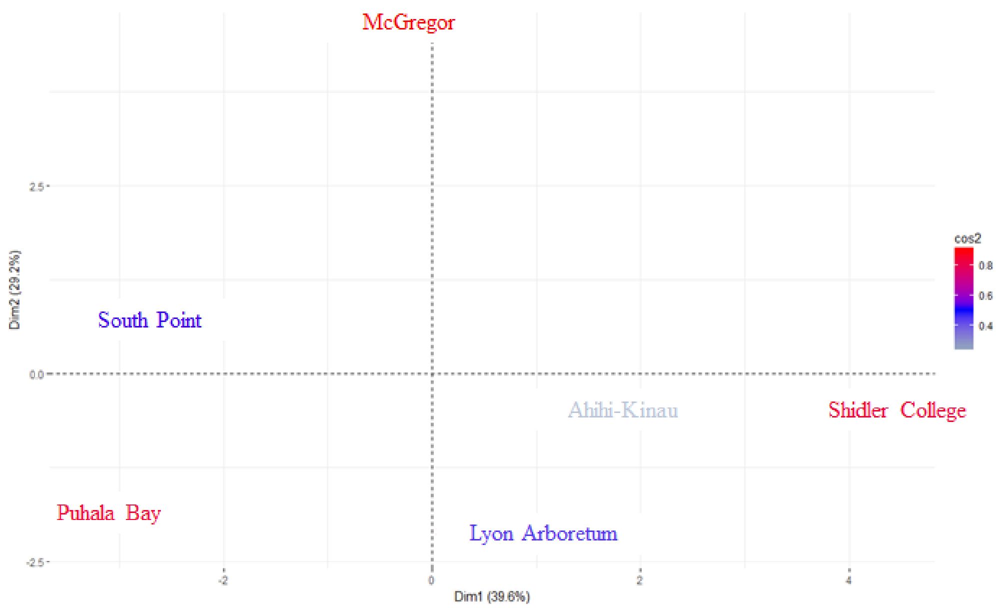


Figure 1.6. Individual factor map of the of the six pa'uohi'iaka accessions generated by the qualitative and quantitative morphologic characters. Colors of accessions represent the strength of each accession in the component.

The individual factor map (Figure 1.6) shows that Shidler College scored high while Ahihi-Kinau scored the lowest in component 1. McGregor and Puhala Bay accessions scored high in component 2. Morphological characters near each accession in the Bi-Plot (Figure 1.5) is what makes it score high in the individual factor map.

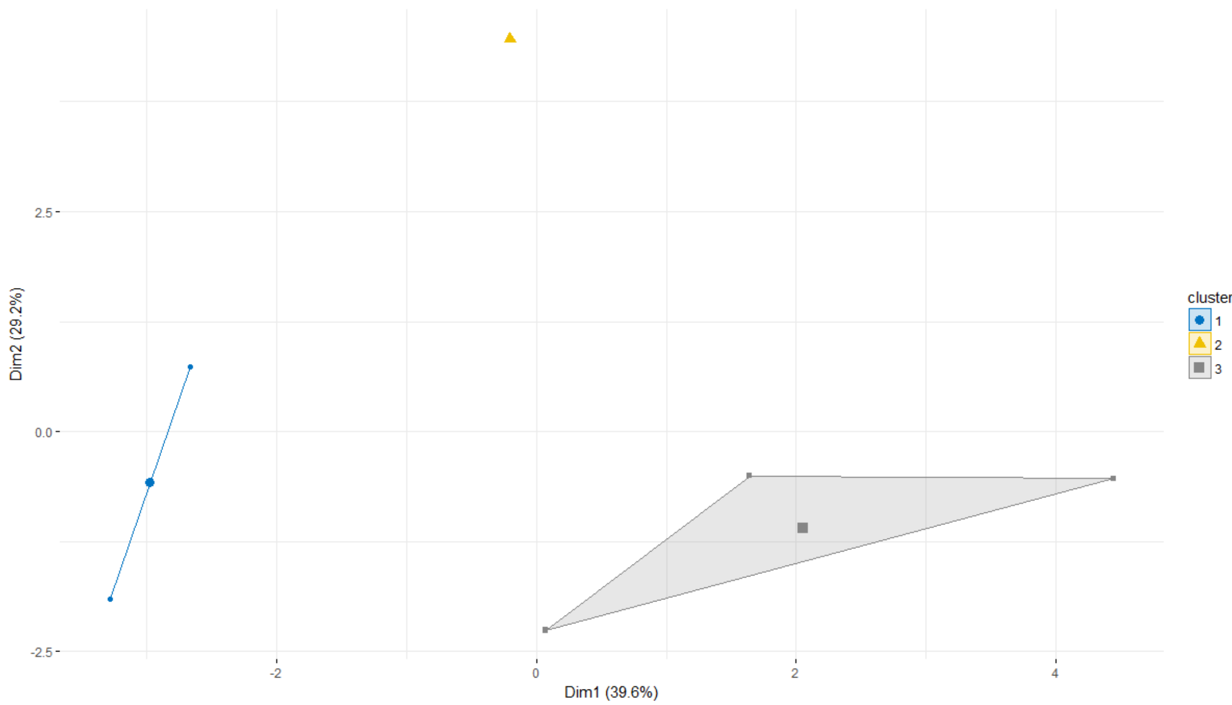


Figure 1. 7. Clustering of the six pa‘uohi‘iaka accessions generated by seventeen qualitative and quantitative morphologic characters. Colors represent each clusters.

Figures 1.7 and 1.8 reveal that the six accessions of pa‘uohi‘iaka fall under three major clusters. The first cluster contained the South Point and Puhala Bay accessions. The second cluster was the McGregor accession and the third cluster comprised of the Ahihi-Kinau, Shidler College and Lyon Arboretum accessions.

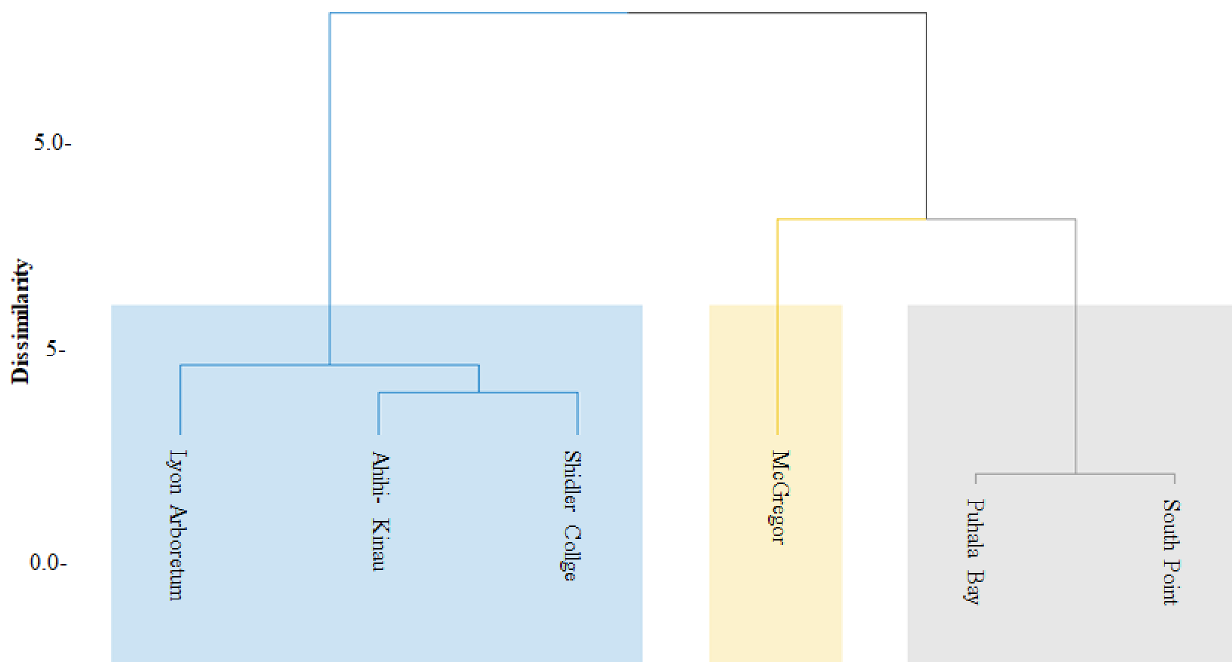


Figure 1.8. Cluster dendrogram of the six pa‘uohi‘iaka accessions generated by seventeen qualitative and quantitative morphologic characters.

Discussion

The results of this study indicate that each of the six accessions has its own unique set of qualitative and quantitative characters. No single morphological character significantly affects the variation of each of the accession but rather a combination of varying set of morphological characters. Morphological characters like leaf shape, leaf length, adaxial and abaxial stem color, length of internodes, length of lateral branches, flower color and number of flowers showed strong correlation and influence on the construction of PC1 and PC2 components. These characters showed a high level of importance as variables in the variation and strength of each accession. These characters also strongly influence the clustering pattern of the six accessions (Figure 1.2, Figure 1.3 and Figure 1.4).

The cluster analysis indicated three major clusters (Figure 1.8). Ahihi-Kinau, Lyon Arboretum and Shidler College formed one cluster. South Point and Puhala Bay accessions formed the second cluster while McGregor appears as a separate cluster. McGregor possesses traits that are unique compared to the South Point and Puhala Bay accessions (Figure 1.5). The distance in dissimilarity suggests that Ahihi-Kinau and Shidler College accessions are likely more similar to each other than to the Lyon Arboretum accession. Ahihi-Kinau and Shidler College are more closely related to Lyon Arboretum than South Point and Puhala Bay are to the McGregor accession cluster.

The bi-plot indicated that the six accessions can be divided into two major groups in terms of leaf shape, density of stem pubescence and leaf pubescence (Figure 1.5). Puhala Bay, South Point and McGregor possess an ovate leaf shape with medium to dense stem pubescence and medium to dense leaf pubescence. Ahihi-Kinau, Lyon Arboretum and Shidler College possess an obovate leaf shape with medium to sparse stem pubescence and non-pubescent leaves. Visually, the non-pubescent-leafed accessions have purplish stems while the pubescent-leafed accessions mostly exhibited green stems. The McGregor accession was an exception since it exhibited a purplish color on the adaxial part of the stem and green color on the abaxial portion of the stem (Figure 1.1).

Among the quantitative characters evaluated, the length of internodes and number of lateral branches were the two ideal traits for selecting accessions suitable for hanging basket use. In the variable factor map and Bi-plot, these two morphological characters belongs to a group that are located on opposing quadrants (Figure 1.2 and Figure 1.5). This fits with our criteria for compact form attributed by shorter internodes and increased number of lateral branches.

Among the six accessions, Puhala Bay, Lyon Arboretum and South Point responded well to pruning by exhibiting a compact form due to shorter internodes and a higher number of uniformly cascading lateral branches (Table 1.3 and Figure 1.3). In the bi-plot, these accessions were located close to the vector of number of lateral branches. This means that this particular trait is being shared by the three selected accessions. In contrast, McGregor, Shidler and Ahihi-Kinau are located on the opposite end (Figure 1.5). The selected accessions were also located on the opposite end of the vector of internode length because to the number of lateral branches (Figure 1.2). Due to these characteristics, Puhala Bay, Lyon Arboretum and South Point were selected for further evaluation as hanging basket plants.

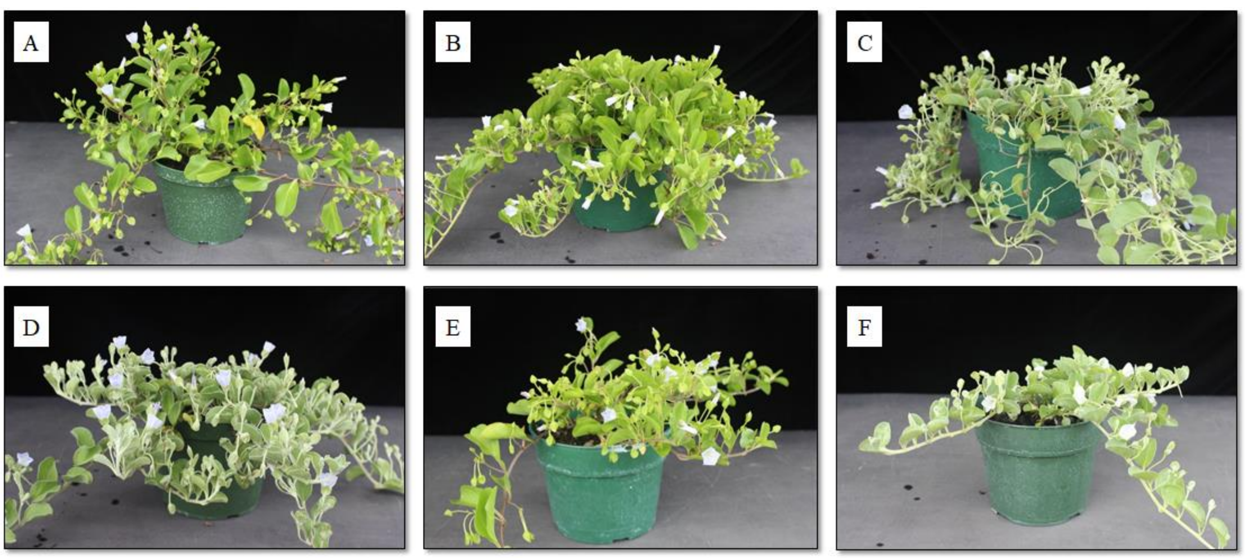


Figure 1.9. Whole plant of six pa‘uohi‘iaka accessions; A) Ahihi-Kina, B) Lyon Arboretum, C)McGregor, D) Puhala Bay, E) Shidler College and F) South Point.

Other accessions such as Ahihi-Kinau are vigorous, but do not have a compact appearance compared to Lyon Arboretum. Ahihi-Kinau could be an ideal landscape ground cover as it grows vigorously like Shidler College, an accession used in landscaping. Shidler College did not exhibit compact growth and might not be suitable for use in hanging baskets.

Among the six accessions, McGregor has the most number of flowers, does not possess compact growth and dense foliage under the conditions of this experiment.

This study revealed that within-species variation exists in pa‘uohi‘iaka collections. Morphological characterization served as a tool for identifying accessions with potential as a hanging basket plant. It also aided in determining the similarities and relationships between accessions. Internode length, number of lateral branches, and flower count are the most suitable characters to use in selecting wild-collected accessions for the purpose of hanging basket production.

CHAPTER 3

EVALUATION OF SINGLE AND FOUR NODE STEM CUTTINGS AS A PROPAGATION MATERIAL FOR SIX ACCESSIONS OF PA‘UOHI‘IAKA (*JACQUEMONTIA SANDWICENSIS* A. GRAY)

Introduction

Stem cuttings are one of the most common propagation methods employed due to low cost (Hartmann et al., 1997). Vegetative propagation through stem cuttings also produces uniform planting materials (Maria and Bona, 2010), which is important in ornamental production. Pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) is an endemic, perennial vine commonly found in coastal areas of the Hawaiian Islands and has the potential to be developed as a hanging basket plant. It is an easy plant to propagate from cuttings due to the presence of pre-formed roots on its stems. The recommended length of stem cuttings for pa‘uohi‘iaka should be 7-10 cm (3 to 4 in) long with two or three nodes per cutting; rooting hormone is not required (Lilleeng-Rosenberger, 1998). For vegetative propagation to become efficient and reliable, numerous factors need to be considered, including standardizing the size of stem cuttings and the rooting response of collections.

Morphological variations exist in pa‘uohi‘iaka (Chapter 1). Each of the six accessions has its own unique set of morphological characters, but an important consideration is a good ability to root. It is essential to evaluate the rooting response of these unique accessions to determine which ones are more responsive to rooting. Multiplying desirable genotypes – selected from natural variability for different purposes is a major issue for plant germplasm improvement and maintenance (da Rocha Correa et al., 2011). Developing an effective rooting protocol is crucial for efficient maintenance of germplasm collections and for generating enough planting material for evaluation studies. Aside from evaluating the rooting response of each accession, testing the feasibility of single node stem cuttings as a propagation material is also essential. If successful, single node cuttings would maximize the number of plants propagated at a given time compared to the current practice. In this study, the rooting response of single node and four node stem cuttings harvested from each of the six pa‘uohi‘iaka accessions were evaluated in two different dates (March and October 2018). The rooting response of stem cuttings with or without leaves was also tested to determine the effect of leaves on root initiation.

Materials and Methods

Effect of number of nodes on rooting of accessions

This study was conducted from March 6 to 27, 2018 and October 2 to 23, 2018 at the Magoon Research Facility, University of Hawaii at Manoa, Honolulu, USA (Latitude: 21.306616, Longitude -157.809925, Elevation: ~48 m above sea level). The purpose of repeating the experiment in October 2018 was to validate the results obtained from March 2018. Six accessions of pa‘uohi‘iaka collected from Oahu, Maui and Hawaii were evaluated in this study (Table 2.1)

Table 2.1. Provenance information of pa‘uohi‘iaka accessions used for the morphological characterization study.

Accession Name/Provenance	Collection Site	Genetic Status
Ahihi-Kinau	Maui Nui Botanical Gardens, Maui	Wild
Lyon Arboretum*	Leeward Community College, Oahu	Cultivated, seed bank accession
McGregor	Maui Nui Botanical Gardens, Maui	Wild
Puhala Bay	Maui Nui Botanical Gardens, Maui	Wild
Shidler College*	Shidler College Business School, Oahu	Cultivated
South Point	South Point, Hawaii	Wild

* unknown provenance

The stock plants of these accessions were maintained under outdoor irrigated condition from October 2017 to October 2018 (see Chapter 1). Stock plants were grown in 2 gallon (7.57 liter) plastic pots filled with a 1:1 by volume mix of coconut coir and 1.90 cm (3/4 inch) diameter cinder. Slow-release fertilizer (Nutricote 13-4.8-9.1, Arysta LifeScience) was also incorporated into the growing media at 6,992 grams/cubic meter (198 grams/cubic feet). Stock plants were grown under full sun and watered twice daily for five minutes through irrigation spray stakes. Each pot received a total of 6.6 liters of water per day. Stem cuttings from the March and October 2018 studies were gathered from the same group of mother plants.

Single node and four node stem cuttings with pre-formed roots were gathered from the mid-portion of lateral branches of each accession. Pedicels were removed and the stem cuttings were planted horizontally in 15 cm (six-inch size) diameter pots with 1:1 by volume perlite and vermiculite. All pre-formed roots were in contact with the rooting medium at the time of planting (Figure 2.1). Pots were placed on a mist bench inside a shaded glass house. Misting was set to operate for 10 seconds every six minutes (Figure 2.2).

The study was laid out in a Split-Split-Plot Design with the two different planting dates (March and October 2018) serving as the main plot. The six different accessions: Ahihi-Kinau, Lyon Arboretum, McGregor, Puhala Bay, Shidler College and South Point, served as the subplot; and the two types of nodal cuttings (four nodes and single node) served as the sub-subplot. Treatment combinations were replicated four times with each replicate consisting of 10 stem cuttings. Average root length, average root number, average number of leaves retained, average number of shoots and percent rooting were calculated 21 days after propagation. Average root length was obtained by calculating the average length of all the roots that initiate directly from the nodes (Appendix Figure 2.3).

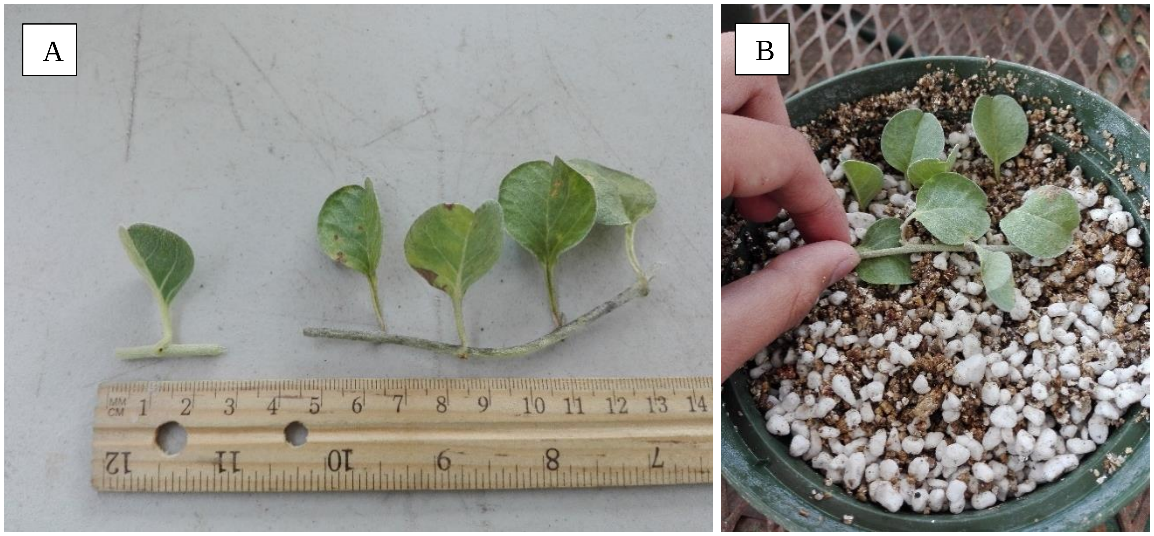


Figure 2.1. Pa‘uohi‘iaka *(Jacquemontia sandwicensis* A. Gray) single (A) and four node (B) stem cuttings planted in 1:1 by volume of perlite and vermiculite

Effect of leaf removal and number of nodes on rooting

This experiment was conducted from February 14 to March 14, 2018, at the Pope Laboratory Greenhouse, University of Hawaii at Manoa, USA (Lat: 21.302576, Long: -157.815111, Elevation: ~30 meters above sea level). The Ahihi-Kinau accession was used for this experiment. Treatments were laid out in a 2x2 Factorial Completely Randomized Design with four replicates with each replication consisting of 10 stem cuttings. Factor A was the number of nodes (single node and four node) of stem cuttings and Factor B was the presence and absence of leaves. Cuttings were rooted in 15 cm (6 inch) pots filled with a 1:1 by volume mix of perlite and vermiculite on a mist bench set to open for 20 seconds every two minutes. Average temperature during the experiment was 22.3°C. Data collected and calculated were the following; average root length, average root number, and percent rooting were recorded 30 days after planting.

Statistical analysis

Analysis of variance (ANOVA) in Statistix 10 statistical software (Analytical Software) was used to determine significant treatment effects or interactions. Assumptions for using ANOVA, e.g. normality and homogeneity of variances, were checked. Significant differences between treatment means were determined using Tukey's Honest Significant Difference (HSD) Test.

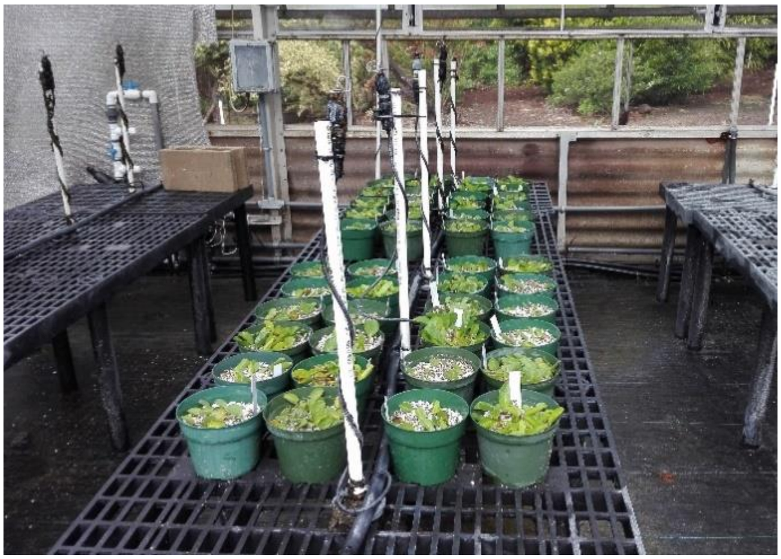


Figure 2.2. Experimental set up of rooting response of six collections of pa'uohi'iaka using single and four node stem cuttings

Results

Effect of number of nodes on rooting of accessions

Average root length

ANOVA did not indicate a significant three-way interaction between propagation dates, accession, and number of nodes (*P=0.3136*). Significant interactions between propagation dates and node (*P=0.0053*), and between propagation dates and accession (*P=0.0004*) were observed. In the interaction between propagation dates and node, no differences in root length between the two dates were observed within single node and within four node cuttings (Figure 2.3). The average root length of four node cuttings was between 6.58 to 7.28 cm. In single node cuttings, the average root length was between 7.66 and 6.18 cm. No significant differences in average root length was observed between the four node and single node stem cuttings, except when root length of four node cuttings recorded in March was compared with root length of single node cuttings recorded in October.

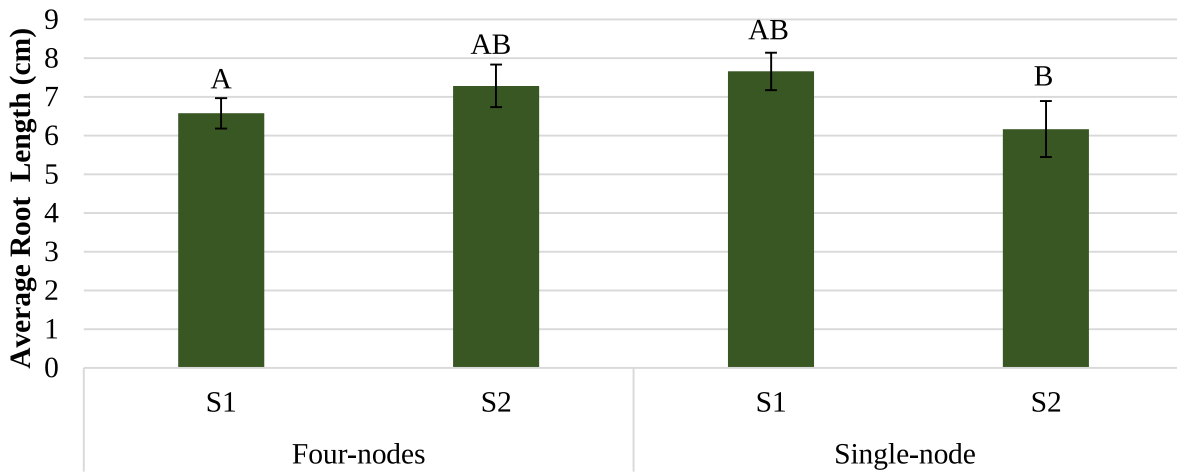


Figure 2.3. Root length of pa‘uohi‘iaka as influenced by propagation dates (S1: March 2018 and S2: October 2018) and node number of stem cuttings. Root lengths and standard errors presented are combined across accessions. Bars with different letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=24.

Table 2.2. Average root length of pa‘uohi‘iaka accessions as influenced by propagation dates, accessions and node number of stem cuttings. Root length and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=8.

Accession	**Average root length (cm)**	
	S1 (March 2018)	S2 (October 2018)
Ahihi-Kinau	6.63 bcd	7.04 bcd
Lyon Arboretum	7.61 bc	9.45 ab
McGregor	5.95 cde	4.15 de
Puhala Bay	7.30 bc	3.16 de
Shidler College	9.56 ab	10.62 a
South Point	5.64 cde	5.91 cde

In the interaction between accession and propagation dates, Shidler College, Lyon Arboretum, Ahihi-Kinau, South Point, and McGregor exhibited similar root lengths between dates (Table 2.2). The Shidler College accession consistently exhibited the longest average root length compared to the other accessions. The root length of the Puhala Bay accession was significantly longer in March 2018 in contrast to October 2018. The South Point and McGregor accessions consistently exhibited the shortest root length.

Average number of roots

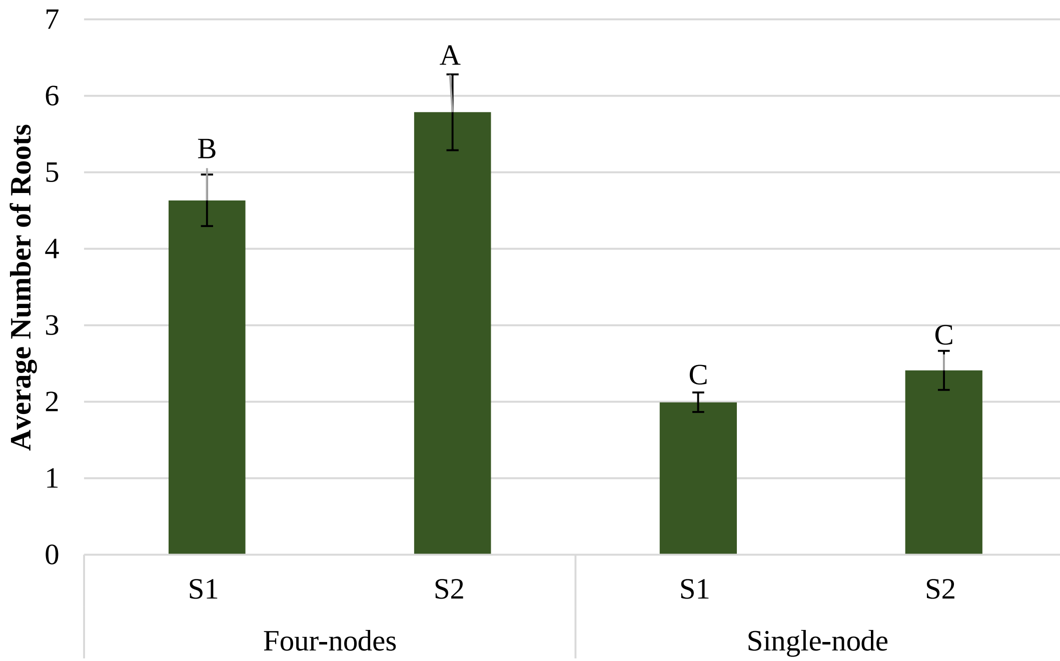


Figure 2.4. Average number of roots of pa‘uohi‘iaka as influenced by propagation dates (S1: March 2018 and S2: October 2018) and node number of stem cuttings. Number of roots and standard errors presented are combined across accessions. Bars with different letters are significantly different using Tukey’s HSD pairwise comparison test at $P<0.05$, n=24.

ANOVA results did not show a significant three-way interaction between propagation dates, number of nodes, and accession (*P=0.6252*). Significant interactions between propagation dates and number of nodes (*P=0.0286*), propagation dates and accession (*P*=0.0001) and accession by nodes (*P<0.01*) were observed. Results observed in the interaction between propagation dates and number of nodes indicate that the four node stem cuttings propagated in October 2018 exhibited a significantly higher number of roots compared to those propagated in March 2018. Average root numbers of single node stem cuttings between the two dates were similar, but lower than those observed in cuttings with four nodes.

Table 2. 3. Average root number of pa‘uohi‘iaka accessions as influenced by propagation dates, accessions and node number of stem cuttings. Number of roots and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey’s HSD pairwise comparison test at P<0.05, n=8.

Accessions	**Average root number**	
	S1 (March 2018)	S2 (October 2018)
Ahihi-Kinau	4.93 b	7.40 a
Lyon Arboretum	3.36 cde	3.18 cde
McGregor	2.44 de	2.42 de
Puhala Bay	2.66 cde	2.91 cde
Shidler College	3.07 cde	4.08 bc
South Point	3.43 bcde	4.60 bc

Results observed in the interaction between propagation dates and accession indicate that there is a significant increase in the average number of roots for Ahihi-Kinau in October 2018 (Table 2.3). Average number of roots of Ahihi-Kinau increased from 4.93 in March 2018 to 7.40 in October 2018. The rest of the accessions maintained the same average number of roots between dates.

Table 2. 4. Average root number of pa‘uohi‘iaka accessions as influenced by node number of stem cuttings. Number of roots and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey's HSD pairwise comparison test at $P<0.05$, n=8.

Accessions	Average root number	
	Four nodes	Single node
Ahihi-Kinau	8.60 a	3.72 c
Lyon Arboretum	4.63 bc	1.90 e
McGregor	3.39 cd	1.46 e
Puhala Bay	3.64 c	1.92 e
Shidler College	5.09 b	2.05 e
South Point	5.90 b	2.12 de

In the interaction between accession and nodal number of cuttings, the four node cuttings of Ahihi-Kinau exhibited the most roots compared to the four node cuttings of all other accessions (Table 2.4). McGregor exhibited the lowest root numbers among the four node cuttings of all accessions. Single node cuttings of all accessions except Ahihi-Kinau, exhibited low root numbers (<3). Figure 2.5 shows the vigorous rooting of Ahihi-Kinau single node and four node cuttings

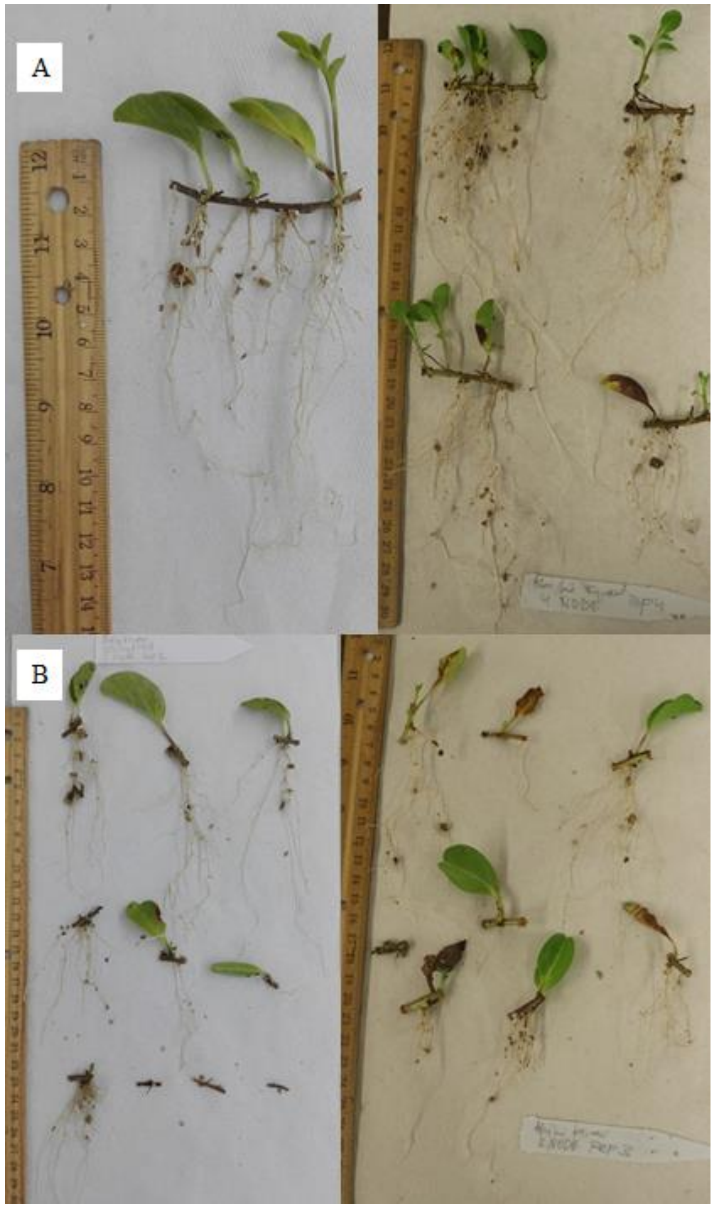


Figure 2. 5. Vigorous rooting of Ahihi-Kinau accession: A) Four node stem cutting and B) Single node stem cutting

Percent rooting

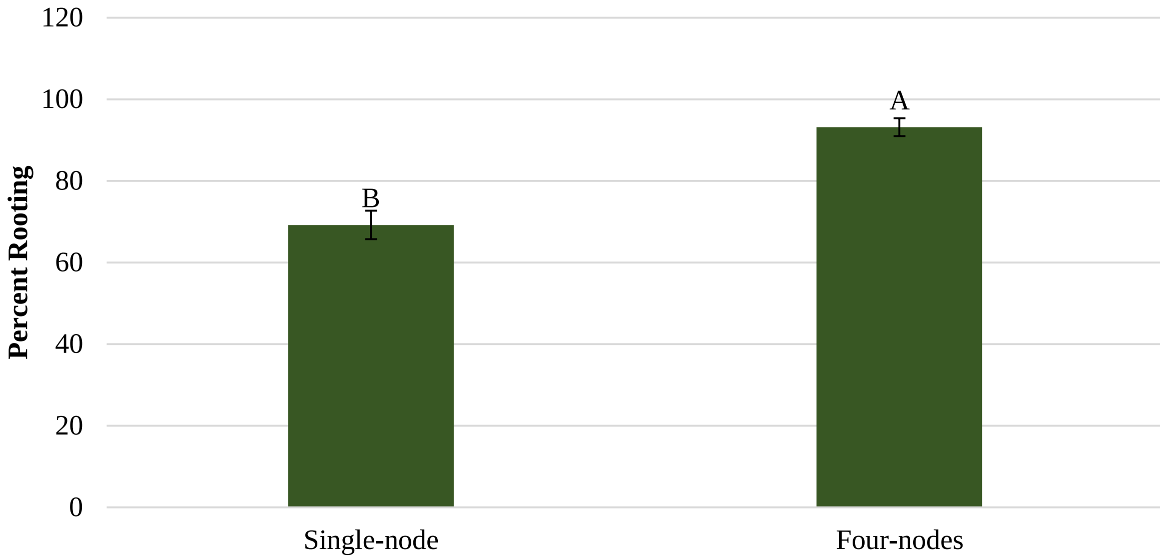


Figure 2.6. Percent rooting of pa‘uohi‘iaka as influenced by node number of stem cuttings. Percent rooting and standard errors presented are combined across accessions. Bars with different letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=48.

ANOVA results did not indicate a three-way interaction between propagation dates, number of nodes, and accession. No significant interaction was also found between the number of nodes and propagation dates, and between the number of nodes and accession. This allowed for the pooling of propagation dates and accessions in each nodal cutting. The percent rooting of four node stem cuttings were significantly higher than single node cuttings (Figure 2.6). A significant interaction between propagation dates and accession was also observed (P<*0.01*) allowing for the pooling of nodal cuttings.

Table 2. 5. Percent rooting of pa‘uohi‘iaka accessions as influenced by propagation dates, accessions and node number of stem cuttings. Percent rooting and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey’s HSD pairwise comparison test at P<0.05, n=8.

Accessions	**Percent rooting**	
	S1 (March 2018)	S2 (October 2018)
Ahihi-Kinau	91.25 a	95 a
Lyon Arboretum	86.25 a	82.5 a
McGregor	80 ab	41.25 c
Puhala Bay	85 a	58.75 bc
Shidler College	91.25 a	98.75 a
South Point	77.5 ab	86.75 a

All accessions except McGregor and Puhala consistently exhibited high percent rooting (>85%) between the two dates, suggesting propagation date effects on the percent rooting of the McGregor and Puhala Bay accessions (Table 2.5). Both accessions exhibited the lowest percent rooting in October at 58.75 % for Puhala Bay and 41.25% for McGregor.

Average number of shoots

ANOVA results did not indicate a three-way interaction between propagation dates, number of nodes, and accession. Significant two-way interactions between propagation dates and accession ($P<0.005$), and between the number of nodes and accession ($P=0.0154$) were observed.

Table 2.6. Average number of shoots of pa‘uohi‘iaka accessions as influenced by propagation dates, accessions and node number of stem cuttings. Number of shoots and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=8.

Accessions	Average number of shoots	
	S1 (March 2018)	S2 (October 2018)
Ahihi-Kinau	0.46 bcd	0.77 ab
Lyon Arboretum	0.52 bcd	0.79 ab
McGregor	0.31 bcd	0.15 d
Puhala Bay	0.58 abcd	0.13 d
Shidler College	0.35 bcd	0.97 a
South Point	0.41bcd	0.49 b

Results observed in the interaction between accession and propagation dates indicated that only Shidler College exhibited a significant increase on the average number of shoots in October 2018 (Table 2.6). The average number of shoots for Shidler College increased from 0.35 in March 2018 to 0.97 in October 2018.

Table 2.7. Average number of shoots of pa‘uohi‘iaka accessions as influenced by node number of stem cuttings. Number of shoots and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=8.

Accessions	Average number of shoots	
	Four nodes	Single node
Ahihi-Kinau	0.92 a	0.31 cde
Lyon Arboretum	0.89 a	0.42 bcde
McGregor	0.31 cde	0.15 de
Puhala Bay	0.43 bcde	0.28 de
Shidler College	0.76 ab	0.56 abcd
South Point	0.67 abc	0.23 de

Results observed in the interaction between accession and number of nodes indicate that the four node cuttings of Ahihi-Kinau, Lyon Arboretum, and South Point exhibited a significantly higher number of shoots in contrast to one-node cuttings of the same accessions (Table 2.7). The shoot numbers were not significantly different between four node and single node stem cuttings of Shidler College, Puhala Bay and McGregor. The shoot numbers from these accessions were comparable to those observed in the one-node cuttings of Ahihi-Kinau, Lyon Arboretum and South Point.

Average number of leaves retained

ANOVA indicated no significant three-way interaction between propagation dates, number of nodes, and accession (*P=0.132*). Significant interactions between propagation dates and node (*P=0.014*), accession and node (*P<0.01*) and propagation dates and accession (*P<0.01*) were observed.

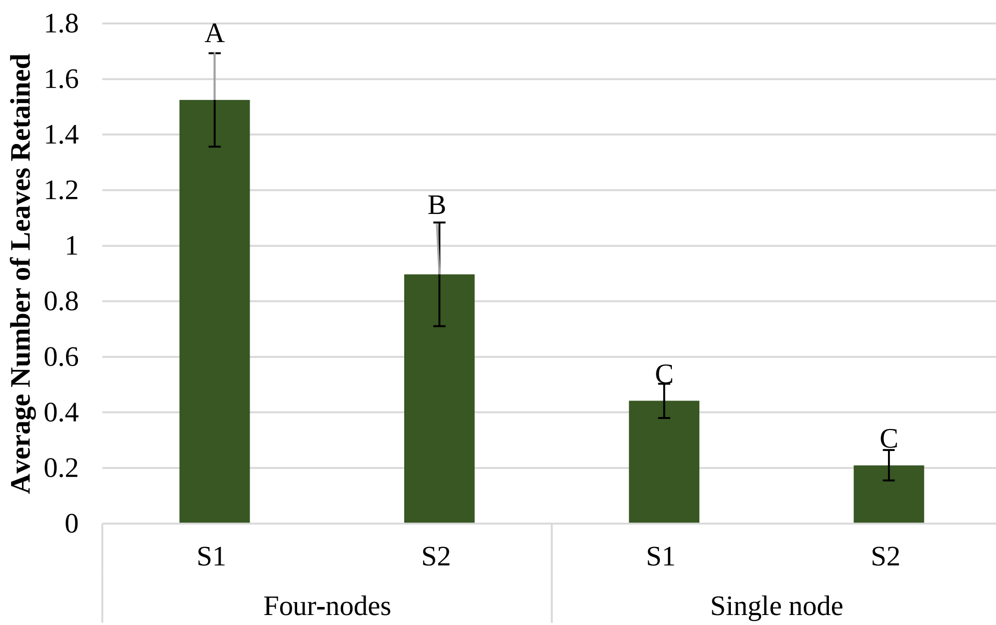


Figure 2.7. Average number of pa‘uohi‘iaka leaves retained as influenced by propagation dates (S1: March 2018 and S2: October 2018) and node number of stem cuttings. Number of leaves retained and standard errors presented are combined across accessions. Bars with different letters are significantly different using Tukey’s HSD pairwise comparison test at P<0.05, n=24.

Results observed in the interaction between propagation dates and number of nodes (Figure 2.7) indicated that in general, four node cuttings had a higher number of leaves retained compared to single node cuttings. In four node cuttings, a significantly lower number of intact leaves was observed in cuttings propagated in October propagated cuttings than in March. In March 2018, the average number of leaves retained in four node stem cuttings was 1.52. In October 2018, the average number of leaves retained was less (0.89). The number of intact leaves between single node cuttings planted on either date was not significantly different.

Table 2.8. Average number of leaves retained by six pa‘uohi‘iaka accessions as influenced by propagation dates, accessions and node number of stem cuttings. Number of leaves and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey's HSD pairwise comparison test at $P<0.05$, n=8.

Accessions	**Average number of leaves retained**	
	S1 (March 2018)	S2 (October 2018)
Ahihi-Kinau	1.3 a	0.82 ab
Lyon Arboretum	1.21 a	0.94 ab
McGregor	0.41 bc	0.01 c
Puhala Bay	1.35 a	0.05 c
Shidler College	1.22 a	1.36 a
South Point	0.41 bc	0.127 c

Results observed in the interaction between accession and propagation dates indicate that in March 2018, South Point and McGregor significantly lost more leaves than the rest of the accessions tested (Table 2.8). In October 2018, South Point, McGregor and Puhala Bay significantly lost more leaves than Shidler College, Ahihi-Kinau and Lyon Arboretum. Between propagation dates, Shidler College, Ahihi-Kinau and Lyon Arboretum exhibited similar leaf numbers. McGregor and South Point also exhibited similar leaf numbers between dates. Puhala Bay had significantly higher leaf retention in March 2018 in contrast to October 2018.

Table 2.9. Average number of leaves retained by pa‘uohi‘iaka accessions as influenced by node number of stem cuttings. Number of leaves retained and standard errors presented are combined across node number of stem cuttings. Values that do not have the same letters are significantly different using Tukey's HSD pairwise comparison test at $P<0.05$, n=8.

Accessions	**Average number of leaves retained**	
	Four nodes	Single node
Ahihi-Kinau	1.78 a	0.33 c
Lyon Arboretum	1.71 a	0.43 bc
McGregor	0.27 c	0.13 c
Puhala Bay	1.05 b	0.35 c
Shidler College	2a	0.58 bc
South Point	0.43 bc	0.10 c

No significant differences on the average number of leaves retained were observed in single node cuttings of all accessions (Table 2.9). Four node stem cuttings of McGregor, Puhala Bay and South Point exhibited significantly less leaves retained compared to four node stem cuttings of Ahihi-Kinau, Lyon Arboretum and Shidler College accessions. Four node and single node stem cuttings of McGregor and South Point accessions similarly exhibited poor leaf retention.

Effect of leaf removal and number of nodes on the rooting of the Ahihi-Kinau accession

No significant two-way interactions between number of nodes and presence of leaves were observed for average root length (Appendix Table 2.5) and percent rooting (Appendix Table 2.6). Both data exhibited significant main effects (i.e. number of nodes and presence/absence of leaves).

Four node stem cuttings exhibited longer root lengths and higher percent rooting compared to single node stem cuttings (Appendix Figure 2.12 and Appendix Figure 2.15). Cuttings with leaves had longer roots and higher percent rooting compared to stem cuttings without leaves (Appendix Figures 2.13 and Appendix Figure 2.14).

Significant two-way interactions were only observed for average root number (*P=0.0085*). Four node cuttings with leaves significantly exhibited the highest number of roots (4.82) compared to other treatments (Figure 2.8). Four node cuttings without leaves, single node cuttings with leaves and single node cuttings without leaves exhibited low root numbers (<2) and were not significant from each other.

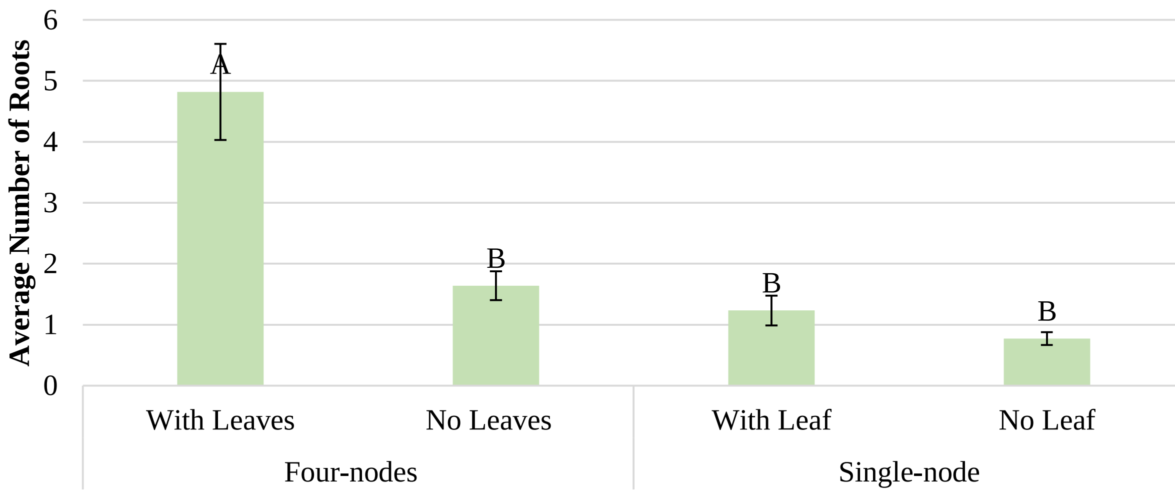


Figure 2.8. Average number of roots of Ahihi-Kinau accession stem cuttings as influenced by number of nodes of stem cuttings and presence and absence of leaves. Bars with different letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=4.

Overall, four node stem cuttings and stem cuttings with leaves exhibited better rooting characteristics compared to single node cuttings and stem cuttings without leaves (Appendix Figure 2.13)

Discussion

Results of the experiments indicate that propagation dates, number of nodes, accession/leaf type and leaf retention affect rooting success of pa'uohi'iaka. The significant interactions observed between propagation dates and accession, and between propagation dates and number of nodes, were due to the malfunction of the mist system in October 2018. This incident resulted in very wet conditions during the rooting period. As a result, root length and percent rooting of the Puhala Bay accession were negatively impacted. This suggests that Puhala Bay is sensitive to overwatering (Figure 2.10) and may be an indication that its provenance may have drier conditions. Hypoxia or oxygen deficiency from excess water may result in a decrease in root growth in most plants (Friend et al., 1994). In contrast, the wet conditions significantly increased average number of roots (Table 2.3) of the Ahihi-Kinau accession, indicating that Ahihi-Kinau favors wetter conditions for root growth. Aside from the propagation dates by accession interaction, the propagation dates by number of node interaction indicated that root number of four node cuttings increased under wetter conditions (October 2018).

The number of nodes as well as leaf bearing nodes also appear to influence rooting success. Single node stem cuttings of all accessions except for Ahihi-Kinau and Shidler College exhibited poor rooting characteristics compared to four node cuttings of all accessions. Four node stem cuttings had better rooting characteristics than single node cuttings because single node stem cuttings contain less leaf and stem tissue. The presence and retention of leaves in nodal cuttings appear to be an essential parameter in determining not only the ability of pa'uohi'iaka stem cuttings to root but it can also dictate the survival of stem cuttings after transplanting. Leaves sustain photosynthesis and replenish the carbohydrates and photosynthates needed to initiate rooting (Tombesi et. al., 2015). There is a positive correlation between high photosynthate content of stem cuttings and rooting because the supply of photosynthates supports adequate rooting (Hamilton et al., 2002). Without the leaves, stem cuttings may still produce roots but not as much as those with leaves. The presence of leaves on stem cuttings influences rooting compounds, such as auxin and co-factors that exert a stimulating effect on rooting (Maria and Bona, 2010). Leaves also hold auxin (Hartmann et al., 2002) and nutritional factors leading to adventitious root initiation (Jarvis, 1986).

The degree of pubescence on the leaves observed in each accession also appear to have an impact on rooting of pa‘uohi‘iaka. In general, accessions with glabrous leaves (Figure 2.9) tend to have significantly higher average number of shoots and number of leaves retained. This happened despite the irrigation malfunction in our experiment, suggesting that Shidler College (glabrous leaf type) responded favorably to wetter conditions. Accessions with medium to densely pubescent leaves (Figure 2.16) tend to have low leaf retention and fewer roots. These observations were further supported when data were re-analyzed into pubescent and glabrous types (Appendix Figures 2.17 to 2.19 and Appendix Tables 2.8 to 2.10). Under mist conditions, the accessions with pubescent leaves turned yellowish and defoliated faster than glabrous types. Survival of rooted cuttings from pubescent accessions also appeared to be low after transplanting because of poor leaf retention (Darel Kenth Antesco, personal observation).

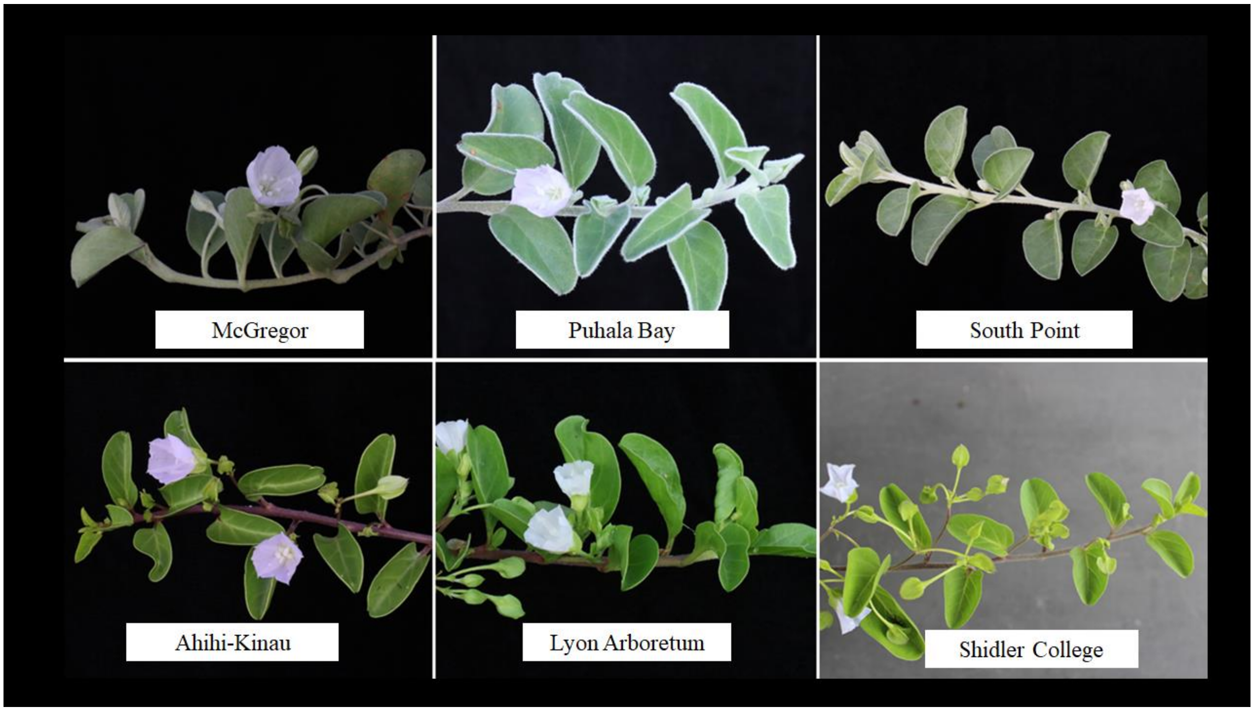


Figure 2. 9. Pubescent (top) and glabrous (bottom) leafed accessions of pa‘uohi‘iaka evaluated in the nodal cutting study.

Figure 2.10. Leaf retention of the Puhala Bay accession after 21 days in the mist bed: A) four node stem cuttings in March 2018, B) single node stem cuttings in March 2018, C) four node stem cuttings in October 2018 and D) single node stem cuttings in October 2018.

Cuttings of many plant species can develop roots but do not survive for a long time after rooting (Hartmann and Kester 1983). This is possibly caused by the inability to recover after transplanting or the failure to adapt to the field environment (Berhe and Negash 1998). In this study, we observed the difficulty of establishing rooted cuttings of the South Point accession after transplanting (Figure 2.11). Although South Point successfully developed roots and shoots under mist conditions, the number of leaves retained in the stem cuttings were low compared to other accessions (Table 2.9). At transplanting, most rooted cuttings of South Point have lost their leaves (Darel Kenth Antesco, personal observation). The depletion of photosynthates stored in the cuttings even before shoots were able to photosynthesize was probably the reason why majority of the cuttings died after potting. Due to the difficulty of achieving enough number of plants, the accession was dropped for hanging basket evaluation.

Figure 2.11. Mortality of South Point rooted cuttings after transplanting

Among the six accessions, Ahihi-Kinau and Shidler College can be considered as the most rooting responsive. These are also the only accessions that successfully rooted using single node stem cuttings. Despite the overwatering incident in October 2018, these accessions exhibited enhanced rooting response by producing significantly higher number of roots (Ahihi-Kinau) or shoots (Shidler College) compared to cuttings propagated in March 2018. The low average number of leaves retained in the McGregor, Puhala Bay and South Point accessions indicated sensitivity of pubescent types to wet conditions. These accessions average less than 1 leaf retained in the four node stem cuttings (Table 2.9). In contrast, glabrous accessions like Ahihi-Kinau, Lyon Arboretum and Shidler College have greater tolerance to wet conditions. Ahihi-Kinau significantly increased average number of roots while Shidler College significantly increased average number of shoots in the second propagation dates (October 2018). In the first propagation date (March 2018), Puhala Bay (pubescent leaf type) responded well to the misting irrigation interval of 10 seconds every six minutes. Other pubescent leaf types like McGregor and South Point accessions responded poorly under this condition, suggesting that these accessions might require a different condition of propagation, i.e. not under mist or less watering intervals. The McGregor accession can be considered as the weakest among the six accessions evaluated for rooting response under mist conditions. It recorded low rooting response for all parameters measured in two propagation dates. Thus, further study focusing on the rooting response of these three accessions on drier or less frequent watering interval is needed to improve the efficiency of maintaining these accessions.

CHAPTER 4

CONCLUSION

Findings in the morphological characterization and rooting response studies generated useful information for identifying pa‘uohi‘iaka hanging basket selections and developing production protocols. Documentation of the traits for each accession provided a snapshot of the morphological variation in pa‘uohi‘iaka. Morphological characters that significantly contributed to the variation of accessions were leaf shape, leaf length, adaxial and abaxial stem color, length of internodes, length of lateral branches, flower color and number of flowers. Among the morphological characters examined, shorter internodes and a higher lateral branch counts were the two most important characters to consider for identifying the suitability of selections for hanging basket use. Cluster analysis of the six accessions revealed three distinct groupings. Accessions with the same leaf shape, stem color and leaf pubescence (i.e. glabrous or pubescent) tend to group together in the same cluster. Identification of these important characters and relationships between accessions provides relevant information that can be used for developing new cultivars in future breeding programs.

The rooting response study revealed that four node stem cuttings were better propagation materials compared to single node stem cuttings. Four node stem cuttings possessed more preformed roots as well as more leaf and stem tissue to support root growth. Leaf retention and rooting of the six accessions also appeared to be influenced by leaf pubescence. The glabrous leafed accessions (Ahihi-Kinau, Shidler College and Lyon Arboretum) rooted well because of a significantly higher number of leaves retained. In contrast, pubescent leafed accessions (McGregor, Puhala Bay and South Point) rooted poorly due to rotting of most leaves. The wet conditions in the mist bench caused the leaves of the pubescent leafed accessions to defoliate, indicating that rooting of pubescent leafed accessions may require drier growing conditions or lower frequency of misting. Leaves support the root initiation of stem cuttings through a continuous supply of photosynthates. It also ensures survival of rooted cuttings after transplanting. Due to poor rooting and survival of South Point, only two accessions, Lyon Arboretum and Puhala Bay), were advanced to the hanging basket trials. The trials evaluating the response of the two accessions to different frequencies of manual pinching are currently ongoing.

Overall, this thesis indicated that inherent variation in native plants can be used for developing ornamental selections for particular uses. It also showed that propagation protocols may vary depending on characteristics of an accession. Further collection is needed to increase and assess the diversity of pa‘uohi‘iaka for future evaluation and breeding programs.

APPENDICES

Appendix Table 1.1 .Analysis of variance (ANOVA) of average leaf length (cm) of six pa‘uohi‘iaka accessions one month after pruning.

Source	df	SS	MS	F	P
Samples	5	1.0674	0.21348		
Accession	5	17.1139	3.42278	13.35	0
Error	25	6.4105	0.25642		
Total	35	24.5919			

Appendix Table 1. 2. Analysis of variance (ANOVA) of average leaf width (cm) of six pa‘uohi‘iaka accessions one month after pruning.

Source	df	SS	MS	F	P
Samples	5	3.0142	0.60284		
Accession	5	4.1742	0.83483	1.27	0.3094
Error	25	16.4846	0.65938		
Total	35	23.673			

Appendix Table 1. 3. Analysis of variance (ANOVA) of average leaf thickness (mm) of six pa‘uohi‘iaka accessions one month after pruning.

Source	df	SS	MS	F	P
Samples	5	0.00865	0.00173		
Accession	5	0.17064	0.03413	8.17	0.0001
Error	23	0.09608	0.00418		
Total	33				

Appendix Table 1.4. Analysis of variance (ANOVA) of average peduncle length (cm) of six pa‘uohi‘iaka accessions one month after pruning.

Source	df	SS	MS	F	P
Samples	5	2.7523	0.55047		
Accession	5	8.637	1.72741	2.21	0.0977
Error	18	14.0378	0.77988		
Total	28				

Appendix Table 1.5. Analysis of variance (ANOVA) of average peduncle diameter (mm) of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	0.11927	0.02385		
Accession	5	0.07221	0.01444	1.24	0.3369
Error	16	0.18654	0.01166		
Total	26				

Appendix Table 1.6. Analysis of variance (ANOVA) of average petiole length (cm) of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	0.06742	0.01348		
Accession	5	0.98982	0.19796	4.82	0.0032
Error	25	1.02618	0.04105		
Total	35	2.08342			

Appendix Table 1.7. Analysis of variance (ANOVA) of average length of internodes (cm) of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	1.41272	0.28254		
Accession	5	4.23672	0.84734	5.66	0.0013
Error	25	3.74108	0.14964		
Total	35	9.39052			

Appendix Table 1.8. Analysis of Variance (ANOVA) of number of lateral branches of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	5.222	1.0444		
Accession	5	174.222	34.8444	3.25	0.0215
Error	25	268.444	10.7378		
Total	35	447.889			

Appendix Table 1.9. Analysis of variance (ANOVA) of average length of lateral branches of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	567.78	113.556		
Accession	5	553.6	110.72	2.59	0.0521
Error	24	1026.16	42.757		
Total	34				

Appendix Table 1.10. Analysis of variance (ANOVA) of average number of flowers of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	13.333	2.6667		
Accession	5	73.333	14.6667	7.75	0.0002
Error	25	47.333	1.8933		
Total	35	134			

Appendix Table 1.11. Analysis of variance (ANOVA) of average floral diameter of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	4.5194	0.90388		
Accession	5	36.5553	7.31107	11.03	0.0001
Error	15	9.9398	0.66266		
Total	25				

Appendix Table 1.12. Analysis of variance (ANOVA) of average number of preformed roots of six pa‘uohi‘iaka accessions one month after pruning.

Source	**df**	**SS**	**MS**	**F**	**P**
Samples	5	403.14	80.628		
Accession	5	748.47	149.694	1.33	0.285
Error	25	2819.36	112.774		
Total	35	3970.97			

Appendix Table 2.1. Analysis of variance (ANOVA) for root length (cm) of six pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018

Source	**df**	**SS**	**MS**	**F**	**P**
Rep (A)	3	8.214	2.738		
Propagation dates (B)	1	3.682	3.6817	6.49	0.0841
Error A*B	3	1.701	0.5671		
Accession (C)	5	324.548	64.9095	21.32	0
B*C	5	96.824	19.3647	6.36	0.0004
Error A*B*C	30	91.318	3.0439		
Nodes (D)	1	0.007	0.0067	0	0.9644
B*D	1	29.018	29.018	8.81	0.0053
C*D	5	13.193	2.6386	0.8	0.5565
B*C*D	5	20.326	4.0651	1.23	0.3136
Error A*B*C*D	36	118.608	3.2947		
Total	95	707.437			

Appendix Table 2.2. Analysis of variance (ANOVA) for number of roots of six pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018

Source	**df**	**SS**	**MS**	**F**	**P**
Rep (A)	3	12.122	4.041		
Propagation dates (B)	1	14.758	14.758	18.64	0.0229
Error A*B	3	2.375	0.792		
Accession (C)	5	141.174	28.235	55.9	0
B*C	5	19.604	3.921	7.76	0.0001
Error A*B*C	30	15.152	0.505		
Nodes (D)	1	217.262	217.262	347.85	0
B*D	1	3.249	3.249	5.2	0.0286
C*D	5	28.057	5.611	8.98	0
B*C*D	5	2.194	0.439	0.7	0.6252
Error A*B*C*D	36	22.485	0.625		
Total	95	478.433			

Appendix Table 2.3. Analysis of variance (ANOVA) for percent rooting of six pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018

Source	**df**	**SS**	**MS**	**F**	**P**
Rep (A)	3	294.8	98.3		
Propagation dates (B)	1	1584.4	1584.4	20.1	0.0207
Error A*B	3	236.5	78.8		
Accession (C)	5	13655.2	2731	18.92	0
B*C	5	7821.9	1564.4	10.84	0
Error A*B*C	30	4331.2	144.4		
Nodes (D)	1	13776	13776	66.01	0
B*D	1	1	1	0	0.9441
C*D	5	2430.2	486	2.33	0.0624
B*C*D	5	530.2	106	0.51	0.7682
Error A*B*C*D	36	7512.5	208.7		
Total	95	52174			

Appendix Table 2. 4. Analysis of variance (ANOVA) table for number of shoots of six pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018

Source	**df**	**SS**	**MS**	**F**	**P**
Rep (A)	3	0.096	0.03199		
Dates (B)	1	0.3049	0.30488	2.39	0.22
Error A*B	3	0.383	0.12766		
Accession (C)	5	2.5506	0.51013	10.85	0
B*C	5	2.8819	0.57638	12.26	0
Error A*B*C	30	1.4105	0.04702		
Nodes (D)	1	3.0353	3.03526	78.42	0
B*D	1	0.0982	0.09818	2.54	0.12
C*D	5	0.6335	0.12671	3.27	0.0154
B*C*D	5	0.2209	0.04418	1.14	0.3567
Error A*B*C*D	36	1.3934	0.03871		
Total	95	13.0081			

Appendix Table 2.5. Analysis of variance (ANOVA) for number of leaves retained of six pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018

Source	**df**	**SS**	**MS**	**F**	**P**
Rep (A)	3	0.6511	0.217		
Dates (B)	1	4.4376	4.4376	54.95	0.0051
Error A*B	3	0.2423	0.0808		
Accession (C)	5	16.434	3.2868	27.37	0
B*C	5	4.5177	0.9035	7.52	0.0001
Error A*B*C	30	3.603	0.1201		
Nodes (D)	1	18.8151	18.8151	131.14	0
B*D	1	0.9401	0.9401	6.55	0.0148
C*D	5	6.5881	1.3176	9.18	0
B*C*D	5	1.3116	0.2623	1.83	0.132
Error A*B*C*D	36	5.1651	0.1435		
Total	95	62.7055			

Sub Study: Effect of leaf removal and number of nodes on the rooting of pa‘uohi‘iaka

Appendix Table 2.6. Analysis of variance (ANOVA) table for root length of pa‘uohi‘iaka propagated from one-node with leaves and no leaves and four node with leaves and no leaves stem cuttings.

Source	**df**	**SS**	**MS**	**F**	**P**
Number of Nodes	1	8.8283	8.8283	16.3	0.0016
Leaves	1	21.9141	21.9141	40.46	0
Number of Nodes * Leaves	1	2.1572	2.1572	3.98	0.0692
Error	12	6.4987	0.5416		
Total	15	39.3984			

Appendix Table 2 7.Analysis of variance (ANOVA) table for percent rooting of pa‘uohi‘iaka propagated from stem cuttings with one-node with leaves and no leaves and four node with leaves and no leaves

Source	**df**	**SS**	**MS**	**F**	**P**
Number of Nodes	1	4225	4225	13.52	0.0032
Leaves	1	4900	4900	15.68	0.0019
Number of Nodes * Leaves	1	25	25	0.08	0.7821
Error	12	3750	312.5		
Total	15	12900			

Appendix Table 2.8. Analysis of variance (ANOVA) for number of roots of pa‘uohi‘iaka propagated from stem cuttings with one node with leaves or no leaves and four nodes with leaves or no leaves .

Source	df	SS	MS	F	P
Number of Nodes	1	13.286	13.286	17.73	0.0012
Leaves	1	19.847	19.847	26.49	0.0002
Number of Nodes * Leaves	1	7.3984	7.3984	9.87	0.0085
Error	12	8.9917	0.7493		
Total	15	49.5232			

Combined data of glabrous and pubescent leaf type accessions

Appendix Table 2. 9. Analysis of variance (ANOVA) table for number of leaves retained of glabrous and pubescent pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018.

Source	df	SS	MS	F	P
Rep	3	0.6511	0.217		
Dates	1	4.4376	4.4376	54.95	0.0051
Error Rep*Dates	3	0.2423	0.0808		
Leaf Type	1	13.5901	13.5901	78.83	0.0001
Dates*Leaf Type	1	1.2421	1.2421	7.2	0.0363
Error Rep*Dates*Leaf Type	6	1.0344	0.1724		
Nodes	1	18.8151	18.8151	130.6	0
Dates*Nodes	1	0.9401	0.9401	6.53	0.0253
Leaf Type*Nodes	1	5.8707	5.8707	40.75	0
Dates*Leaf Type*Nodes	1	0.1785	0.1785	1.24	0.2874
Error Rep*Dates*Leaf Type*Nodes	12	1.7289	0.1441		
Error	64	13.9745	0.2184		
Total	95	62.7055			

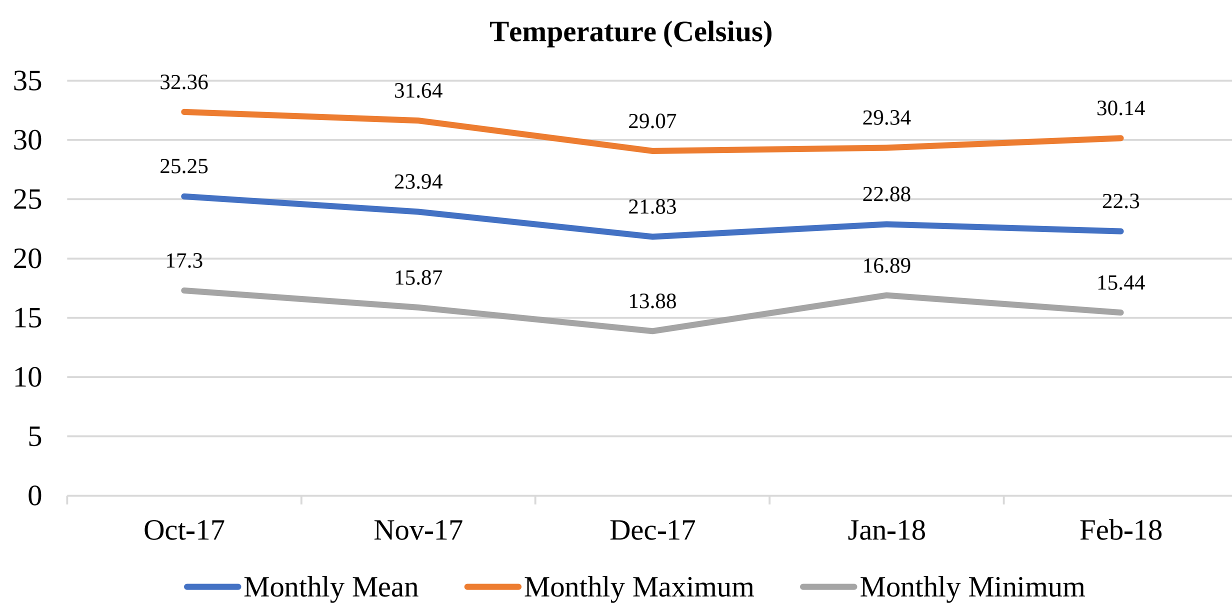
Appendix Table 2. 10. Analysis of variance (ANOVA) table for number of roots of of glabrous and pubescent pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018.

Source	df	SS	MS	F	P
Rep	3	12.122	4.041		
Dates	1	14.758	14.758	18.64	0.0229
Error Rep*Dates	3	2.375	0.792		
Leaf Type	1	38.153	38.153	191.97	0
Dates*Leaf Type	1	2.331	2.331	11.73	0.0141
Error Rep*Dates*Leaf Type	6	1.192	0.199		
Nodes	1	217.262	217.262	364.79	0
Dates*Nodes	1	3.249	3.249	5.45	0.0377
Leaf Type*Nodes	1	6.923	6.923	11.62	0.0052
Dates*Leaf Type*Nodes	1	0.01	0.01	0.02	0.899
Error Rep*Dates*Leaf Type*Nodes	12	7.147	0.596		
Error	64	172.91	2.702		
Total	95	478.433			

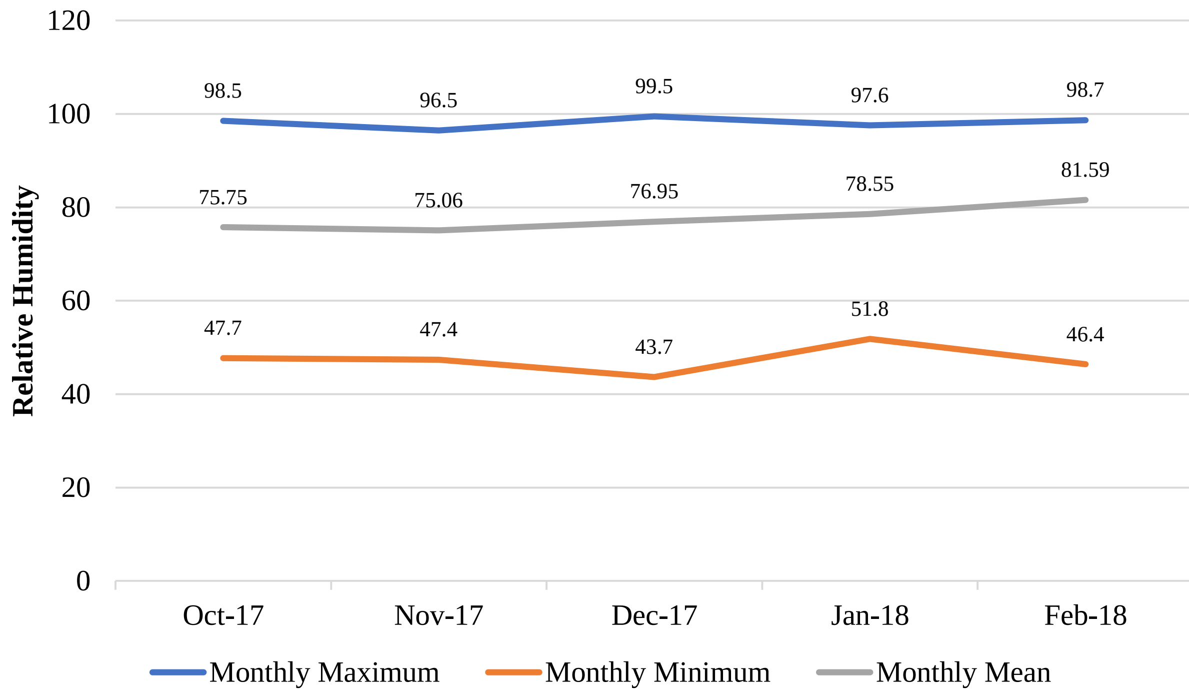
Appendix Table 2. 11. Analysis of variance (ANOVA) table for root length of glabrous and pubescent pa‘uohi‘iaka accessions propagated from one-node and four node cuttings in March and October 2018.

Source	df	SS	MS	F	P
Rep	3	8.214	2.738		
Dates	1	3.682	3.682	6.49	0.0841
Error Rep*Dates	3	1.701	0.567		
Leaf	1	235.188	235.188	111.83	0
Dates*Leaf	1	53.76	53.76	25.56	0.0023
Error Rep*Dates*Leaf	6	12.618	2.103		
Nodes	1	0.007	0.007	0	0.9728
Dates*Nodes	1	29.018	29.018	5.29	0.0402
Leaf*Nodes	1	0.465	0.465	0.08	0.7759
Dates*Leaf*Nodes	1	14.369	14.369	2.62	0.1314
Error Rep*Dates*Leaf*Nodes	12	65.792	5.483		
Error	64	282.624	4.416		
Total	95	707.437			

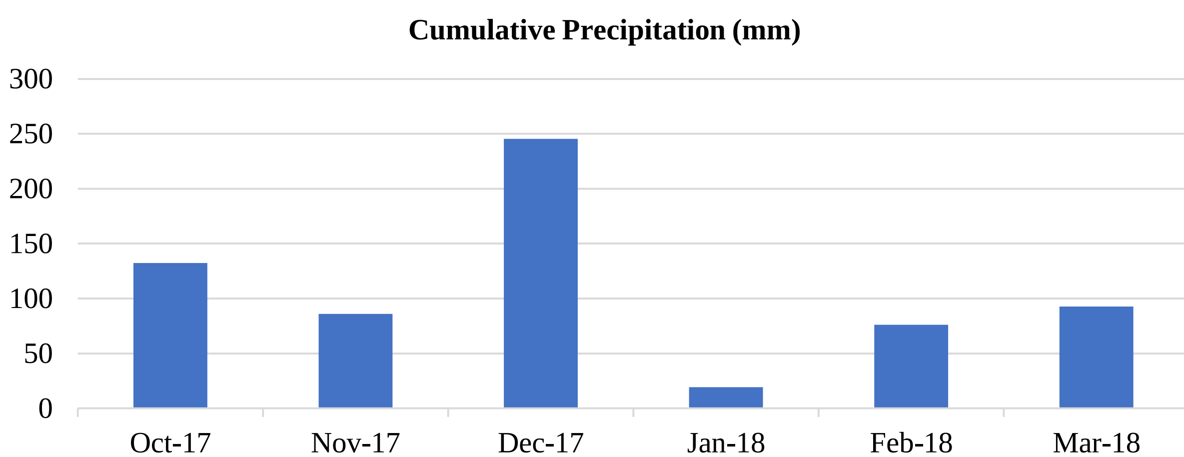
Appendix Figure 1.1. Potted pa‘uohi‘iaka plants before pruning to four inches from the base



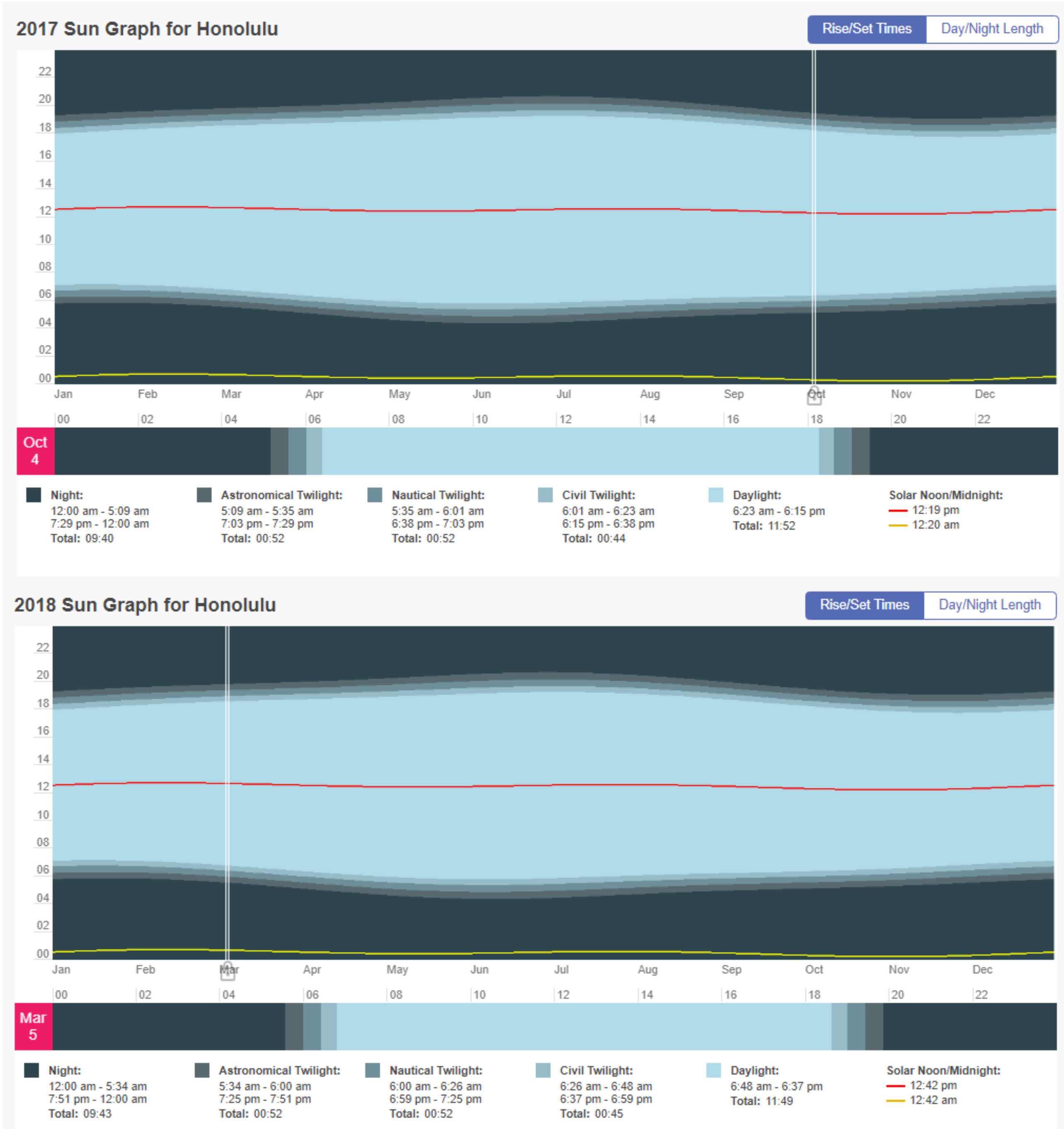
Appendix Figure 1.2. Monthly mean, maximum and minimum temperature at Magoon Research Facility from October 2017 to March 2018.



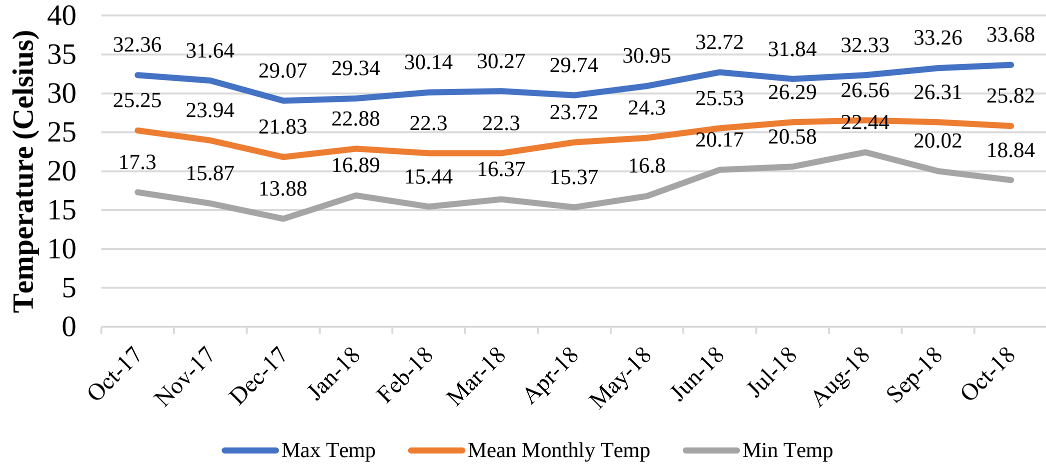
Appendix Figure 1.3. Monthly mean, maximum and minimum relative humidity at Magoon Research Facility from October 2017 to March 2018



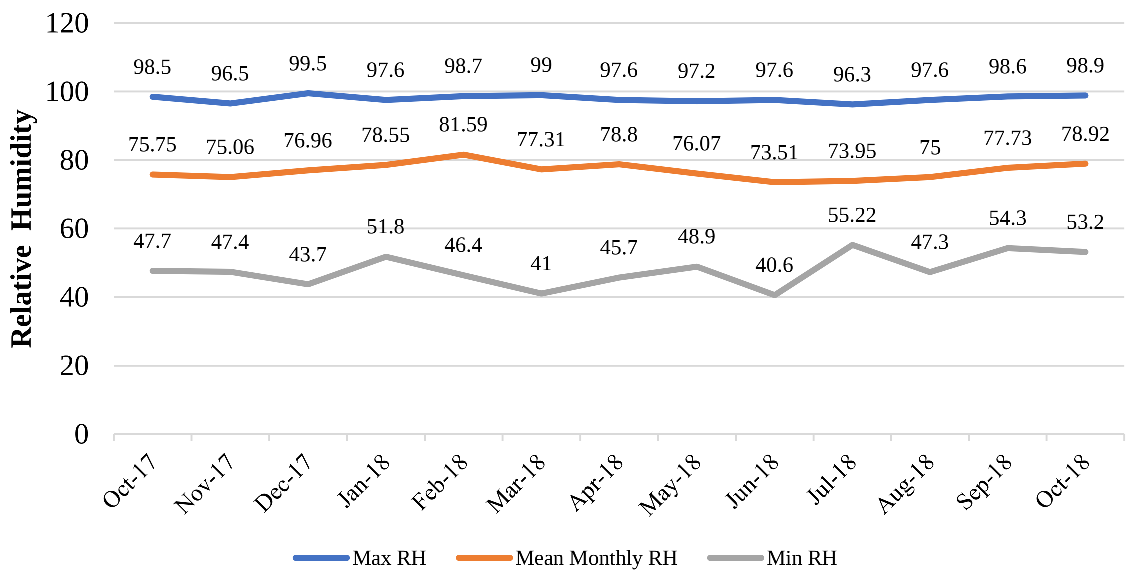
Appendix Figure 1.4.Cumulative monthly precipitation (mm) at Magoon Research Facility from October 2017 to March 2018.



Appendix Figure 1.5. Day length graph for Honolulu, USA from October 2017 to March 2018. Source: www.timeanddate.com/sun/usa/honolulu?month=4&year=2017.

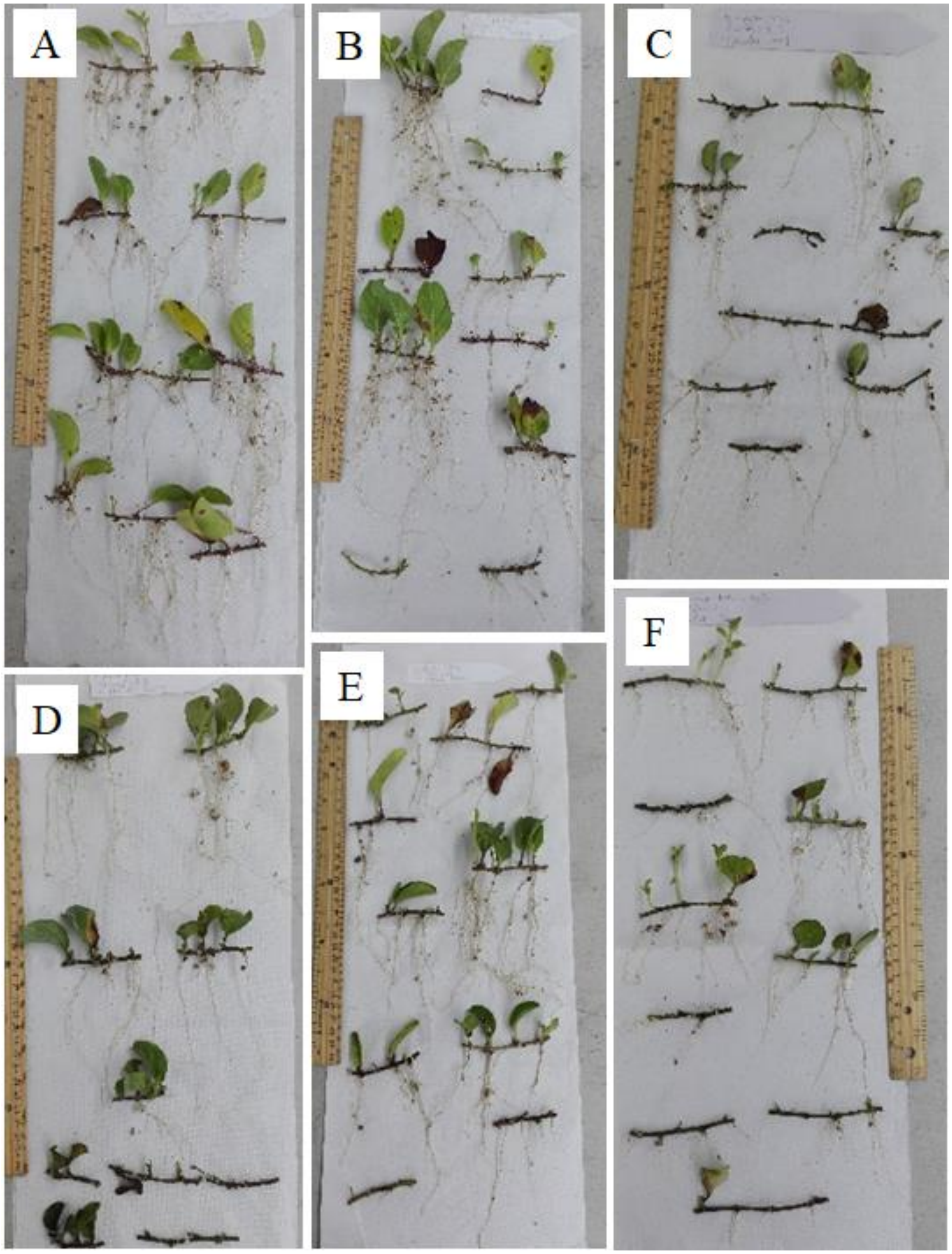


Appendix Figure 2.1. Maximum, mean and minimum monthly temperature at the Magoon Research Facility, Honolulu, Hawaii (~48 m above sea level) from October 2017 to October 2018.

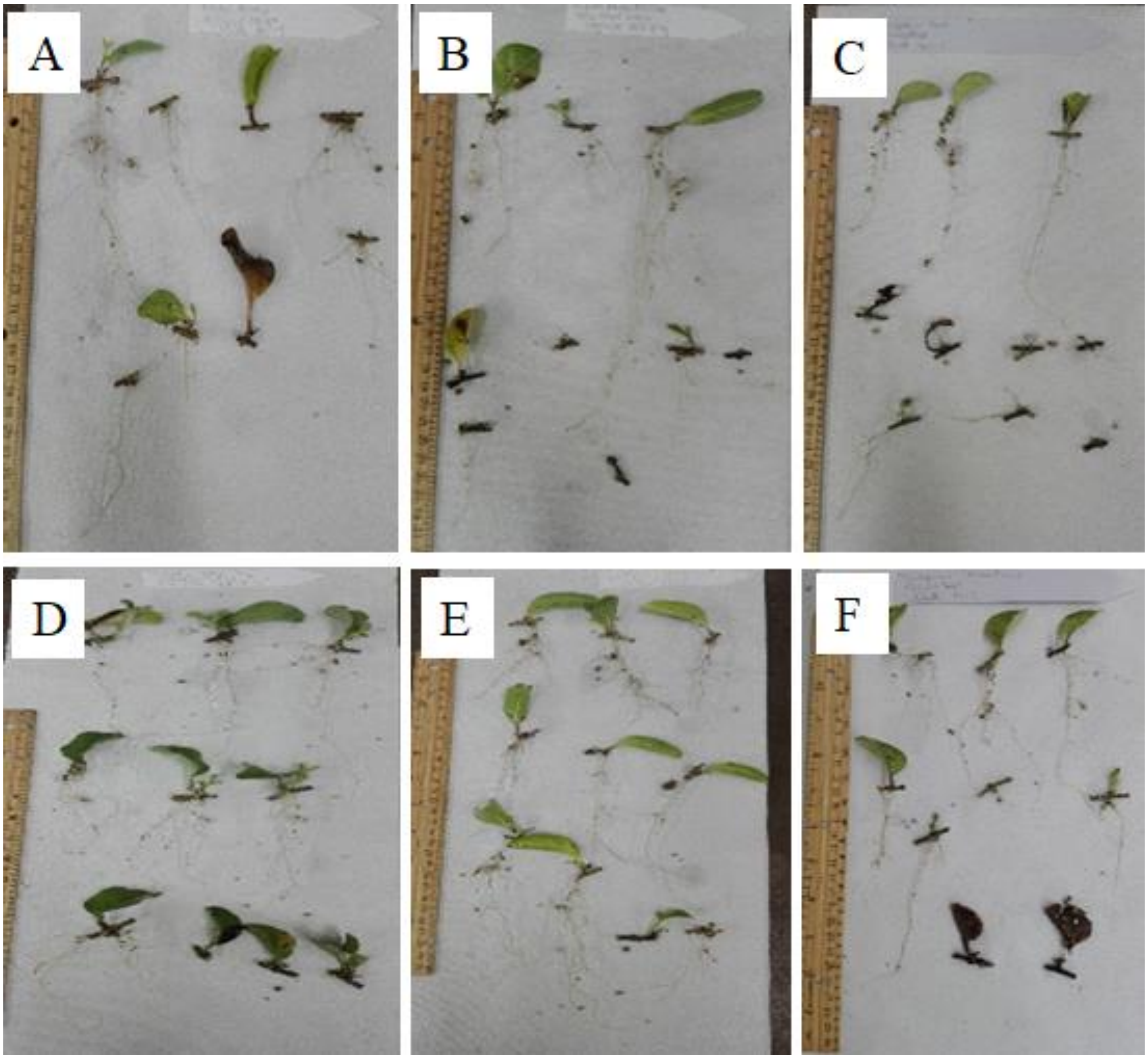


Appendix Figure 2.2. Maximum, mean and minimum monthly relative humidity at the Magoon Research Facility, Honolulu, Hawaii (~48 m above sea level) from October 2017 to October 2018.

Appendix Figure 2.3. Rooting at the nodes of pa‘uohi‘iaka stem cuttings.



Appendix Figure 2.4. Rooting of four node stem cuttings of six pa'uohi'iaka (*Jacquemontia sandwicensis* A. Gray) accessions propagated in March 2018: A) Ahihi-Kinau, B) Lyon Arboretum, C) McGregor, D) Puhala Bay, E) Shidler College, and F) South Point.

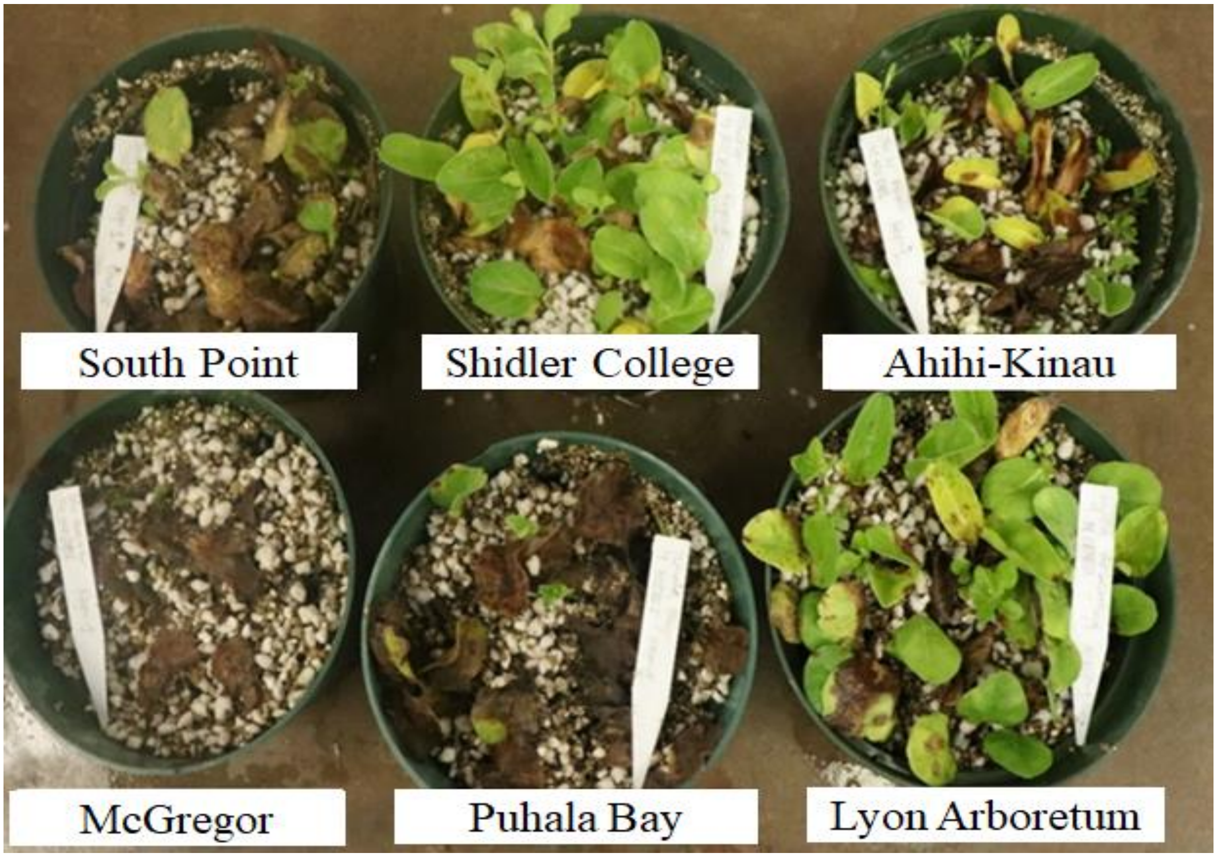


Appendix Figure 2. 5. Rooting of single node stem cuttings of six pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) accessions propagated in March 2018: A)Ahihi-Kinau, B) Lyon Arboretum, C) McGregor, P) Puhala Bay, E) Shidler College, and F) South Point

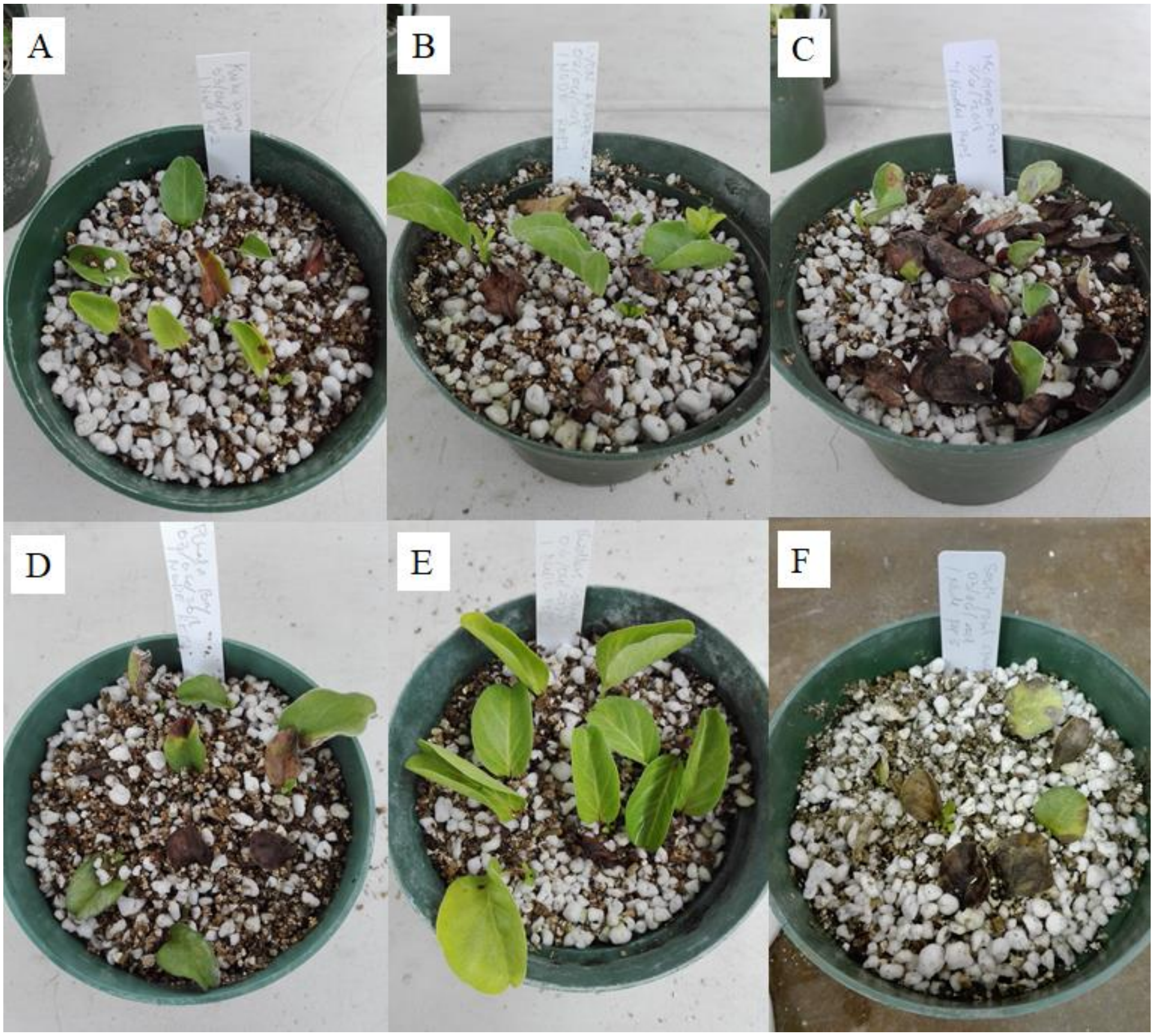
Appendix Figure 2. 6. Rooting of four node stem cuttings of six pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) accessions propagated in October 2018: A) Ahihi-Kinau, B) Lyon Arboretum, C) McGregor, D) Puhala Bay, E) Shidler College, and F) South Point.

Appendix Figure 2. 7. Rooting of single node stem cuttings of six pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) accessions propagated in October 2018: A)Ahihi-Kinau, B) Lyon Arboretum, C) McGregor, D) Puhala Bay, E) Shidler College, and F) South Point.

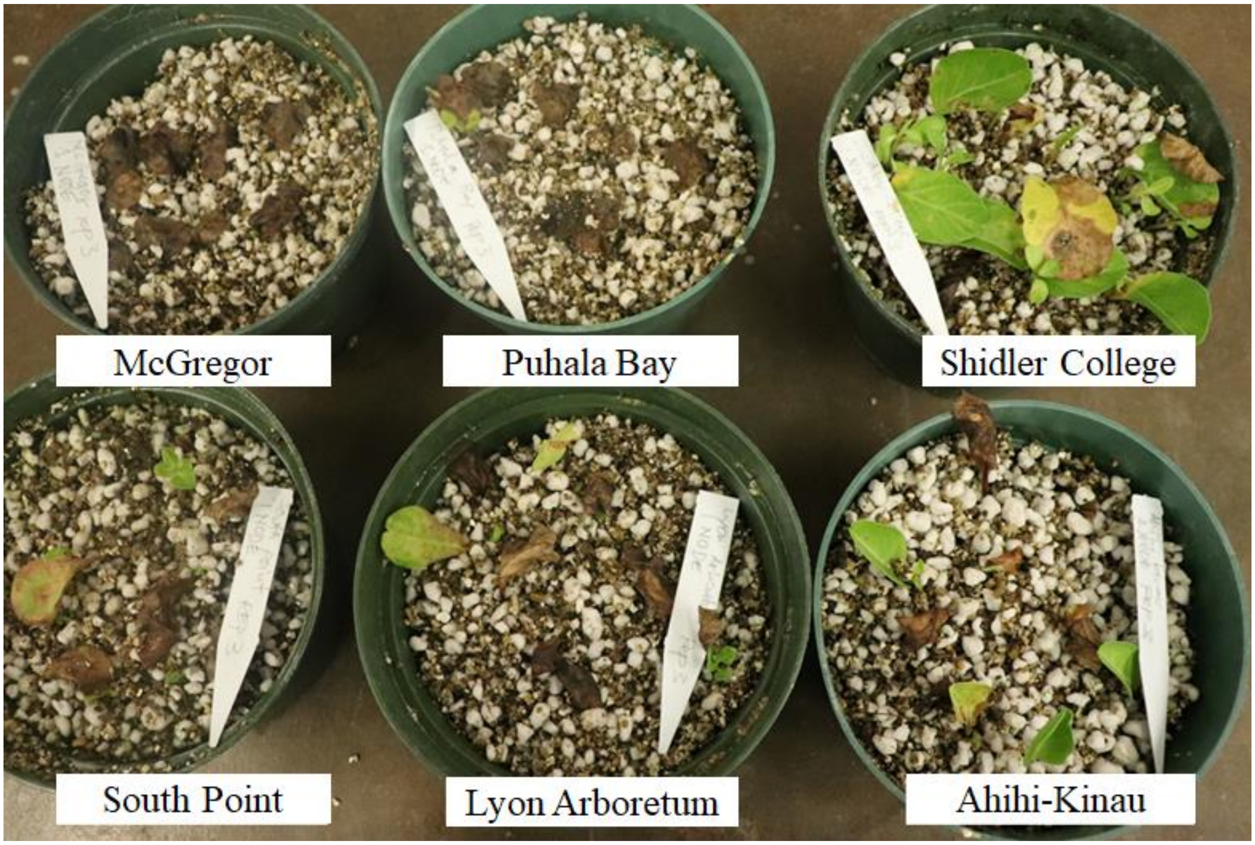
Appendix Figure 2.8. Leaf retention of four node stem cuttings of six pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) accessions 21 days after propagation under the mist bench. Cuttings were propagated in March 2018. A) Ahihi-Kinau, B) Lyon Arboretum, C) McGregor, D) Puhala Bay, E) Shidler College, and F) South Point



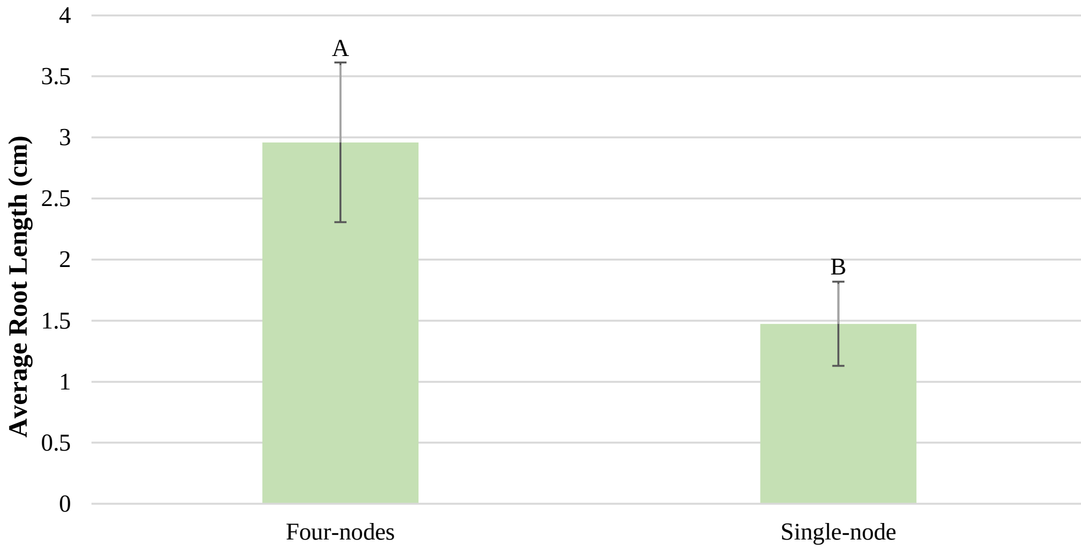
Appendix Figure 2. 9. Leaf retention of the six accessions of pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) four node stem cuttings for second propagation date (October 2018).



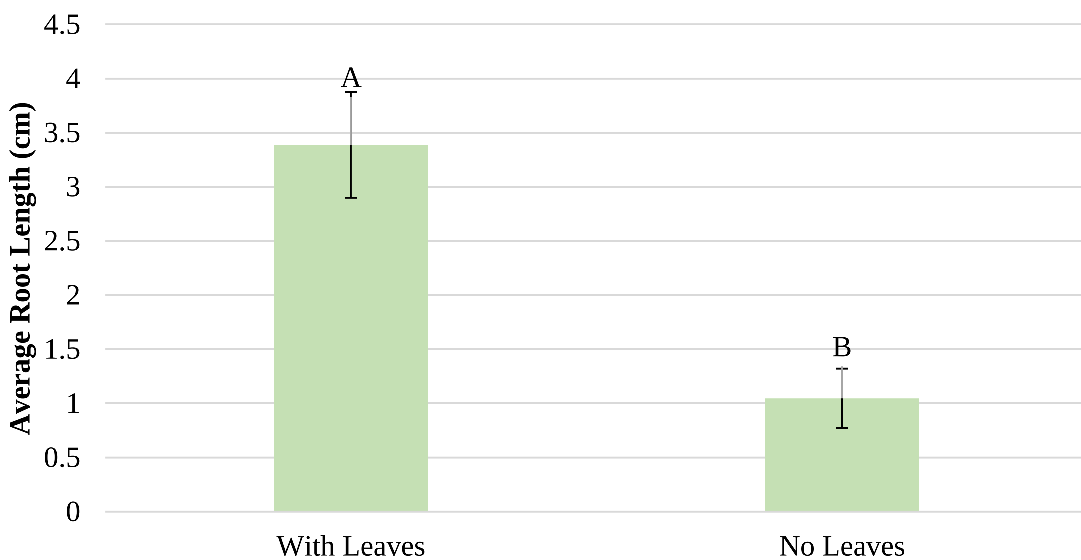
Appendix Figure 2.10. Leaf retention of the six accessions of pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) single node stem cuttings from first propagation dates (March 2018) of propagation. A) Ahihi- Kinau, B) Lyon Arboretum, C) McGregor, D) Puhala Bay, E) Shidler College, and F) South Point.



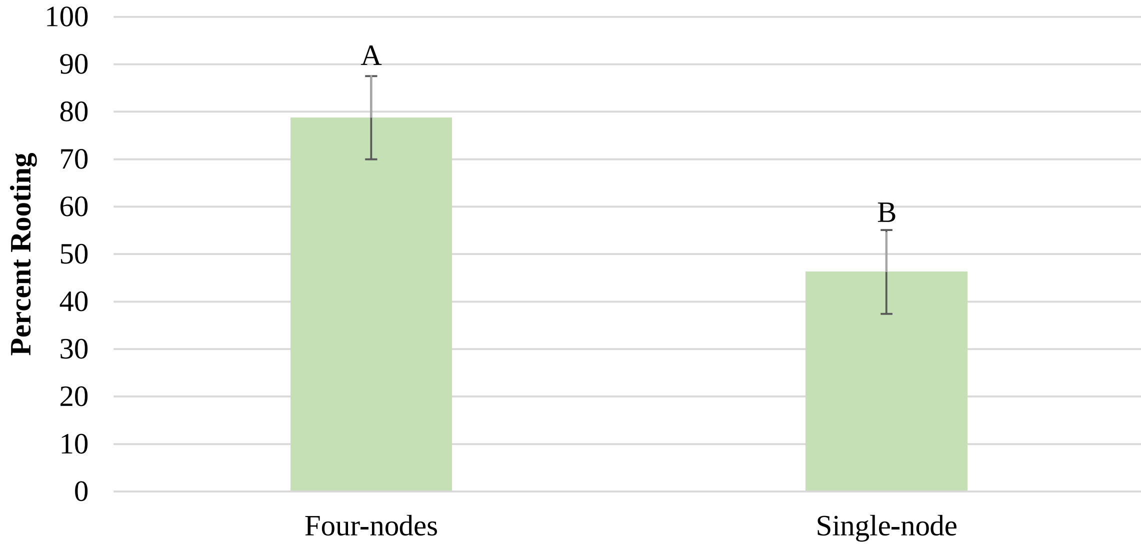
Appendix Figure 2.11. Leaf retention of the six accessions of pa‘uohi‘iaka (*Jacquemontia sandwicensis* A. Gray) single node stem cuttings from first (March 2018) and second propagation dates (October 2018) of propagation.



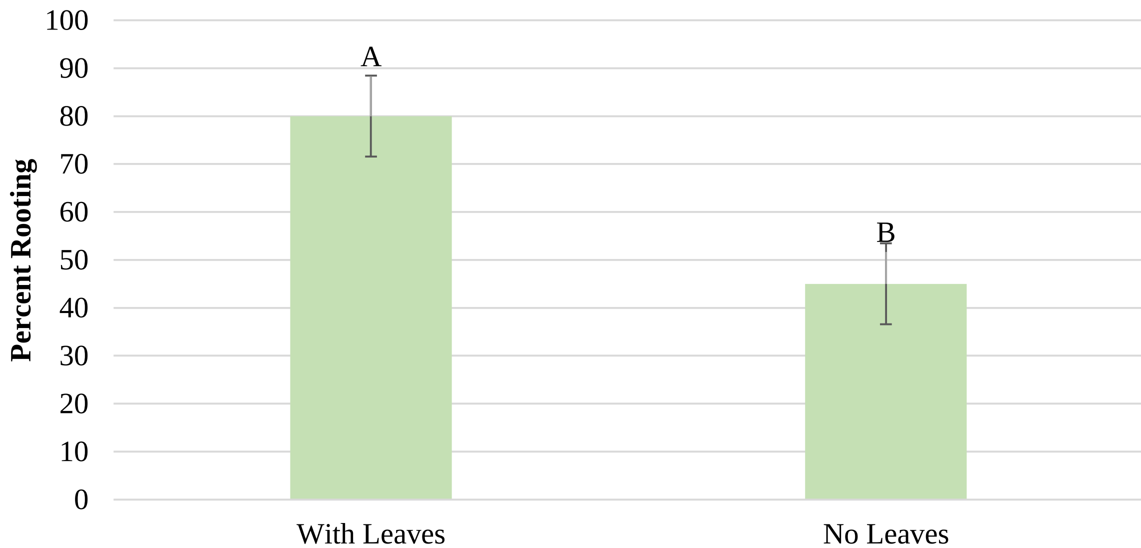
Appendix Figure 2.12. Average root length (cm) of Ahihi-Kinau stem cuttings as influenced by number of nodes. Root length and standard errors presented are combined across stem cuttings with or without leaves. Bars that are not the same letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=8.



Appendix Figure 2.13. Average root length (cm) of Ahihi-Kinau accession single node stem cuttings as influenced by presence and absence of leaves. Root length and standard errors presented are combined across stem cutting lengths. Bars that are not the same letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=8.

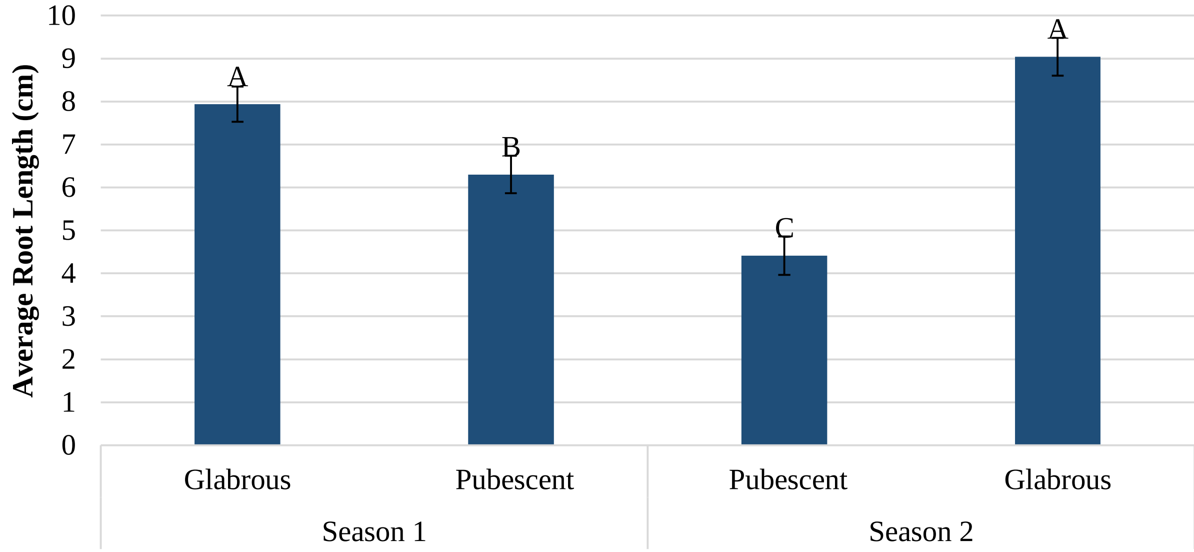


Appendix Figure 2. 14. Percent rooting of Ahihi-Kinau stem cuttings as influenced by number of nodes. Percent rooting and standard errors presented are combined across stem cuttings with or without leaves. Bars that are not the same letters are significantly different using Tukey's HSD pairwise comparison test at $P<0.05$, $n=8$.

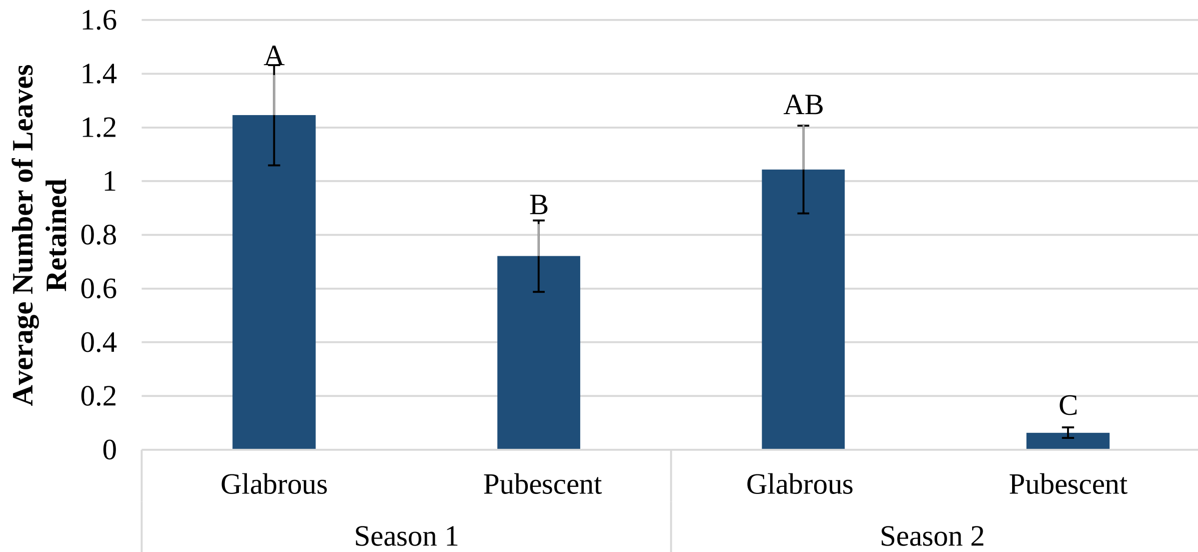


Appendix Figure 2.15. Percent rooting of Ahihi-Kinau accession single node stem cuttings as influenced by and presence and absence of leaves. Percent rooting and standard errors presented are combined across stem cutting lengths. Bars that are not the same letters are significantly different using Tukey's HSD pairwise comparison test at $P<0.05$, $n=8$.

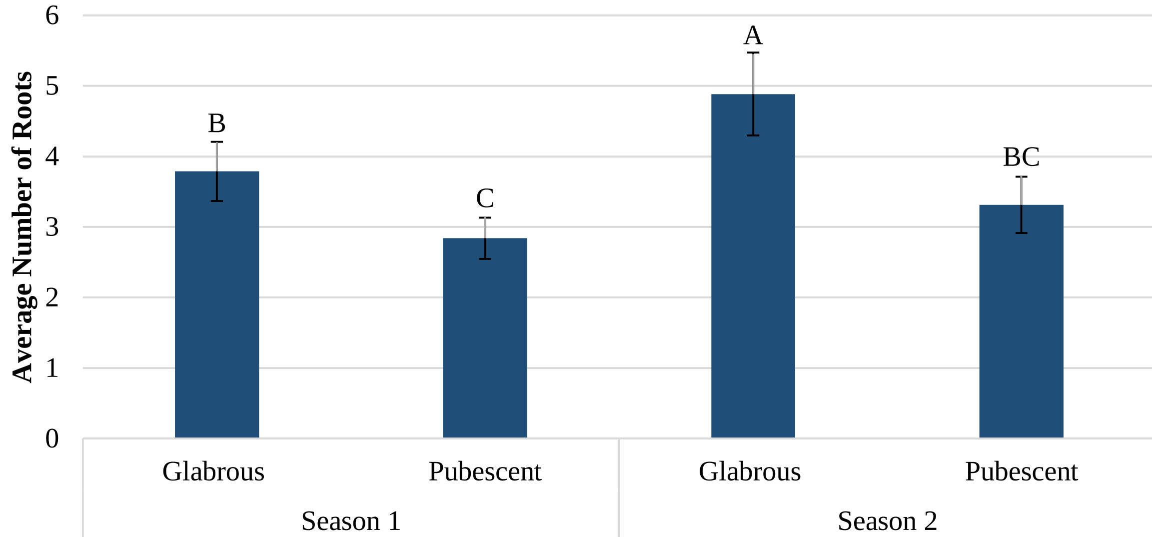
Appendix Figure 2.16. Rooting of Ahihi-Kinau stem cuttings: A) four nodes and no leaves, B) Four nodes with leaves, C) single node with leaf and D) single node without leaf.



Appendix Figure 2.17. Combined data on the average root length of pa‘uohi‘iaka as influenced by propagation dates (S1: March 2018 and S2: October 2018) and leaf type of accessions. Number of leaves retained and standard errors presented are combined across leaf type of accessions. Bars that are not the same letters are significantly different using Tukey’s HSD pairwise comparison test at P<0.05, n=24.



Appendix Figure 2.18. Combined data on the average number of leaves retained of pa‘uohi‘iaka as influenced by propagation dates (S1: March 2018 and S2: October 2018) and leaf type of accessions. Number of leaves retained and standard errors presented are combined across leaf type of accessions. Bars that are not the same letters are significantly different using Tukey’s HSD pairwise comparison test at P<0.05, n=24.



Appendix Figure 2.19. Combined data on the average number of roots of pa‘uohi‘iaka as influenced by propagation dates (S1: March 2018 and S2: October 2018) and leaf type of accessions. Number of leaves retained and standard errors presented are combined across leaf type of accessions. Bars that are not the same letters are significantly different using Tukey's HSD pairwise comparison test at P<0.05, n=24.

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