Investigations into the Morphological, Agronomic, and Nutritional Diversity within Breadfruit (*Artocarpus,* Moraceae) as a Resource for Food Security

by

Andrew Maxwell Phineas Jones

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Abstract

Global food security is one of the most pressing issues facing humanity in the 21st century, with the number of undernourished people reaching an unprecedented high of over 1 billion. The problem is most acute in rural areas in tropical climates. Breadfruit (*Artocarpus*, Moraceae), a high-yielding tropical staple food crop, has been identified under the International Treaty on Plant Genetic Resources for Food and Agriculture for its potential to impact food security. The Breadfruit Institute at the National Tropical Botanical Garden houses the largest breadfruit collection in the world which represents a vast diversity of botanical and nutritional characters developed through millennia of traditional breeding. Breadfruit exhibits a great degree of morphological variability with cultivars that produce small 500g seeded fruit to those that produce large 3.5 kg seedless fruit. Variation is also expressed in the 57 characteristics evaluated in this study, with deep implications regarding the history of breadfruit domestication and the utilization of this crop to bolster food security. Evaluation of agronomic diversity has classified breadfruit into 10 seasonality groups, including non-seasonal, early, and late season cultivars. Informed cultivar selection based on these data will allow the fruiting season to be extended, and year round production will be possible. Further, 94 cultivars of breadfruit along with two related species, *Artocarpus camansi* and *Artocarpus mariannensis*, were evaluated for nutritional quality in fresh fruit and flour. Breadfruit is a good source of calcium, copper, iron, potassium, magnesium, has similar levels of protein as many other tropical staple crops, and many cultivars produce pro-vitamin A carotenoids. Individual cultivars have been identified that would provide 20%-25% of the recommended daily adult requirement for protein, approximately 23.5% calcium, 97.4% copper, 19.2% iron, 48.1% potassium, 115.8% magnesium, 33.6% manganese, 0.6% sodium, 53.5% phosphorous, and 21.0% zinc of the recommended daily intake

of a female between 19-30 years old, and enough pro-vitamin A carotenoids to fulfill over 60% of the minimum daily vitamin A requirement of adults. Together these data show the immense diversity present within breadfruit germplasm and provide a foundation to utilize this variability to provide food security in the tropics.

Preface

The work contained in this thesis was conducted as part of a collaborative project between the Breadfruit Institute at the National Tropical Botanical Garden and the University of British Columbia Okanagan. Versions of Chapter 2-7 are in preparation, or have been submitted and are in various stages of the publication process. The submission information and my contribution to each Chapter are presented below.

- Chapter 2 has been accepted for publication: **Jones, A.M.P.**, Ragone, D., Tavana, N.G., Bernotas, D., Murch, S.J. Beyond the Bounty: Breadfruit (*Artocarpus altilis*) for Food Security and Novel Foods in the 21st Century. Ethnobotany Research and Applications. In press.

  This Chapter is a comprehensive review of breadfruit with an emphasis on its nutritional content and potential for food security. I was responsible for writing the majority of the manuscript. The co-authors provided specific expertise with respect to ethnobotany, economics, breadfruit germplasm and ecology, and general editorial review of the manuscript.

- Chapter 3 has been submitted and is currently under review: **Jones, A.M.P.**, Murch, S.J., Wiseman, J., and Ragone, D. Morphological diversity in breadfruit (*Artocarpus*, Moraceae): Insights into domestication, conservation, and cultivar identification. American Journal of Botany.

  The data for this Chapter were collected by Dr. Ragone at the Breadfruit Institute of the National Tropical Botanical Garden. I was responsible for developing the approaches to data analysis, the statistical analysis of the data, interpreting the results, composing the manuscript, and developing the multi-access cultivar identification key.

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  The data for this Chapter were collected by Dr. Ragone at the National Tropical Botanical Garden over the period 1996-2005. I was responsible for designing the approaches to data analysis, conducting the statistical analysis, interpreting the results, and composing the manuscript.

- Chapter 5 has been submitted and is currently under review: **Jones, A.M.P.,** Ragone, D., Lane, A., Murch, S.J. High Yield and Protein Cultivars of Breadfruit (*Artocarpus altilis*): Developing an Underutilized Crop for Food Security and Novel Foods. Journal of Food Composition and Analysis.

  I was responsible for the experimental design, sample collection, quantification of protein, statistical analysis and interpretation, and composing the final manuscript for publication. My co-authors provided valuable assistance in designing the experiments, sample collection, and revising the final manuscript.

- Chapter 6 has been submitted and is currently under review**: Jones, A.M.P.**, Ragone, D., Aiona, K., Murch, S.J. Breadfruit (*Artocarpus altilis*): An underutilized crop with potential to address the 'hidden hunger' of micronutrient deficiency. Journal of Food Composition and Analysis.

  Soil analysis data were coordinated by Mr. Aiona at the National Tropical Botanical Garden. I was responsible for the experimental design, sample collection, analysis of fruit mineral content, statistical analysis and interpretation, and composing the final manuscript for

publication. My co-authors provided valuable assistance in designing the experiments, sample collection, and revising the final manuscript.

- Chapter 7 is currently in preparation and will be submitted in the near future.

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Chapter 1: Introduction

Ensuring global food security is one of the most pressing issues facing humanity in the 21st century. The term "Food security" was defined at the World Food Summit in Rome, 1996, as "when all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life"(World Bank, 2007). During this meeting, representatives from 185 countries pledged to reduce world hunger to 50% of 1990-1992 levels by the year 2015. However, since that time there has been an unfortunate increase in hunger worldwide (FAO, 2008) and estimates of the number of undernourished people have increased to a record high of 1.02 billion, approximately 1 in 6 people (FAO, 2009a). One of the underlying causes of this increase is the continually expanding global population and static resources available to produce the necessary food. It is estimated that within the next few decades we will need to increase food production by approximately 50% just to maintain the current level of food security, which will need to be achieved with little new agricultural land available. Approximately 80% of the world's undernourished population lives in the tropics, and 70% live in rural areas of developing nations and depend primarily on agriculture for their livelihood (World Bank, 2007). As such, agricultural development in the tropics must play a leading role in the alleviation of world hunger and to increase global food security. However, it is also important to recognize that food security entails not only enough quantity of food, but that the available food is nutritious. Currently, billions of people suffer from various micro-nutrient deficiencies worldwide, leading to a significant amount of mortality, morbidity, and economic losses. Any intervention to address food security needs to address both of these issues.

About 95% of the world's food energy is derived from only 30 of the 7000 recorded plant species used for food (Williams and Haq, 2002). Reliance on so few species brings into question the capacity of our current agricultural systems to meet the growing demand for food in the face of an ever increasing global population, the changing climate, or new/re-emerging plant diseases. Increasing agricultural biodiversity through crop diversification has the potential to improve the robustness of our agricultural production systems and the sustainability of the world food supply (Williams and Haq, 2002). Neglected and underutilized crops represent a relatively untapped resource to diversify agricultural biodiversity and enhance food security. Many underutilized crops species are well adapted to various climatic conditions and are often very important regionally. However, by definition these crops are currently cultivated to a limited extent and there is a general lack of basic information on crop diversity, agronomy, and nutritional composition.

Breadfruit (*Artocarpus altilis* Parkinson (Fosberg)) is a traditional staple crop grown for its starchy fruit throughout Oceania (Ragone, 1997). It is one of 35 crop species identified in the International Treaty on Plant Genetic Resources for Food and Agriculture for their potential to enhance food security and interdependence (FAO, 2009b). Although it has been introduced throughout the tropics, it remains an underutilized crop grown on a limited scale in most areas. Breadfruit is estimated to yield 6 t/ha of edible dry weight (Bowers, 1981). This is an impressive yield compared to the current globally predominant staple crops with typical yields of 4.11 t/ha for rice, 4 t/ha of corn, and 2.6 t/ha of wheat (Calpe, 2007; FAO, 2009c; FAO, 2009d). Breadfruit is also a relatively low-input crop and a key component of some agroforestry systems that may be suitable for sustainable production in resource-poor regions (Fownes and Raynor, 1991). Despite the high yields, and its potential for low-input sustainable production systems,

breadfruit remains a marginal crop outside of the Pacific region (Ragone, 1997). Millennia of selective breeding by indigenous peoples of Oceania have resulted in enormous variability in morphological, agronomic, and nutritional characteristics among cultivated varieties (Zerega et al., 2004; Zerega et al., 2006). However, as with many underutilized crops there is very little information on the diversity found among cultivars in breadfruit germplasm. A first step towards fully utilizing breadfruit as a more prominent staple on a global scale is to assess the diversity that is present within the species.

The breadfruit germplasm repository maintained by the National Tropical Botanical Garden is the largest and most diverse *ex situ* collection in the world with more than 325 accessions originating from more than 17 Pacific island groups, the Philippines, Indonesia, and the Seychelles (Ragone, 1997). Within this collection are representatives from both of the putative wild relatives, *A. camansi* Blanco and *A. mariannensis* Trécul, seeded diploid *A. altilis*, seedless diploid and triploid *A. altilis*, early generation *A. altilis* × *A. mariannensis* hybrids, and highly domesticated *A. altilis* × *A. mariannensis* hybrids covering the complete process of domestication and representing vast geographic regions (Ragone, 2001; Ragone and Wiseman, 2007; Zerega et al., 2005). This diverse group of breadfruit cultivated in a geographically isolated region provides an invaluable resource to study phenotypic diversity within breadfruit. The following studies were conducted to evaluate the botanical and nutritional diversity present within this collection to provide insights into crop domestication, help guide future conservation efforts, and as a resource to provide food security in the tropics. The specific objectives of this thesis are to:

I. Use standardized morphological descriptors (Ragone and Wiseman, 2007) to compare and contrast breadfruit cultivars in order to:

a. Identify cultivars that exhibit desirable traits for farmers, consumers and for processing into flour
b. Provide insights into the morphological changes that occurred during domestication and further breeding of the crop.
c. Determine the amount and geographical distribution of morphological diversity within breadfruit and identify regions with distinct morphologies to guide future conservation efforts.
d. Produce a multi-access breadfruit cultivar identification key to identify known cultivars and new cultivars with unique characteristics.

II. Compare the diversity in breadfruit seasonality to:
    a. Identify germplasm with complementary fruiting seasons to extend the breadfruit season and achieve year round production.
    b. Develop a predictive model to determine the changes in the fruiting season when planted in a new location.

III. Evaluate the diversity in the nutritional composition of breadfruit to:
    a. Identify elite, nutrient-dense cultivars with potential to improve food security
    b. Compare various geographic origins and species to guide future germplasm screening programs.

Together, these studies provide the most comprehensive evaluation of the amount and geographical distribution of phenotypic diversity present within breadfruit germplasm. Elite cultivars identified here will provide a foundation to utilize this rich resource for agricultural development to improve food security in the tropics. Understanding the geographical

distribution of this diversity will be invaluable for future germplasm screening programs, conservation efforts, and provide insights into crop domestication.

Chapter 2: Breadfruit (*Artocarpus altilis*) for Food Security and Novel Foods in the 21st Century[1]

Breadfruit: An Historical Perspective

While much of the temperate world may know breadfruit as an obscure plant seen on tropical vacations or in movies and books, the potential of this plant as a valuable food resource has long been recognized. The Spanish collected breadfruit and introduced it to the Philippines during the 17th century, but it was not until the 1700s that more widespread dissemination of the crop outside of Oceania occurred (Barrau, 1976; Ragone, 1997; Smith et al., 1992). This spread was spurred by the writings of Sir Joseph Banks who recommended the introduction of breadfruit to the Caribbean and other tropical colonies as a source of food after seeing it on his voyage on HMS Endeavour from 1768-1771 (Banks, 1962). After his failed attempt to introduce Tahitian breadfruit to the Caribbean following the famed mutiny on the HMS Bounty, Captain William Bligh led a subsequent expedition and successfully transported more than 600 breadfruit plants to Jamaica and St. Vincent in 1772 (Powell, 1977; Ragone, 1997). Soon after, French contemporaries introduced breadfruit to many of their tropical colonies. Breadfruit is now cultivated to a limited extent in over 80 countries worldwide including regions of Africa, Australia, South America, South and South-East Asia.

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Breadfruit is a well known part of daily life in much of Oceania (Melanesia, Micronesia and Polynesia) where it has been cultivated as a staple crop for 2000-3000 years (Zerega et al., 2004, 2005) and plays a significant role in many aspects of daily life. There are many legends about the origin of breadfruit from various parts of Oceania (Ragone, 1991) that often describe the origins of the breadfruit tree arising from a man or god, and providing sustenance during times of famine. The following example was originally recorded on Raiatea, based on an oral account in 1887 (Henry and Orsmond, 1928; Ragone, 1991):

"During a time of famine long ago, red clay was the only food. A Raiatean man took his wife and four children into the mountains where they hid in a cave and ate ferns. He told his wife to go outside in the morning and she would see "my hands become leaves, the trunk and two branches my body, and the round fruit my head and the core inside, my tongue." In the morning, a beautiful tree stood as her husband had foretold. That valley is now called *Tuauru*, the place of breadfruit. She roasted the fruit, soaked it in a nearby stream and peeled it. She fed her family, but did not first make a customary offering to the king. When she prepared the fruit, pieces of the core and peel washed down stream. Servants of the king were catching shrimp in the stream. They found and ate the pieces. They were curious about this strange food and searched until they found the tree. They asked the woman what it was and she replied *uru*. She explained how it had arisen from the body of her husband who wanted to feed his family. The servants admired the tree which was covered with fruit of all the cultivars. The tree was taken down from the mountain and planted in her family's *marae*. A root was broken off and taken to the island of Tahaa where it grew. Ripe fruits were taken to the king and he liked it so much he ordered his servants to bring the tree and its owner to him. While they were transplanting the

tree, woman begged for some roots and planted them in a valley which became known as *Maiore*. The family wept for their lost tree, but new trees soon arose from the roots left behind."

Throughout Oceania there are hundreds of unique cultivars of breadfruit exhibiting great diversity in many attributes (Morton, 1987; Ragone, 1997). For example, there are 132 cultivars documented from Vanuatu (Walter, 1989), 70 from Fiji (Koroveibau, 1967; Morton, 1987), 50 from Pohnpei (Fownes and Raynor, 1991; Ragone and Raynor, 2009), more than 30 from Tahiti (Wilder, 1928) and over 40 from Samoa (Ragone et al., 2004). Cultivars are named and valued based on morphological characteristics, cooking and storage qualities of the fruit, and agronomic characteristics. Cultivars in Samoa are generally named using a binomial system, composed of the generic name '*ulu*, the local name for breadfruit, followed by a specific epithet that often provides a description of the fruit or cultivar in general (Ragone et al., 2004). However, the general term 'ulu is often not included for cultivars in which it is implicitly understood. For example, Ma'afala, the most commonly known cultivar in Samoa, is not typically referred to as 'ulu ma'afala, but it is understood and may be referred to as such if needed (Ragone et al., 2004). A similar naming system is used in Pohnpei where the generic name for breadfruit is *mahi* and most cultivar names are binomials with *mei* or *mein* included as part of the name, e.g., *Meiuhpw* or *Meinpadahk*. Only a few names, such as *Lipet* or *Luhkual*, are monomials. Pohnpeians classify all breadfruit into two types based on skin texture: (I) smooth (*meiniwe*) or (II) rough (*meinsahrek*) (Ragone and Raynor, 2009).

As indigenous peoples in Oceania are becoming more modernized and shifting away from traditional foods in exchange for imported crops, much of the information about cultivars and breadfruit in general is being lost; for example a 20-29 year old Samoan knows an average of 6.1 cultivars while a 60-69 year old Samoan knows an average of 9.2 (Ragone et al., 2004).

Many other aspects of traditional knowledge such as cultivation and storage techniques are also threatened by cultural erosion (Lee et al., 2001; Ragone et al., 2004).

Often overshadowed by its value as a food crop, breadfruit has long been valued for a variety of medicinal and other secondary applications. The wood is used in the construction of homes and canoes, is prized for its resistance to termites, and is often used for wood carvings (Ragone, 1991). The sticky latex has been used as birdlime, as an adhesive/caulking in canoes and in traditional medicine. The bast fibers were used for the production of cloth and cordage, with specific cultivars desirable due to the high-quality cloth that they produced. Various parts of the plant, including crushed leaves, latex, bark, and roots, have been used to treat a variety of ailments ranging from skin conditions to high blood pressure (Table 2-1). Recent research from Indonesia has reported the patenting of phytochemicals isolated from leaf tissue of breadfruit trees for the prevention of stroke and cardiovascular diseases (Sagita, 2009). The use of breadfruit in medicine developed in traditional breadfruit growing regions in Oceania and was spread, or more likely developed independently, in areas where it is a relatively recent introduction such as the Caribbean and Taiwan (Lin et al., 1992; McIntosh and Manchew, 1993). The widespread use of this plant species in medicine, the possible independent development of these practices in isolated regions, and more recent empirical evidence suggest validity in its value in traditional medicine and potential for modern drug discovery.

Ecological Requirements

Breadfruit is well adapted to the wet tropics, doing best at temperatures ranging from 21-32 °C with an annual rainfall of 1525-2540 mm and adequate drainage (Ragone, 1997, 2006a). Cooler temperatures often result in low yields and increased plant mortality (Lebegin et al.,

2007). Although breadfruit requires relatively high levels of rainfall, it can survive droughts of 3-4 months after the tree is established (Elevitch and Wilkinson, 2000). Tolerance to soil salinity is known to vary among *A. altilis*, *A. mariannensis* and hybrid cultivars (Ragone, 1997, 2006a; Ragone and Manner, 2006). *Artocarpus mariannensis* is often found on small atolls and is reportedly more tolerant of soil salinity as well as salt spray from the ocean than *A. altilis*. However, this difference has not been quantitatively evaluated, nor have comparisons between *A. altilis* and *A. altilis* × *A. mariannensis* hybrids.

Taxonomy and Botanical Description

Breadfruit is a tropical tree in the genus *Artocarpus* in the Moraceae (mulberry/fig) family (Figure 2-1; (Fosberg, 1960; Jarrett, 1959)). The genus is comprised of approximately 50-60 species native to South/South-East Asia, and Australasia (Kanzaki et al., 1997; Zerega et al., 2004). It is sometimes further divided into two subgenera, *Artocarpus*, which includes breadfruit, and *Pseudojaca* (Kanzaki et al., 1997). The common name 'Breadfruit' typically refers to the species *Artocarpus altilis* (Parkinson) Fosberg, but is occasionally used in reference to *A. camansi* or *A. mariannensis*. *Artocarpus altilis* is classified as a 'cultigen', a domesticated species of plant that is not found in the wild, domesticated from breadnut (*A. camansi*) (Zerega et al., 2004, 2005). *Artocarpus camansi* is native to Papua New Guinea and potentially the Moluccas and Philippines (Coenen and Barrau, 1961; Zerega et al., 2004). *Artocarpus mariannensis*, another closely related species, is native to the Marianna Islands and Palau. Physical characteristics and Amplified Fragment Length Polymorphism (AFLP) analyses suggest that many varieties of breadfruit are interspecific hybrids of *A. altilis* × *A. mariannensis* (Fosberg, 1960; Zerega et al., 2004, 2005). *Artocarpus altilis* × *A. mariannensis* hybrid cultivars

are found throughout Micronesia, but are absent from Melanesia and Polynesia with the exception of a few recently introduced varieties (Fosberg, 1960; Zerega et al., 2004). The existence of hybrid cultivars was originally proposed by Fosberg and later supported based on human migration patterns (Fosberg, 1960; Zerega et al., 2004) as well as morphological and molecular analysis (Zerega et al., 2005). Phylogenetic analysis based on morphological characteristics and molecular evidence demonstrated that the three species belong in a closely related monophylic group within the genus (Zerega et al., 2004, 2005, 2006).

Seeded varieties of breadfruit are commonly found in and around Papua New Guinea and throughout Melanesia, while seedless cultivars predominate in the Polynesian islands (Ragone, 2001; Zerega et al., 2004). As human populations migrated throughout the Pacific islands, they developed and maintained their own unique cultivars. This has given rise to hundreds of distinct phenotypes often specific to individual islands. The distribution and development of these cultivars has been used to help elucidate human migration throughout Polynesia and Micronesia from Melanesia (Zerega et al., 2004).

Artocarpus altilis (Parkinson) Fosberg

Artocarpus altilis has previously been referred to as *A. incisa, A.* and *A. communis*, however, *A. altilis* is generally accepted as the appropriate nomenclature (Fosberg, 1941, 1960; Ragone, 1997). *Artocarpus altilis* is a moderately large evergreen tree generally growing from 15-20 m, but sometimes reaching over 30 m tall (Niering, 1963; Ragone, 1997, 2006a). During development, terminal buds are encased by two large stipules up to 30 cm long that senesce as the buds emerge (Ragone, 1997). Leaf morphology is highly variable between cultivars and to some degree even within an individual tree. The leaves are large, measuring between 22.8-90

cm long and 20-50 cm wide (Morton, 1987; Zerega et al., 2005), are broadly obovate to broadly ovate in shape, and vary from almost entire to deeply lobed with pinnate venation (Ragone, 1997). The abaxial surface of the leaf is often pubescent, with white or reddish-white trichomes found primarily along the veins. White latex is produced throughout the plant in mostly unarticulated laticifers, and is exuded upon mechanical injury (Harvey, 1999).

Breadfruit trees are monoecious, with inflorescences comprised of about 1500-2000 individual florets connected to the receptacle (Figure 2-1B). Male inflorescences are elongated and club shaped, measuring 12.5-30 cm in length (Morton, 1987; Ragone, 2006a; Zerega et al., 2005). Female inflorescences are globose and develop into a multiple fruit referred to as a syncarp (Jarrett, 1976). Male inflorescences appear earlier than female inflorescences providing a temporal separation preventing self pollination (Heard, 1999; Sharma, 1965). It is suspected that breadfruit is wind pollinated due to the reported lack of scent; however some researchers have reported a distinct floral aroma (Heard, 1999; Ragone, 2006a). Additionally, bees have been observed visiting flowers and fruit, but their significance regarding pollination is not known (Brantjes, 1981). Hand pollination of breadfruit inflorescences is sometimes done to improve fruit set (Heard, 1999). As with leaves, fruit morphology displays a great diversity in size, shape and other attributes. Generally, fruit are about 12 cm long, 12-20 cm wide and weigh 1-2 kg, but some cultivars can produce fruit weighing up to 6 kg (Figure 2-1C;Ragone, 1997, 2006a; Zerega et al., 2005). The skin varies in colour from light green to yellow at maturity while the flesh can range from creamy white to yellow. The individual sections of the fruit surface can be relatively flat or conical rising up to 5 mm above the fruit surface. Thus, the fruit can be smooth, bumpy, or spiked (Ragone, 2006a; Zerega et al., 2005). Many cultivars of *A. altilis* are triploid, and produce no seeds (3n=2x=~84) (Murch et al., 2007; Ragone, 2001), while others are diploid

(2n=2x=~56) and produce few to several seeds. *A. camansi* and *A. mariannensis* are both seeded diploids with 2n=2x=~56 (Ragone, 1997, 2001).

Artocarpus camansi Blanco

Artocarpus camansi, commonly referred to as seeded breadfruit or breadnut, resembles *A. altilis* to a large extent. Some of the distinguishing characteristics of breadnut are that the leaves are highly pubescent, covered in straight pale trichomes, the fruit contain numerous achenes (Bennett and Nozzolillo, 1987; Roberts-Nkrumah, 2002), and the fruit surface is covered in flexible spines measuring 5-12 mm (Ragone, 2006a; Reeve, 1974; Zerega et al., 2005). The principal difference is the high number of large, hard-coated seeds prevalent in the fruit. A key to differentiate these species has been developed (Zerega et al., 2005).

Artocarpus mariannensis Trécul

Artocarpus mariannensis, known as Dugdug, Ebechei and other common names in various regions is also similar to breadfruit in many respects, but can be distinguished using the aforementioned key (Ragone and Manner, 2006; Zerega et al., 2005). Some of the distinguishing characteristics include entire leaf margins or 3-7 lobes in the distal third of the leaf, abundance of reddish brown trichomes on the midrib and abaxial veins, and the fruit is often irregularly shaped with dark green skin, even when ripe. Also, the individual fruit that comprise the syncarp are not fused except near the receptacle and near the skin (Ragone and Manner, 2006). Distinguishing dugdug (*A. mariannensis*) from breadfruit (*A. altilis*) is relatively easy to the well-trained eye, however, *A. mariannensis* x *A. altilis* hybrids can display intermediate characteristics making their identification more complex (Fosberg, 1960; Ragone and Manner, 2006).

Genetic Diversity and Germplasm Conservation

Breadfruit is included as one of the 35 priority crops listed in the International Treaty on Plant Genetic Resources for Food and Agriculture for their potential impact on food security and food interdependence (FAO, 2009b). Furthermore, The Global Crop Diversity Trust has developed a conservation strategy that identifies breadfruit as one of the high priority crops (Ragone, 2007). Genetic diversity is greatest in Micronesia and Melanesia where seeded cultivars predominate, with less diversity found among the seedless triploid cultivars that typify the Polynesian cultivars (Ragone, 2001; Zerega et al., 2005). However, despite the more limited genetic diversity within Polynesia, there is a high degree of morphological diversity and many distinct cultivars. The breadfruit trees introduced outside of Oceania are primarily derived from a select few seedless cultivars from eastern Polynesia and represent a very small amount of the existing diversity (Ragone, 2007). Breadnut, *A. camansi*, has also been introduced outside of its native range and is cultivated on a small scale in the Caribbean, South America, South-East Asia and parts of Africa (Ragone, 2006b). As with breadfruit, the introduced breadnut plants represent a very small amount of the genetic potential that exists. Little work has been conducted on cultivar development or comparison in breadnut (Ragone, 2006b). Genetic diversity of breadfruit is manifested as phenotypic variation in leaf size and shape, seasonality of production, disease resistance, salinity tolerance, nutrient content, fruit size, shape, flavour, and texture. It is likely that breadfruit yields, nutrient content, shelf life and palatability could be improved with detailed descriptions and judicious selection of cultivars to be planted. It may also be possible to expand suitable breadfruit growing areas by identifying cultivars that display traits such as salinity and drought tolerance that would allow them to grow in traditionally marginal environments (Ragone, 2007).

Oceanic societies have shifted away from subsisting on locally produced traditional crops, incorporating larger amounts of imported/introduced food such as rice and wheat (Englberger et al., 2007a; Morton, 1987; Ragone, 2007). For example, between 2001-2003 wheat and rice accounted for approximately 20 percent of the average Samoan diet and 40 percent of the average Fijian diet (FAO, 2007). This dramatic shift in dietary intake indicates a reduced emphasis on the cultivation and the subsequent *in situ* conservation of the immense number of cultivars that exist. Breadfruit trees are also susceptible to natural phenomena such as hurricanes and droughts. With fewer trees being cultivated the probability of losing genetic diversity during a natural disaster increases significantly. Many cultivars are endemic to individual islands and relatively isolated events can represent tragic genetic and cultural erosion.

In order to preserve the genetic diversity of breadfruit, several *ex situ* germplasm collections have been established (Ragone, 2007). However, many of these collections lack proper cultivar identifications and some have diminished due to lack of funding and maintenance (Ragone, 2007). The largest active germplasm collection is located in the Kahanu Garden, Hana, Hawaii, managed by the Breadfruit Institute (BFI), of the National Tropical Botanical Gardens (NTBG). This collection is comprised of 265 trees representing about 120 well-documented cultivars. However, *ex situ* germplasm collections remain vulnerable to natural disasters, disease, and other deleterious events (Murch et al., 2007; Shi et al., 2007). In order to add a measure of security to the NTBG collection and enable large scale cultivar dissemination, the collection is being replicated *in vitro* (Murch et al., 2007; Shi et al., 2007). These efforts are complicated by the high rate of endophytic bacterial and fungal contamination in wild trees (Murch et al., 2007; Rouse-Miller and Duncan, 2000; Tuia et al., 2007) and differential responses to inductive stimuli requiring optimized regeneration protocol for each cultivar (Shi et

al., 2007). Despite these difficulties, 17 cultivars have been successfully incorporated into an *in vitro* collection, with an additional 24 in the preliminary stages.

Breadfruit Phytochemistry

Plants produce a wide range of phytochemicals loosely defined as secondary metabolites, compounds not usually necessary for basic metabolism but often function to attract animals or prevent infection, parasitism and predation (Simpson and Ogorzaly, 2000). The *Artocarpus* genus is known to produce a large number of secondary metabolites, and is specifically rich in phenylpropanoids such as flavonoids and flavones (Nomura et al., 1998). *Artocarpus altilis* (breadfruit) is no exception with over 130 compounds identified in various organs of the tree, more than 70 of which derived from the phenylpropanoid pathway (Table 2-2). Many of the isolated compounds have been found to exhibit biological activity including inhibition of platelet aggregation, anti-bacterial activity, anti-fungal properties, inhibition of leukemia cells and as an anti-tumor agent (See table 2-2). These data support the claim that the breadfruit tree may be an effective medicine with the potential to treat an assortment of medical conditions. Although some ethnobotanical information regarding traditional alternative uses of breadfruit exists (Navarro et al., 2007; Ragone, 1997), the literature is vague regarding methods of preparation, the degree of cultivar specificity, and other details that may be required in order to successfully identify useful products/compounds.

Nutritional Composition

Breadfruit is most often eaten as a staple food due to the high level of carbohydrates found in the flesh of the fruit (Figure 2-2A; Morton, 1987; Ragone, 1997; Ragone and Cavaletto, 2006; Wootton and Tumaalii, 1984). In addition to being a valuable source of carbohydrates,

breadfruit is also high in fiber, some vitamins and minerals. The nutrient composition of breadfruit reported in the literature is highly variable and extensive studies of the many existing cultivars have not been conducted. For example, the reported protein content of the fresh fruit varies by almost four-fold, reported fat content varies by more than a factor of 20, and a similar trend is seen in most of the micronutrients (Table 2-3). This extreme variation in nutritional content may be a result of differences in the maturity of the fruit tested, production systems, environmental factors, inconsistent analytical methods, and cultivars tested (Englberger et al., 2003a; Ragone and Cavaletto, 2006; Wootton and Tumaalii, 1984). There is a need to conduct a comprehensive survey of representative cultivars in a controlled manner to determine the degree of variability among cultivars and identify elite genotypes.

Breadfruit is most often consumed fresh, used as a starchy vegetable. One of the biggest limiting factors for large scale production and international trade is the perishable nature of the fruit. In typical conditions, the fruit will begin to deteriorate in approximately 5 days (Worrell et al., 2002). The shelf life of breadfruit can be extended up to 3-4 weeks using controlled atmosphere storage maintained at 16°C with 5% oxygen, 5% carbon dioxide (Sankat and Maharaj, 2007). The use of controlled atmosphere storage has significant potential to aid in scaling up breadfruit production and export, but these facilities are currently not available to growers in many regions that cultivate the crop. Traditionally, many Oceanic societies preserve breadfruit through a process of pit fermentation (Atchley and Cox, 1985); however, this has not spread into new areas and may not be readily incorporated into diverse international diets.

In order to increase the shelf-life of breadfruit and create a product that can be incorporated into a variety of diets, the production of flour is an ideal approach. Breadfruit flour has been successfully used in stiff porridges (Mayaki et al., 2003), infant formulas (Esparagoza

and Tangonan, 1993), extruded products (McHugh et al., 2007), bread (Ayodele and Oginni, 2002; Esuoso and Bamiro, 1995; Nochera and Caldwell, 1992), cake (Ayodele and Oginni, 2002), pancakes (Ayodele and Oginni, 2002) and biscuits (Nnam and Nwokocha, 2003; Olaoye et al., 2007; Omobuwajo, 2003). For some items such as infant formulas, stiff porridges and extruded products, breadfruit flour was found to be ideal, and produced a high quality product (Esparagoza and Tangonan, 1993, Mayaki et al., 2003, McHugh et al., 2007). In products where breadfruit was used in place of wheat flour, the quality of the products often suffers when a high proportion of breadfruit flour is used. This effect is most pronounced in traditional levened wheat breads where products containing more than 10% breadfruit flour suffer from cracking and crumbling, but in some studies was still acceptable at rates of up to 30% (Ayodele and Oginni, 2002; Esuoso and Bamiro, 1995; Nochera and Caldwell, 1992). In the production of biscuits, a higher level of breadfruit flour could be used, up to 67%, before product quality suffered (Nnam and Nwokocha, 2003; Olaoye et al., 2007; Omobuwajo, 2003). The differential performance of breadfruit flour relative to wheat flour is likely due to the intrinsic differences in their chemical and physical properties. In comparison to wheat flour, breadfruit flour is relatively high in total ash and crude fiber, but low in protein (Esuoso and Bamiro, 1995; Olaoye et al., 2007). The relative mineral and vitamin profile is difficult to ascertain due to the high level of variation observed, and is likely dependent upon the cultivar used in the flour preparation. Although the bulk of research has been conducted on flour produced entirely from the fruit's flesh, the peel and core can be included during processing. Flour produced from whole fruit is higher in ash, fiber, protein, and has a higher bulk density than flour produced from just the fruit flesh (Adewusi et al., 1995; Mayaki et al., 2003). These differences are analogous

to the production of whole wheat vs. refined wheat flours, and they may both find applications in the future.

Breadfruit has also been investigated as an alternative source of starch for industrial and pharmaceutical purposes. Seedless breadfruit cultivated in Venezuela yielded 18.5g/100g (DW) of starch (Rincón and Padilla, 2004). The breadfruit starch exhibited higher levels of water absorption, solubility and swelling power than starch obtained from corn or amaranth. The gelatinization temperature of the starch was 73.3°C, and it was highly stable during heating and cooling cycles. Modification of the starch using oxidation, acetylation, annealing, or heat-moisture treatments can be used to alter some of these functional properties (Adebowale et al., 2005). In general, these modifications resulted in reduced gelling activity, solubility, pasting temperature, peak viscosity, hot paste viscosity and cold paste viscosity. Starch extracted from breadfruit has shown promising results in the pharmaceutical industry as an alternative to corn starch as a tablet binder (Adebayo and Itiola, 2003) and tablet disintegrant (Adebayo et al., 2008). Breadfruit provides a ready alternative source of starch for a variaety of industrial and pharmaceutical applications.

In addition to the starchy flesh, some cultivars also produce edible seeds. This is especially significant in breadnut (*A. camansi*), where the seed accounts for the bulk of the fruit and is the most commonly ingested portion. The breadnut seed is fairly high in carbohydrates, but contains significantly higher levels of fat and protein than breadfruit flesh (Table 2-1). Breadnut can also be used to produce flour, resulting in a product rich in protein, similar or higher than that found in wheat (Esuoso and Bamiro, 1995; Oshodi et al., 1999). However, very few accessions have been studied to date and more work is required to evaluate the potential of the breadnut as a food resource (Ragone, 1997).

Agronomic Considerations

Plant Propagation

Although some cultivars of breadfruit produce viable seeds, they do not survive desiccation and cannot be stored for long periods of time (Rowe-Dutton, 1976; Zerega et al., 2004). Additionally, breadfruit is an out-crossing species and seeds do not grow true to type making seed propagation an undesirable method of propagation when a specific cultivar is wanted (Ragone, 2007). Traditionally, breadfruit is clonally propagated using root suckers, root cuttings, or air layering (Ragone, 1997, 2006a). These methods are suitable for small scale local production, but are insufficient to meet the current global demand for planting material (Moustache and Moustache, 2007; Roberts-Nkrumah, 2007). Further, shipment of root cuttings between countries is not always practical as roots can carry fungi and bacteria that spread disease and specialized agricultural permits are often required, for example, breadfruit plants being imported into the USA, Fiji, and Canada must be bare root, accompanied by a phytosanitary certificate and imported to a facility holding a valid Plant Protection permit. Many of the plants do not survive this type of shipment and losses of breadfruit propagules in this cross-border process are about 60% (Murch et al., 2008). As a result, the limited amount of modern distribution of breadfruit throughout the world that exists is a slow and cumbersome process.

Large-scale mass propagation using plant tissue culture provides an alternative method for the rapid, large-scale production of breadfruit plants in a sterile controlled environment (Figure 3; Murch et al., 2007, 2008). In brief, shoot tips and other small buds (Figure 2-3A) are surface sterilized to remove any fungi and bacteria (Figure 2-3B) before being cultured in a complete medium containing sugars, vitamins, minerals and a gelling agent (Figure 2-3C).

Optimization of the type and concentration of plant growth regulators in the media induces the proliferation of shoots (Figure 2-3D) or roots (Figure 2-3E). Plantlets are subcultured into temporary immersion bioreactor vessels (Figure 2-3F) for the growth of entire plants about 10 cm tall in a sterile, controlled environment in about 6-8 weeks. These plantlets can then be shipped to destinations around the world or acclimatized to a soil environment for planting (Figure 2-3G). This process allows for production of thousands of plants that are almost identical clones of the original tree (Figure 2-3H & I). The sterile nature of this technique ensures that resulting propagules are free from insects and disease, reducing the risk to the grower, importation restrictions, and often eliminating the need for quarantine.

Acclimatization and Cultivation of Trees

Breadfruit cultivation is a time-honored tradition in much of Oceania. Young breadfruit trees are traditionally planted in small pits supplemented with compost; no further fertilization is used (Ragone, 1997). They are planted during the rainy season to ensure adequate water for successful establishment. Breadfruit trees are a key component of various low input agro-forestry systems (Elevitch and Wilkinson, 2000; Raynor and Fownes, 1991). Coconut trees are often used as the upper canopy, breadfruit trees are found in the lower canopy and smaller shrubs and herbaceous crops comprise the understory (Mueller-Dombois and Fosberg, 1998). In other agroforestry systems, breadfruit is used as the upper canopy, with smaller crops grown underneath. Trees grown in agro-forestry systems are generally spaced relatively far apart to accommodate the understory crops, which results in a lower yield than would be obtained using an intensive monoculture production system (Elevitch and Wilkinson, 2000). Breadfruit grown in such a setting in Pohnpei produces yields in the range of 6.67 t/ha of fresh fruit (Fownes and

Raynor, 1991). Depending on the cultivar used and environmental conditions this would translate to approximately 0.5-1.9 t/ha of dried fruit flesh. The lower yield obtained in a mixed cropping system is compensated for by the additional harvest of spices, essential oil plants, fruit, roots/tubers, vegetables, herbs, coffee or other commodities grown in the understory. Understory crops, specifically annual and short lived perennials, are especially useful during the early establishment phase to provide income and/or food before the tree begins to bear fruit. Agro-forestry also diversifies production and reduces the risk of catastrophic losses due to diseases or natural disasters that kill the tree crop. Breadfruit trees are also commonly found as "backyard trees" growing in the gardens of individual residences (Gbčhounou, 2007).

Modern Breadfruit Production

For better or worse, modern agriculture has shifted primarily to monoculture production systems. Although breadfruit is a major staple crop in many countries, large scale cultivation is virtually non-existent. The majority of breadfruit is still obtained from small growers, and consumed locally. Very little breadfruit is exported, with the Caribbean being a primary source, exporting around 1500 tonnes/year (Roberts-Nkrumah, 2007). Some of the primary factors preventing large-scale production of breadfruit are the highly perishable nature of the fruit, lack of planting material, and a lack of marketing and distribution networks (Roberts-Nkrumah, 2007).

Information on optimal fertilization regimes, pruning/training, planting density and other practices for modern orchard production have not been extensively evaluated, but there is some preliminary information (Coronel, 1990; Goebel, 2007; Lebegin et al., 2007; Webster, 2006). Soil tillage, specifically deep tilling, has been conducted prior to orchard establishment (Lebegin

et al., 2007). However, this is not traditionally required and may not be necessary in all locations and soil types. Likewise, fertilizer has been applied, but the nutrient requirements of the tree are not well known and application of fertilizer should be conducted based on a soil nutrient analysis. Planting densities ranging from 83.3-333 trees/ha have been used but even at 83.3 trees/ha the plants were too crowded upon maturity; lower planting densities have been recommended (Goebel, 2007; Lebegin et al., 2007). The practice of planting sturdy trees such as *Syzygium* spp., *Casuarina* spp., and coconuts (*Cocos nucifera* (L.)) at the edge of the orchard has been recommended to minimize damage caused by high winds (Goebel, 2007). To date, mechanical harvesters have not been utilized for the cultivation of breadfruit and hand harvesting remains the only viable option. It is estimated that over 50% of the fruit may be lost due to the difficulty of harvesting fruit from large trees (Roberts-Nkrumah, 2007).

Yield Potential

The moisture content of the fruit ranges from 62.7-89% (Table 1) and individual trees produce between 50 and 900 fruit per season depending on environmental conditions, tree size and cultivar (Lorens and Englberger, 2007; Marte, 1986; Ragone, 1997, 2006a). Fruit generally weigh 1-2 kg, but can reach up to 6 kg (Ragone, 1997, 2006a; Ragone and Cavaletto, 2006). The edible portion of the fruit accounts for approximately 70-75% of the fruit, with the skin and receptacle accounting for the remaining portion (Ragone, 1997). Based on these factors the estimated yield of breadfruit ranges from 4 t/ha to 50 t/ha with edible dry weight yields of up to 14 t/ha. Bowers (1981) reported that 6 t/ha of edible dry matter production for breadfruit is a reasonable estimate (Sauerborn, 2002). For perspective, the average global yield of irrigated

modern rice is 4.1 t/ha with an estimated upper limit of approximately 10 t/ha using modern cultivars in intensive agricultural systems (Calpe, 2007; FAO, 1999).

The Potential of Breadfruit

Hunger is a problem that has ravaged human civilization since pre-history. With the current rate of population growth, food shortages are imminent unless dramatic increases in food production are achieved. Many of the countries that suffer from high levels of undernourishment are found in tropical climates that are suitable for breadfruit production. The significance of this is apparent when one compares the yield of breadfruit to other commonly grown staple crops such as rice, corn and wheat. Some cultivars of breadfruit also contain significant amounts of essential vitamins and minerals (Englberger et al., 2003a, Ragone and Cavaletto, 2007). Breadfruit offers an opportunity to significantly increase food production in regions of the world that need it the most in a sustainable manner, and could play a substantial role in averting a crisis.

In addition to its potential in fighting hunger, breadfruit has significant economic potential worldwide once the obstacles to developing a global market are overcome. With adequate product development and marketing, processed breadfruit could have huge potential value as a grain substitute, cattle feed, latex and lumber. The global market for grain is enormous. Projections for grain consumption estimate that 2.4 billion tons of grain will be consumed by 2015 with a value of approximately $600 billion (Bruinsma, 2003). This is an average increase of 27 million tons ($7 billion) per year. As breadfruit emerges as a substitute for grain based foods, the value of the worldwide breadfruit crop could easily swell to billions of dollars in light of these economic trends.

Even in the absence of these trends, breadfruit would have significant growth potential in developed markets. Since the nutritional value of breadfruit flour has several advantages over cereal grains, an obvious application for the processed carbohydrate is as a nutritional supplement for products designed to appeal to the growing health conscious consumer group. A specific advantage of flour produced from breadfruit is that it is gluten free, giving breadfruit flour a unique market niche for those who suffer from Celiac disease and gluten allergies which affect approximately 1 in 133 people within the USA (Fasano et al., 2003). The market for gluten-free products in the USA alone was approximately $700 million in 2007 and growing with a projected value of $1.7 billion by 2010 (Cureton, 2007). Currently, gluten free products are considered expensive, presenting opportunity for gluten free flours such as breadfruit.

Breadfruit, and some of the by-products of breadfruit processing, can also be used as livestock feed (Ragone, 1997). According to the National Corn Growers Association, over one half of all corn grown in the USA is fed to livestock within the USA and overseas (www.NCGA.com). With the price of corn reaching over $6 per bushel in 2008 (Tenenbaum, 2008), the economic potential for an alternative livestock feed such as breadfruit is substantial. There are several secondary and tertiary products that may eventually develop from a global breadfruit market. Although the economic potential of these products may be less obvious than using breadfruit as a food source for people or livestock, examination of these possibilities reveals the true magnitude of breadfruit's global economic potential. These products are summarized below:

1) Ethanol. Ethanol can be made from breadfruit, by-products of breadfruit flour production, or from various parts of the tree (Ilori et al., 1996). In 2006, approximately 40 billion litres of ethanol biofuel was produced, utilizing about 50%

of Brazil's sugarcane, and 20% of the American corn harvest (World Bank, 2008). The demand for ethanol biofuel is increasing, and breadfruit could provide what appears to be an economically viable alternative feedstock (Ilori et al., 1996).

2) Latex. Latex derived from breadfruit has many traditional applications (Ragone, 1997) and may find modern uses and be sold as a by-product. Since the latex can be harvested without detriment to the tree, this is complementary to production of food products.
3) Wood. Wood from breadfruit trees has been used locally for generations (Ragone, 1997). It is resistant to moisture and pests, and performs well in furniture, boats, and other applications. Although harvesting the wood obviously cannot be a primary objective since it precludes fruit production, it is a possible source of additional income for growers as trees become less productive.
4) Carbon credits. Since the Kyoto protocol of 1997, capitalizing on carbon sequestration is as easy as selling carbon credits on the Chicago Climate Exchange (CCX), the European Climate Exchange (ECX) or the Global Carbon Exchange (GCX) (Capoor and Ambrosi, 2008). Since breadfruit trees are proficient carbon sinks, and the breadfruit farming industry will directly address the ongoing deforestation problem in tropical regions, corporations in developed nations may use breadfruit as a way to offset their carbon emissions as cap-and-trade programs gain steam. This is a growing market and has grown from a value of $10 billion in 2005 to approximately $64 billion in 2007 (Capoor and Ambrosi, 2008).

Overall, the economic impact of a global breadfruit market could be fundamental in helping less developed tropical nations improve their standard of living and per capita income.

Currently, regions where breadfruit can be grown are among the poorest regions in the world. In addition, the tendency for these countries to import grains from developed nations like the U.S. exacerbates the cycle of poverty. By developing a global market for an environmentally sustainable crop, these regions would strengthen their positions in the global economy as well as improving economic conditions locally.

Table 2-1: Traditional medicinal uses of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg).

Location	Plant Part	Preparation	Use	Reference
Vanuatu	Latex	Mix equal amount of latex from *Ficus adenosperma* and *Artocarpus*	Menorrhagia	(Bourdy and Walter, 1992)
Vanuatu (Mota Lava)	Latex	Not documented	Diarrhea	(Navarro et al., 2007)
Pacific Islands	Latex	Rubbed into skin	Broken bones and sciatica	(Ragone, 1997)
Pacific Islands	Latex	Diluted and taken internally	Diarrhea, dysentry and stomach aches	(Ragone, 1997)
Pacific Islands	Latex and/or crushed leaves	Rubbed into skin	Skin ailments and fungal infections	(Ragone, 1997)
Pacific Islands	Latex and/or crushed leaves	Latex and/or juice from crushed leaves	Ear infections	(Ragone, 1997)
Western Pacific	Leaf bud and latex	Chew and swallow from one to five fresh leaf buds, then drink one small glass of fresh latex. Repeat one to three times per day, until recovery is complete	Ciguatera poisoning	(Bourdy et al., 1992)
Western Pacific	Leaf Buds	Mix fresh leaf buds together with coconut oil	Ciguatera poisoning	(Lobel, 1979)
Trinidad and Tobago	Leaves	Not documented	Hypertension	(Lans, 2006)
Suriname	Leaves	Not documented	Fever	(Bipat et al., 2008)
Rotuma	Leaves	Not documented	Oral inflammation and pain	(McClatchey, 1993)
Taiwan	Leaves	Not documented	Liver disease and fever	(Ragone, 1997)
West Indies	Leaves	Tea made from yellowing leaves	High blood pressure, and perhaps	(Ragone, 1997)
Vanuatu	Young leaves	Not documented	Headaches	(Navarro et al., 2007)
Vanuatu (Aneithyum)	Young leaves	Not documented	Urinary infections	(Navarro et al., 2007)
Vanuatu (Mota Lava),	Male inflorescence	Burned	Mosquito repellant	(Navarro et al., 2007)
Not documented	Male inflorescence	Roasted, powdered and rubbed on gums	Aural pain relief	(Ragone, 1997)
Not documented	Root	Not documented	Purgative	(Ragone, 1997)
Not documented	Root	Mascerated and used as a poultice	Skin ailments	(Ragone, 1997)
Western Pacific	Shoots	Fluid pressed from the shoots	Ciguatera poisoning	(Weiner, 1984)
Samoa	Leaves	The juice from the chewed or crushed leaf petiole is dripped into the afflicted eye	Injured eyes	(Whistler, 2001)
Samoa	Bark/root	An infusion from the scraped bark or roots is sometimes taken as a potion	Urinary tract problems	(Whistler, 2001)
Samoa	Small branches	The smoke from a hollow, burning breadfruit twig is sometimes blown into the anal area of an infant for treating	*Ila*	(Whistler, 2001)
Vanuatu (Mota Lava)	Unknown	Not documented	Black magic, stop the rain	(Navarro et al., 2007)

Table 2-2: Identity and reported biological activity of compounds identified from Breadfruit.

Compound	Plant Part	Reported Biological Activity	Reference
Cyclomorusin	Unspecified		Nomura et al., 1998
Cyclomulberrin	Unspecified		Nomura et al., 1998
Engeletin	Unspecified		Nomura et al., 1998
Artocarpin	Unspecified		Hakim et al., 2006
artoindonesianin B	Unspecified		Hakim et al., 2006
artoindonesianin F	Unspecified		Hakim et al., 2006
Artonin E	Unspecified	Arachidonate 5-lipoxygenase inhibition; Inhibit leukemia cells	Nomura et al., 1998
Artonin E	Unspecified		Hakim et al., 2006
Artonin F	Unspecified		Nomura et al., 1998
Artonol A	Unspecified		Nomura et al., 1998
Artonol B	Unspecified		Nomura et al., 1998
Artonol B	Unspecified		Hakim et al., 2006
Artonol C	Unspecified		Nomura et al., 1998
Artonol D	Unspecified		Nomura et al., 1998
Artonol E	Unspecified		Nomura et al., 1998
Chaplasin	Unspecified		Hakim et al., 2006
cycloartobiloxanthone	Unspecified		Hakim et al., 2006
Cycloartocarpin	Unspecified		Hakim et al., 2006
prenylflavanoids (3 structures)	Unspecified		Lu et al., 2007
1,2-cyclohexanediol	Fruit (fresh and cooked)		Iwaoka et al., 1994
	(fresh & cooked)		
1: oxyresveratrol	Fruit	Anti-fungal; anti-oxidant	Amarasinghe et al., 2008
1-methylbutyl acetate	Fruit (cooked)		Iwaoka et al., 1994
1-octen-3-01	Fruit (fresh and cooked)		Iwaoka et al., 1994
1-penten-3-01	Fruit (fresh and cooked)		Iwaoka et al., 1994
2,3-butanediol	Fruit (fresh and cooked)		Iwaoka et al., 1994
2-cyclohexenone	Fruit (fresh and cooked)		Iwaoka et al., 1994
2-heptanol	Fruit (fresh and cooked)		Iwaoka et al., 1994
2-heptanone	Fruit (fresh and cooked)		Iwaoka et al., 1994
3β-acetoxyolean-12-en-11-one	Fruit		Amarasinghe et al., 2008
3-hydroxy-2-butanone	Fruit (fresh and cooked)		Iwaoka et al., 1994
5-ethyl2(5H)-furanone	Fruit (fresh and cooked)		Iwaoka et al., 1994
20,40,5,7-tetrahydroxy-6-(3-	Fruit		Iwaoka et al., 1994
benzyl alcohol	Fruit (fresh and cooked)		Iwaoka et al., 1994
Butanol	Fruit (fresh and cooked)		Iwaoka et al., 1994
Chloroform	Fruit (fresh and cooked)		Iwaoka et al., 1994
cinnamic alcohol	Fruit (fresh and cooked)		Iwaoka et al., 1994
cis-3-hexenol	Fruit (fresh and cooked)		Iwaoka et al., 1994
cis-3-hexenyl acetate	Fruit (fresh and cooked)		Iwaoka et al., 1994
cycloartenyl acetate	Fruit		Amarasinghe et al., 2008
Cyclopentanol	Fruit (fresh and cooked)		Iwaoka et al., 1994
diethylene glycol monoethyl	Fruit (fresh and cooked)		Iwaoka et al., 1994
ethyl 3-hydroxybutyrate	Fruit (fresh and cooked)		Iwaoka et al., 1994
Hexanal	Fruit (fresh and cooked)		Iwaoka et al., 1994
hexanoic acid	Fruit (fresh and cooked)		Iwaoka et al., 1994
Hexanol	Fruit (fresh and cooked)		Iwaoka et al., 1994
hexyl acetate	Fruit (fresh and cooked)		Iwaoka et al., 1994
isoamyl alcohol	Fruit (fresh and cooked)		Iwaoka et al., 1994
Isoartocarpesin	Fruit		Amarasinghe et al., 2008
Moracin M	Fruit	Anti-fungal; anti-oxidant; cytotoxic;	Amarasinghe et al., 2008
Norartocarpetin	Fruit		Amarasinghe et al., 2008
Norartocarpanone	Fruit		Amarasinghe et al., 2008
octanoic acid	Fruit (fresh and cooked)		Iwaoka et al., 1994
phenylpropyl alcohol	Fruit (fresh and cooked)		Iwaoka et al., 1994
Sitosterol	Fruit		Amarasinghe et al., 2008
sitosterol b-D-glucopyranoside	Fruit		Amarasinghe et al., 2008
trans-2(or 4)-	Fruit (fresh and cooked)		Iwaoka et al., 1994
trans-2-hexenol	Fruit (fresh and cooked)		Iwaoka et al., 1994
trans-2-pentenal	Fruit (fresh and cooked)		Iwaoka et al., 1994
trans-3-hexenol	Fruit (fresh and cooked)		Iwaoka et al., 1994
y –valerolactone	Fruit (fresh and cooked)		Iwaoka et al., 1994
2-cyclohexenol	Fruit (cooked)		Iwaoka et al., 1994

Compound	Plant Part	Reported Biological Activity	Reference
2-ethenyl-2-butenal (tentative)	Fruit (cooked)		Iwaoka et al., 1994
3-cyclohexenol	Fruit (cooked)		Iwaoka et al., 1994
3-hexene-2,5-diol (tentative)	Fruit (cooked)		Iwaoka et al., 1994
3-hexene-2,5-diol (tentative)	Fruit (cooked)		Iwaoka et al., 1994
amyl alcohol	Fruit (cooked)		Iwaoka et al., 1994
Benzaldehyde	Fruit (cooked)		Iwaoka et al., 1994
benzyl acetate	Fruit (cooked)		Iwaoka et al., 1994
cis-2-hexenal	Fruit (cooked)		Iwaoka et al., 1994
Ethanol	Fruit (cooked)		Iwaoka et al., 1994
ethyl acetate	Fruit (cooked)		Iwaoka et al., 1994
ethyl benzoate	Fruit (cooked)		Iwaoka et al., 1994
methyl acetate	Fruit (cooked)		Iwaoka et al., 1994
Toluene	Fruit (cooked)		Iwaoka et al., 1994
trans-2-hexenal	Fruit (cooked)		Iwaoka et al., 1994
Vanillin	Fruit (cooked)		Iwaoka et al., 1994
y- hexalactone	Fruit (cooked)		Iwaoka et al., 1994
dimethylbenzenepropionic acid	Fruit (cooked)		Iwaoka et al., 1994
2-butanone	Fruit (fresh)		Iwaoka et al., 1994
2-methyl-4-pentenal	Fruit (fresh)		Iwaoka et al., 1994
2-methylbutyric acid	Fruit (fresh)		Iwaoka et al., 1994
2-pentanol	Fruit (fresh)		Iwaoka et al., 1994
2-pentanone	Fruit (fresh)		Iwaoka et al., 1994
butyric acid	Fruit (fresh)		Iwaoka et al., 1994
cyclohexyl benzene	Fruit (fresh)		Iwaoka et al., 1994
ethyl butyrate	Fruit (fresh)		Iwaoka et al., 1994
ethyl palmitate	Fruit (fresh)		Iwaoka et al., 1994
trans,trans-2,4-heptadienal	Fruit (fresh)		Iwaoka et al., 1994
trans-3-hexenoic acid	Fruit (fresh)		Iwaoka et al., 1994
AC-3-1	Inflorescence		Nomura et al., 1998
AC-3-2	Inflorescence		Nomura et al., 1998
AC-3-3	Inflorescence		Nomura et al., 1998
AC-5-1	Inflorescence		Nomura et al., 1998
AC-5-2	Inflorescence		Nomura et al., 1998
Cycloaltilisin 6	Inflorescence Stipule		Patil et al., 2002
Cyclyaltilisin 7	Inflorescence Stipule		Patil et al., 2002
Frutackin	Seeds	Chitin binding, anti-fungal	Trindade et al., 2006
artocarpin dichoromethane	Root Bark		Chantrapromma et al.,
artomunoxanthentrione	Root Bark		Nomura et al., 1998
Artomunoxanthone	Root Bark		Nomura et al., 1998
artomunoxanthotrione epoxide	Root Bark		Nomura et al., 1998
Cycloartomunin	Root Bark		Nomura et al., 1998
cycloartomunoxanthone	Root Bark		Nomura et al., 1998
Cyclocommunin	Root Bark		Nomura et al., 1998
Cyclocommunol	Root Bark		Nomura et al., 1998
dihydrocycloartomunin	Root Bark		Nomura et al., 1998
dihydroisocycloartomunin	Root Bark		Nomura et al., 1998
friedelan-3-ol	Root Bark		Fun et al., 2007
Friedelin	Root Bark		Fun et al., 2007
artochamins B	Root Cortex	Anti-platelet	Wang et al., 2006
artochamins D	Root Cortex	Anti-platelet	Wang et al., 2006
artocommunol CC	Root Cortex		Wang et al., 2006
artomunoflavonone	Root Cortex		Wang et al., 2006
artomunoisoxanthone	Root Cortex		Wang et al., 2006
cyclocomunomethonol	Root Cortex		Wang et al., 2006
dihydroartomunoxanthone	Root Cortex	Anti-platelet	Wang et al., 2006
Cycloaltilisin	Stem		Chen et al., 1993
Isocyclomorusin	Stem		Chen et al., 1993
Isocyclomuberrin	Stem		Chen et al., 1993
artonin V	Stem Bark		Nomura et al., 1998
KB-2	Stem Bark	Inhibit leukemia cells	Nomura et al., 1998
Morusin	Stem Bark	Anti-tumour	Nomura et al., 1998;
2-geranyl-2',3,4,4'-	Heart Wood	5a-Reductase inhibition	Shimizu et al., 2000
geranyl dihydrochalcones (9 structures)	Leaves		Wang et al., 2008

Table 2-3: Reported nutrient composition of breadfruit mesocarp, mesocarp flour, fresh seed, and seed flour. ND = Not Detected, - = Not reported. Data compiled from (Englberger et al., 2003a; Englberger et al., 2007a; Huang et al., 2000; Mayaki et al., 2003; Morton, 1987; Oshodi et al., 1999; Ragone, 1997; Ragone and Cavaletto, 2006; Webster, 2006; Wootton and Tumaalii, 1984).

	Fresh Fruit		**Breadfruit flour**		**Fresh Seeds**		**Seed flour**	
	Min	Max	Min	Max	Min	Max	Min	Max
Moisture content (%)	62.7	89.16	2.55	21.02	35.08	61.9	8	8
kcal (per 100g)	105	138	-	-	-	-	-	-
Protein (%)	0.6	2.24	2.9	6.6	5.25	13.3	13.8	19.96
Fat (%)	0.1	2.36	1.8	2.8	2.5	5.59	3	12.79
Carbohydrates (%)	21.5	33	66.6	77.3	26.6	44.03	15.95	64.5
Starch (%)	20.1	20.1	53.4	75.7	-	-	-	-
Sugars (%)	2.9	2.9	2.17	31.8	-	-	-	-
Total fiber (%)	0.9	7.37	2.84	10.7	1.34	2.14	1.7	3.87
Soluble fiber (%)	ND	2.81	-	-	-	-	-	-
Minerals								
Ash (%)	0.56	1.2	1.69	4.5	1.5	5.58	3.42	3.5
Sodium (mg/100g)	1	70	-	-	ND	ND	0.29	0.29
Magnesium (mg/100g)	20	34	-	-	ND	ND	0.08	0.08
Phosphorous (mg/100g)	0.04	79	-	-	0.35	189	0.16	0.37
Potassium (mg/100g)	283	480	-	-	-	-	0.7	0.7
Calcium (mg/100g)	0.05	30	-	-	0.11	48.3	0.12	0.18
Iron (mg/100g)	0.29	2.4	-	-	2.3	3.87	-	-
Copper (mg/100g)	0.08	0.08	-	-	-	-	-	-
Boron (mg/100g)	0.52	0.52	-	-	-	-	-	-
Zinc (mg/100g)	0.07	0.13	-	-	-	-	-	-
Vitamins								
Vitamin B1 (mg/100g)	0.09	0.15	-	-	-	-	-	-
Vitamin B2 (mg/100g)	0.02	0.05	-	-	-	-	-	-
Vitamin B3 (mg/100g)	0.75	1.4	-	-	-	-	-	-
Vitamin C (mg/100g)	1.6	34.4	-	-	1.9	22.6	-	-
β-carotene (µg/100g)	ND	19.8	-	-	-	-	-	-
Retinol equivalents (µg/100g)	ND	157	-	-	-	-	-	-
Lutein (µg/100g)	38.6	119.7	-	-	-	-	-	-
Thiamin (mg/100g)	0.07	0.28	-	-	0.13	0.33	0.18	0.18
Riboflavin (mg/100g)	0.03	0.1	-	-	0.08	0.1	0.84	0.84
Niacin (mg/100g)	0.5	1.96	-	-	1.8	3.54	2.6	2.6

Figure 2-1: Morphology and growth of breadfruit (*Artocarpus altilis*) trees. (A) Trees grow from 30 – 60' tall and live for 35 – 50 years or longer when properly maintained. (B) Male (bottom arrow) and female inflorescences (top arrow) develop at the terminal apex of individual branches. (C) Mature fruit range from about 0.5 kg to over 3 kg in weight and often exude a sticky white latex when first harvested.

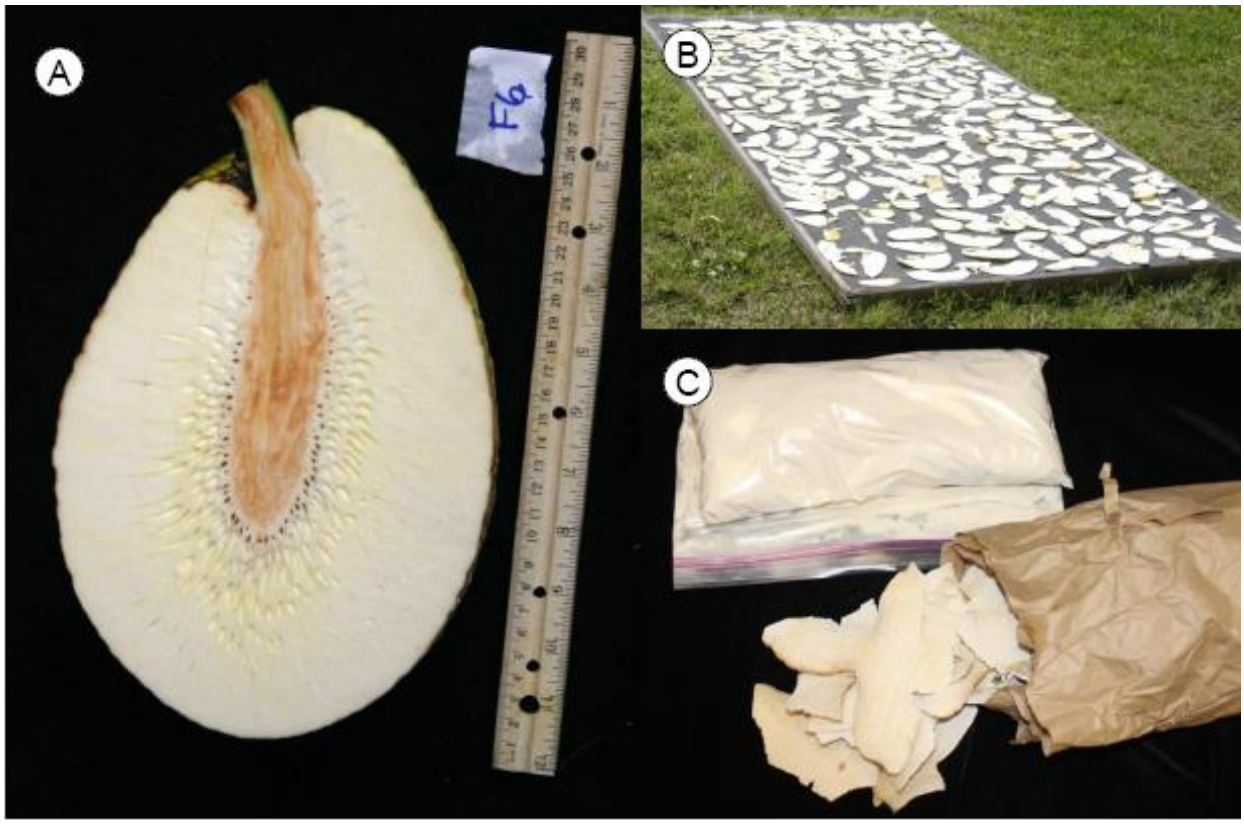


Figure 2-2: Preparation of flour from breadfruit (*Artocarpus altilis*). (A) Mature fruit are peeled, halved and cored prior to slicing. (B) Slices are dried on simple wire racks in sunlight or under a roof until fully dehydrated (C) Dry slices can be ground into a fine powder using a variety of mills and burr grinders.

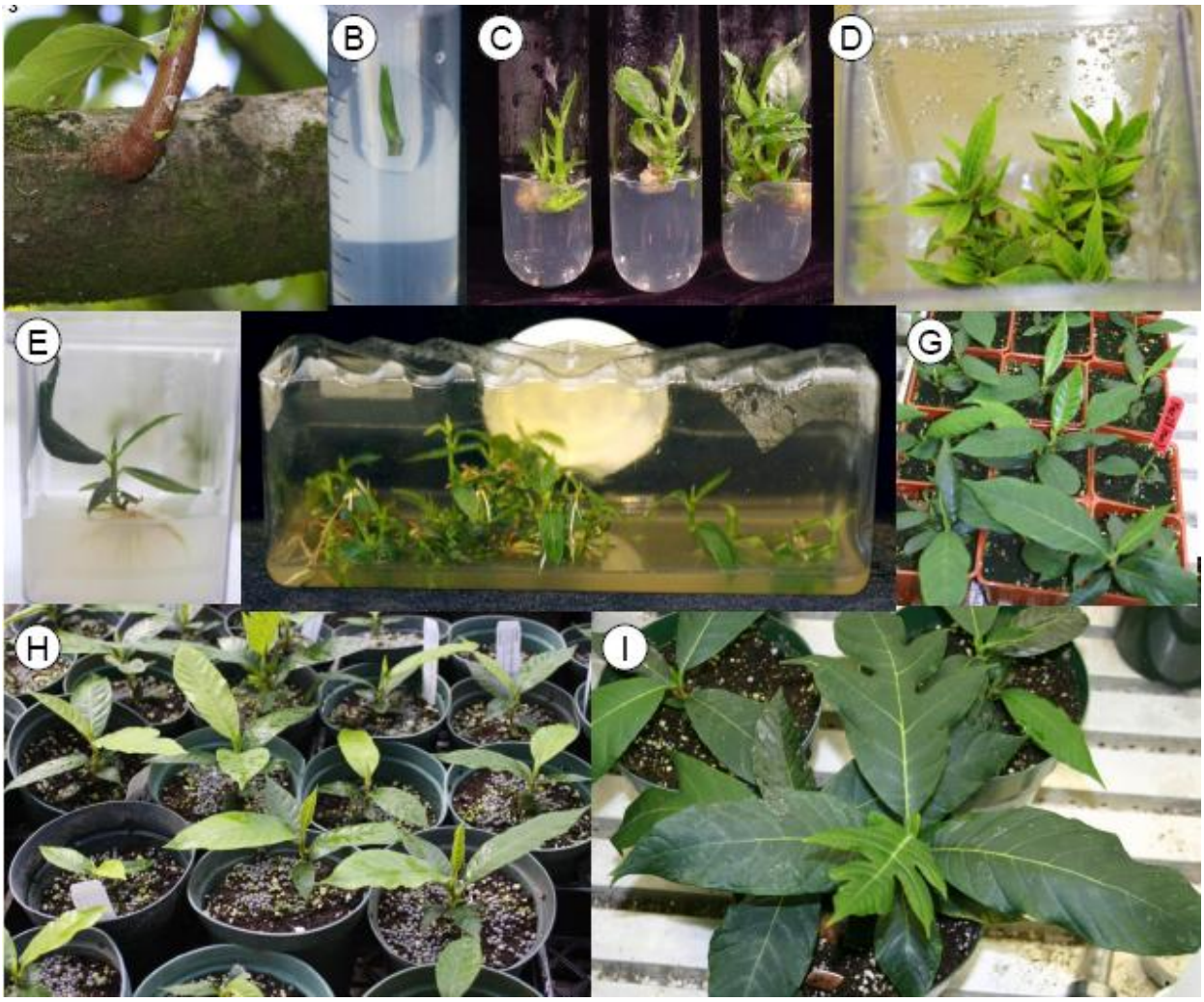


Figure 2-3: *In vitro* production of Breadfruit (*Artocarpus altilis*). (A) Young buds from branches of mature trees provide the source materials for regeneration in tissue culture. (B) Buds are disinfected of bacteria, fungi and other contaminants prior to culture onto a sterile medium containing sugar, vitamins, minerals, a protein gelling agent and optimized concentrations of plant hormones (C) Within 4 -6 weeks, multiple shoots develop on each of the sterile buds (D) Shoot proliferation continues for 3-4 months leading to a population of clones of the original plant in sterile tissue culture (E) Individual shoots are subcultured onto a rooting medium for development of roots and whole plants (F) Rooted plants can be grown to a height of 3-4 inches in sterile, controlled environment bioreactors (G-I) In vitro-grown plants can be transferred to a growth cabinet or acclimatized in a greenhouse for production of mature plants suitable for distribution and planting in the natural environment.

Chapter 3: Morphological Diversity in Breadfruit (*Artocarpus*, Moraceae): Insights into Domestication, Conservation, and Cultivar Identification[2]

Introduction

Breadfruit is an important staple crop traditionally cultivated throughout Oceania (Ragone, 1997). The long history and importance that this crop plays in the traditional way of life in Oceania is evident by the impact it has had on linguistics. For example, in Pohnpei, "Rahk" is a term used to delineate the season of abundant food, more specifically referring to the breadfruit season (Sakiyama, 1998). This ancient relationship between breadfruit and humans has resulted in the development of over 2000 named cultivars (Ragone, 1995). However, the recent trend towards urbanization and the incorporation of non-traditional foods have reduced the reliance on breadfruit in many regions of Oceania. As the reliance on breadfruit wanes, the threat of genetic erosion increases and many of these geographically restricted cultivars face the real threat of disappearing forever.

Breadfruit is one of 35 crops identified for their importance for food security and interdependence included in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGR) and it is classified as a priority crop by the Global Crop Diversity Trust (FAO, 2009b; http://www.croptrust.org/main/lprioritycrops.php). In order to develop a

[2] A version of this chapter has been submitted for publication and is currently under review: Jones, A.M.P., Murch, S.J., Wiseman, J., and Ragone, D. Morphological diversity in breadfruit (*Artocarpus*, Moraceae): Insights into domestication, conservation, and cultivar identification. The American Journal of Botany

methodical approach to germplasm conservation of breadfruit it is important to first have detailed information on the amount of diversity that is present, how this diversity is geographically distributed, and to develop methods to identify morphologically unique accessions.

Several authors have recorded morphological descriptions of breadfruit cultivars in a number of different island groups (Koroveibau, 1967; Parham, 1966; Ragone, 1988, 1995, 2007; Ragone and Wiseman, 2007; Sasuke, 1980; Wilder, 1928). However, these studies did not use standardized methodology for data collection and were primarily focused on limited geographical areas. The use of standardized measurements is important for the description of all plant species, but is particularly vital for the evaluation of breadfruit due to the high level of variability expressed even among clones of the same cultivar and branches within an individual tree (Ragone, 1995). For example, leaf characteristics such as the number of lobes, degree of dissection, size, and shape can vary between young shoots and older branches (Ragone, 1995). Likewise, as the fruit develops the skin texture, colour, amount of latex, and other morphological traits often change (Ragone, 1995). This morphological variability necessitates the use of standardized guidelines for data collection and makes direct comparison of previous studies problematic.

A set of 60 standardized morphological characteristics for the description of breadfruit morphology were recently developed (Ragone and Wiseman, 2007). Provenance information and observation data for 183 accessions using these descriptors are available in the USDA National Germplasm Resources Information Network (GRIN) (http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?217). The current study uses 57 of these descriptors to describe and contrast the morphology of 222 breadfruit trees growing in a field genebank at the National

Tropical Botanical Garden in Hawaii. This repository is the largest and most diverse breadfruit collection in the world and represents trees from 34 Pacific islands spanning Melanesia, Micronesia, and Polynesia, the Philippines, Indonesia, and the Seychelles now growing in a geographically restricted area. This study represents the most comprehensive morphological comparison of breadfruit conducted to date and provides insights into the complex heritage and domestication of breadfruit, identifies how the morphological diversity is geographically distributed, and is being used to develop a multi-access cultivar identification key to identify cultivars, clarify vernacular nomenclature, and guide future germplasm conservation initiatives.

Materials and Methods

Breadfruit Germplasm Repository at the National Tropical Botanical Garden

The National Tropical Botanical Garden (NTBG) maintains the largest and most diverse collection of breadfruit in the world (Ragone, 1997, 2007). Over 270 accessions of breadfruit (*Artocarpus altilis* and *A. altilis* x. *A. mariannensis* hybrids), breadnut (*A. camansi*), and dugdug (*A. mariannensis*) (Figure 3-1) originating from 34 Pacific islands, the Philippines, Indonesia, and the Seychelles (Ragone, 1997) are conserved in the genebank at the Kahanu Garden in Maui (20°47'57.07"N, 156°02'18.42"W). The collection is situated at an elevation of 15 m, receives a mean maximum temperature of 27.1°C, mean minimum temperature of 19.7°C, and an average of 2051mm of rain each year (Western Regional Climate Center; http://wrcc.dri.edu/). The soil within the collection is classified as Hana Very Stony Silt Clay Loam (http://websoilsurvey.nrcs.usda.gov). This soil is derived from volcanic ash, is typically well draining, slightly/moderately acidic and contains approximately 8% organic matter in the surface horizon. Root-restrictive obstructions generally occur at a depth of 1.5m. The soil surface layer

is subtended by a base of deep lava. Soil nutrient analysis of this location during the study period can be found in Chapter 6.

Data Collection

A total of 57 morphological characteristics were measured using previously standardized descriptors to compare and contrast 222 accesions of breadfruit (Ragone and Wiseman, 2007). The 222 trees included 153 accessions of *Artocarpus altilis*, 33 accessions of late generation *A. altilis* × *A. mariannensis* hybrids, 27 accessions of early generation *A. altilis* × *A. mariannensis* hybrids, 4 accessions of *A. camansi*, and 5 accessions of *A. mariannensis*. The descriptors include 18 quantitative and 11 qualitative leaf characteristics, 13 quantitative and nine qualitative fruit descriptors, two quantitative and two qualitative seed traits, and two quantitative measurements of the male inflorescences as described in Table 3-1. Leaf descriptors were measured from fully expanded leaves located three nodes from the distal end of mature branches to provide a standardized stage of development. Fruit descriptors were taken from mature, but not yet ripe fruit (Worrell et al., 1998). Descriptors of male inflorescences were taken when the flowers were mature and had dehisced. Seed descriptors were taken from fully developed seeds found in the mature fruit. Each descriptor was measured 10 times for each breadfruit accession providing a mean and distribution about the mean for quantitative traits and a distribution for qualitative traits.

Statistical Analysis

All statistical analyses were conducted using JMP 8.0.2 (SAS institute, Cary, NC); see appendix 1-1 for details of analyses. For each quantitative descriptor the mean and distribution

about the mean was calculated for each accession. Accessions were grouped based on their region of origin (Melanesia, Micronesia, Western and Eastern Polynesia) and species (*A. altilis*, *A. camansi*, *A. mariannensis*, and *A. altilis* × *A. mariannensis*) to calculate a mean and distribution for each group. For grouping trees into the regions of origin, only domesticated species were included, while accessions of *A. camansi*, *A. mariannensis*, and the early generation *A. altilis* × *A. mariannensis* were omitted in order to determine the changes that occurred during the domestication process. Melanesia included 27 breadfruit accessions, 11 from Fiji, 8 from the Solomon Islands, and 8 from Vanuatu. Western Polynesia included 35 accessions, 9 from Rotuma, 14 from Samoa, and 2 from Tonga. Eastern Polynesia was comprised of 74 accessions, 10 from the Cook Islands, 2 from Hawaii, 9 from the Marquesas Islands, and 53 from the Society Islands. Micronesia included 45 accessions, 5 from Chuuk, 3 from Kiribati, 4 from the Mariana Islands, 7 from Palau, 24 from Pohnpei, and 2 from Yap. For each of these groupings, a general linear model (GLM) was used to conduct a multi-variate analysis of variance (MANOVA) of the quantitative descriptors to determine the significance of the overall model with the grouping as the independent variable and the descriptors as the dependent variables. For each trait, a general linear model was used to conduct an analysis of variance (ANOVA) evaluate if there was significant variation among the groups for that specific characteristic. Where significant differences were detected by the ANOVAs, student's means separations using Tukey's adjustment were conducted.

Linear discriminant analysis was conducted using 29 of the quantitative descriptors with the aforementioned groupings to determine if the species and geographic origin of unknown trees could be identified based on this set of descriptors. Fruit stem length, petiole length, seed length,

seed diameter, and male inflorescence descriptors were omitted from this analysis due to missing data for some of the accessions.

Cultivar Identification Key

Even prior to Linneaus, plants have been identified and classified on the basis of the identification of characteristic descriptors, often assembled as a botanical key. The majority of botanical keys are dichotomous with a process of sequential characteristics narrowing the potential identity of the specimen. In preliminary experiments, a wide variety of different statistical and visual tools were explored with the overall objective of creating a dichotomous botanical key that would accurately identify breadfruit cultivars. However, there is a high degree of variability in morphology even within a single cultivar making discrete dichotomous separations difficult. Ultimately, cultivar identification could not be achieved using this approach. Instead, datasets of both quantitative and qualitative descriptors were used to create a multi-access identification key on a Lucid 3.3 platform (Centre for Biological Information Technology; www.lucidcentral.com) using Lucid3 Builder for data compilation and Lucid3 Player to create a descriptor-based searchable key that will be freely available on the Breadfruit Institute's website (figure 3-2; http://www.ntbg.org/breadfruit/).

Results

Morphological Comparison of Species

Statistically significant differences were found among *Artocarpus altilis, A. camansi, A. mariannensis,* early generation *A. altilis* × *A. mariannensis* hybrids, and domesticated *A. altilis* ×

A. mariannensis hybrids for the majority of descriptors evaluated (Table 3-2; Figure 3-3, 3-4). Breadnut, *A. camansi,* is the putative wild progenitor of *A. altilis* and typically has large dark green or brownish, oval fruit covered in long flexible spines that weigh an average of 1317 g (SE±106.3). The fruit contain more seeds than any of the other species with 34.5 (SE ± 0.92) showing in a longitudinal section, and have a large central core measuring about 13.5 cm (SE±0.51) long and 5.3 cm (SE±0.15) wide. Breadnut has large leaves with an average length of 56.5 cm (SE±1.4) and an average width of 40.5 cm (SE±1.14). The leaves typically have about 7.9 (±0.28) lobes per leaf and are covered in trichomes on the upper and lower surface.

Breadfruit, *A. altilis*, exhibited a higher degree of variability than breadnut, but on average produces fruit of similar size with a mean weight of 1482 g (SE±15.1). However, the fruit of *A. altilis* are either few seeded, or in many cases seedless, with an average of 1.6 (SE±0.13) seeds showing in a longitudinal section of a fruit. The core is significantly smaller than *A. camansi* measuring an average of 10.4 cm (SE±0.07) long and 4.2 cm (SE±0.02) in diameter. The shape of the fruit is much more diverse than breadnut and includes cultivars with round, oval, oblong, ellipsoid, heart-shape, and irregular-shaped fruit. Most breadfruit cultivars also have a raised or flattened, elongated collar that is generally absent in breadnut. The texture of the fruit is variable, but is most often smooth, smooth with raised sections, or covered with small sharp spikes. Skin color is light green or yellow-green more often than the dark green and brown displayed by breadnut. Leaves of *A. altilis* are significantly smaller than *A. camansi* with an average length of 41.0 cm (SE±023) and an average width of 33.5 cm (SE±0.18). The leaves have an average of 7.1 (SE±0.05) lobes each and while they have significantly fewer trichomes than those of *A. camansi*, they are still moderately pubescent.

Artocarpus mariannensis is a closely related species that is capable of hybridizing with seeded *A. altilis*. This species produces a much smaller fruit, with an average weight of 444 g (SE±130.2). The fruit are seeded, with about 3.0 (SE±1.06) seeds showing in a longitudinally sliced fruit. While this number of seeds is statistically similar to what is found in *A. altilis* it must be recognized that the fruit in which they are produced is much smaller making the fruit proportionally more heavily seeded. The fruit are irregular in shape and the skin texture varies from rounded pebbly to flat pebbly. The outside of the fruit remains dark green even when ripe, but the fruit pulp is more yellow than either *A. altilis* or *A. camansi*. The leaves of *A. mariannensis* are significantly shorter than those of *A. altilis* or *A. camansi* with an average of 28.8 cm (SE±1.25) long and 18.0 cm (SE±1.02) wide. The leaves have significantly fewer lobes, with an average of 1.2 (SE±0.25) per leaf and are less dissected with a dissection ratio of 0.43 (SE+/00.020) compared to 0.67 (SE±0.023) and 0.74 (SE±0.004) for *A. camansi* and *A. altilis,* respectively. The leaves have significantly fewer trichomes on the adaxial and abaxial surface of the leaf lamina, and the hairs that are present tend to have more of a red pigment than *A. altilis* or *A. camansi.*

Interspecific hybrids between *A. altilis* and *A. mariannensis* were divided into two groups, early generation hybrids that have occurred naturally where the two species grow in close proximity, and highly domesticated cultivars that have been bred for hundreds or thousands of years (Fosberg, 1960; Ragone, 2007). The early generation hybrids produce fruit that most closely resemble its *A. mariannensis* parent. The fruit have an average weight of 459 g (SE±57.1), and produce a similar number of seeds with an average of 1.7 (SE±0.49) showing in a longitudinal section of fruit. Like its *A. mariannensis* parent, the fruit are irregularly shaped and have a flattened or rounded pebbly texture. However, the fruit of the early hybrids sometimes

turn a light green to yellow-green color at maturity. Unlike the fruit characteristics, the leaves of early generation hybrids tend to resemble their *A. altilis* parent to a higher degree with some traits being intermediate. On average, the leaves are similar in size to *A. altilis* at 41.2 cm (SE±0.55) long and significantly wider at 35.6 cm (SE±0.35). They have an average number of 6.4 (SE±0.11) lobes per leaf and a dissection ratio of 0.69 (SE±0.009), both traits are intermediate between the two parent species but closer to *A. altilis*. The amount of trichomes on the adaxial side of the leaf is intermediate between the two parent species, but is greater than either on the abaxial surface. The trichome color varies from white to red.

The second group of interspecific hybrids represents the morphological changes that have occurred over many generations of human selection. The fruit of these domesticated hybrids are much larger than *A. mariannensis* or the early generation hybrids and similar to that of *A. altilis* and *A. camansi* with an average weight of 1457 g (SE±32.0). Like cultivars of *A. altilis,* the majority of the advanced hybrids are seedless, with an average of 0.4 (SE±0.27) seeds showing in a longitudinally sliced fruit, and have relatively small core dimensions with an average length of 12.0 cm (SE±0.15) and diameter of 3.6 cm (SE±0.05). The color of the fruit pulp is intermediate between *A. altilis* and *A. mariannensis*. The shape of hybrid fruit is similar to *A. altilis* in that it varies widely, however, a larger proportion of hybrid cultivars produce irregularly shaped fruit indicative of their *A. mariannensis* heritage. The texture of the hybrid fruit is most often flattened to rounded pebbly like *A. mariannensis,* but some cultivars have the sharp spiked fruit observed in some cultivars of *A. altilis*. The leaves of domesticated hybrid cultivars are longer, but slightly more narrow than *A. altilis* with an average length of 44.8 cm (SE±0.48) and width of 31.2 cm (SE±0.39). The leaves have an average of 6.3 (SE±0.10) lobes; intermediate between the parent species but closer to *A. altilis*. The hybrids have the greatest dissection ratio

of all species at 0.78 (SE±0.008). The amount of trichomes on the adaxial side of the leaves is intermediate between the parent species, while the amount on the abaxial side is lower than either.

Discriminant analysis of the quantitative descriptors indicates that all five of these groups are morphologically distinct at 95% confidence (Figure 3-5A; See appendix 1). Using this approach, it would be possible to differentiate *A. camansi* and *A. mariannensis* from one another and from the remaining groups, but the high degree of morphological variation within each group makes it impossible to use this method alone to differentiate *A. altilis* from either group of hybrids. Overall, 13.9% of the cultivars were misclassified using this analysis, mostly among the *A. altilis* and the hybrid cultivars. Discriminant analysis of the same traits with *A. altilis* and the hybrids combined in a single group resulted in only 1.3% of the cultivars being misclassified, and the most commonly misclassified were early generation hybrids classified as *A. mariannensis*. As such, discriminant analysis using these traits is capable of discriminating species, but is not able to adequately distinguish *A. altilis* from *A. altilis* × *A. mariannensis* hybrids. This analysis also confirms that the early generation hybrids are morphologically intermediate between the two parent species. While this is also true for the late generation hybrids, they are morphologically more similar to the *A. altilis* parent.

Morphological Comparison by Region of Origin

Significant differences were found among cultivars of breadfruit (both *A. altilis* and *A. altilis* × *A. mariannensis* hybrids combined) grouped by their geographic origin for the majority of the descriptors (Table 3-2; Figure 3-3, 3-4). Some trends are discussed below.

Breadfruit, *A. altilis*, was first domesticated in Melanesia and this region presumably has the most ancient cultivars (Ragone, 1997). The breadfruit cultivars found in Melanesia produce the largest fruit, weighing an average of 1593 g (SE ±37.7). The fruit have relatively large cores at 11.3 cm (SE±0.18) long and 4.9 cm (SE±0.05) in diameter and are typically seeded with an average of 4.0 (SE±0.12) seeds showing in a longitudinally sliced fruit. The fruit pulp tends to be more yellow than cultivars from other regions, and the mature fruit exude a relatively high amount of latex upon being cut in half. The skin texture is variable, but the two most common textures are rough sandpapery, or sharp raise spikes. The leaves are a similar length among locations with the exception of Micronesian cultivars which have slightly longer leaves. Melanesian cultivars have leaves of similar width as Western Polynesia, slightly more narrow than eastern Polynesican or Micronesian cultivars. However, Melanesian cultivars have significantly more lobes than cultivars from any other region with an average of 8.2 (SE±0.10) per leaf. Melanesian cultivars have a similar amount of trichromes on the adaxial surface of the leaf as western Polynesian cultivars, and more than cultivars from eastern Polynesia. The adaxial trichromes are also significantly longer than those found on the leaves of cultivars from other regions. The abaxial surface of the leaf has more trichomes than Eastern Polynesian or Micronesian cultivars, but less than Western Polynesian cultivars, however, the abaxial trichromes of Melanesian cultivars are also longer than other regions.

The breadfruit cultivars originating from Western Polynesia produce significantly smaller fruit than those in Melanesia, with an average weight of 1378 g (SE±36.9). The fruit have cores of similar length at 10.9 cm (SE±0.17) but are slightly more narrow with an average diameter of 4.4 cm (SE±0.05) and a similar number of seeds with an average of 3.2 (SE±0.10). The fruit pulp is significantly less yellow than the Melanesian cultivars, but the fruit exude a similar

amount of latex when cut in half. The textures of the outside of the fruit are most often sandpapery, flattened pebbly, or smooth with irregularly raised sections and they sometimes have sharp spikes, although this is less frequent than in Melanesian cultivars. The leaves of Western Polynesian cultivars have fewer lobes than Melanesian cultivars with an average of 7.7 (SE±0.10) lobes per leaf. The adaxial leaf surface has a similar number of trichomes, and the abaxial surface has more than the Melanesian cultivars. However, the trichromes of both surfaces tend to be shorter than those of their Melanesian counterparts.

Cultivars from Eastern Polynesia produce larger fruit than Western Polynesia, similar to those originating from Melanesia with an average weight of 1497g (SE±22.5). The core is smaller than Melanesian or Western Polynesian cultivars measuring 10.3cm (SE±0.11) long and 4.0 cm (0.03) wide. Cultivars in this region are predominantly seedless with some producing a few seeds with an average of 0.5 (SE±0.07) seeds showing in a fruit sliced longitudinally. The pulp is significantly less yellow than fruit from Melanesia or Western Polynesia, and the fruit exude less latex when sliced in half. The most common skin texture of fruit from this region is smooth with irregularly raised sections, followed by sandpapery, and a few bear the sharp spikes more common in Melanesia. The leaves have fewer lobes than Melanesian or Western Polynesian cultivars with an average of 6.7 (SE±0.06) and have fewer trichomes on both the abaxial and adaxial surfaces of similar length to Western Polynesian and shorter than Melanesian cultivars.

Micronesian cultivars produce fruit smaller than Melanesian cultivars, similar to cultivars from Western and Eastern Polynesia with an average weight of 1461 g (SE±28.9). The core of the fruit is similar in length to that of Eastern and Western Polynesian cultivars at 11.0 cm (SE±0.14), but is narrower than either with a diameter of 3.7 cm (SE±0.04). Micronesian

cultivars are most often seedless, or have very few seeds with an average of 0.2 (SE±0.09) showing in a longitudinally sliced fruit. The flesh color is similar to cultivars from Western Polynesia, less yellow than those from Melanesia but more yellow than Eastern Polynesian cultivars. At maturity, fruit of Micronesian cultivars exude significantly less latex than fruit from than any other region when sliced in half. While fruit shape varies among cultivars from all regions, there is a greater tendency for Micronesian cultivars to produce irregularly shaped fruit. They also most often have flattened to rounded pebbly fruit textures, with spiked, sandpapery, and smooth being less common than in other regions. The leaves of Micronesian cultivars are larger than those from the other regions with an average length of 43.6 cm (SE±0.41) and width of 34.1 cm (SE±0.34). However, their leaves have the lowest number of lobes with an average of 6.05 (SE±0.08) and are the most widely spaced. The adaxial surface of the leaves have a similar amount of trichomes as the Melanesian and Polynesian cultivars. The abaxial surface has relatively few trichomes, similar to Eastern Polynesian cultivars.

Discriminant analysis of the quantitative descriptors indicates that cultivars from Melanesia and Western Polynesia are similar to each other, but distinct from Eastern Polynesian and Micronesian cultivars at a confidence level of 95% (Figure 3-5C). However, due to the high degree of morphological variability within each of these regions, 23.3% of the cultivars were misclassified using these characteristics alone. As such, these descriptors can provide some clues to the origin of an unknown cultivar, but cannot provide a definitive answer.

Using Descriptors to Distinguish Cultivars

Initial efforts were made to create a dichotomous key for the identification of unknown breadfruit cultivars but were unsuccessful due to the seasonality of the species, variability of

some descriptors within a single tree or between trees of the same cultivar, and overlap between different cultivars, making a discrete separation problematic. When it became apparent that a dichotomous key would not serve the purpose of breadfruit cultivar identification well, a multi-access key was developed using Lucid 3.3 as a platform. A database containing the 57 morphological descriptors for 222 accessions of breadfruit in the NTBG collection was developed using the Lucid Builder system. For qualitative traits, the most frequent state for each cultivar was entered as the common score, and less frequent states were entered as rare scores. In cases where two states were equally frequent they were both entered as common scores. For quantitative traits the maximum and minimum of the 10 values recorded were entered into the database as the extreme maximum and minimum scores.

The Lucid system allows the user to begin with the descriptor characteristic that is most available and to enter the data in any order they choose (Figure 3-2). As the user enters more data, the system can be set to eliminate the cultivars in the database that do not match, or to rank the cultivars based on their similarity to user-entered data. At any time, the user will be able to select a potential match and view larger images and be directed to a detailed description of the cultivar. As new cultivars are identified they can be added to the database so that it can continually grow and improve over time. A prototype version of the key has been field tested and will be available online at http://www.ntbg.org/breadfruit/.

Discussion

The National Tropical Botanic Garden (NTBG) maintains the largest and most diverse collection of breadfruit in the world with accessions collected throughout the crop's traditional range (Ragone, 2007). Within this collection exist accessions representative of the entire

domestication process including the two wild progenitor species, *Artocarpus camansi* and *A. mariannensis*, seeded diploid and seedless triploid *A. altilis*, early generation inter-specific hybrids between *A. altilis* and *A. mariannensis*, and highly domesticated advanced *A. altilis* × *A. mariannensis* hybrids (Ragone, 1997, 2001, 2007; Figure 3-6). Breadfruit is most often asexually propagated, and its spread and domestication is intimately linked to human migration patterns (Zerega et al., 2004, 2006). As humans migrated out of Melanesia and colonized the Pacific Islands, breadfruit and other crops collectively referred to as "canoe plants" were carried with them. Each time a new island group was colonized the settlers would select their favourite cultivars of each crop to bring with them. This process of repeated bottlenecks appears to have resulted in heavy selection pressure evident by the high level of morphological diversity observed in breadfruit. A detailed morphological comparison of the NTBG's collection of breadfruit germplasm described herein provides new insights into the domestication of this species and how it is related to human migration, provides a framework for cultivar classification, and identifies regions and accessions with distinct morphological traits that will help guide future germplasm conservation initiatives.

Previous morphological comparisons and more recent molecular evidence suggests that breadfruit, *Artocarpus altilis* (Parkinson) Fosberg, is a cultigen originally derived from the wild species *Artocarpus camansi* Blanco (Ragone, 1997; Zerega et al., 2004). *Artocarpus camansi* Blanco is native to Papau New Guinea, and possibly the Moluccas (Ragone, 1997). The initial domestication of *A. altilis* from *A. camansi* likely occurred in Papua New Guinea or the surrounding islands before continuing as it was moved eastward (Zerega et al., 2004, 2006). The domestication syndrome exhibited by breadfruit is interesting in that the average fruit size has not increased relative to the wild progenitor species (Table 3-2). Many species, including the

closely related jackfruit, *A. heterophyllus*, have significantly larger fruit than their wild counterparts (Khan et al., 2010). However, while domesticated breadfruit is similar in size and weight to breadnut, the fruit has significantly fewer seeds and in many cases is seedless (Table 3-1). This is the major change that enabled the transition from a seed/nut crop into the important starchy staple that it is today. Other important differences in fruit morphology that occurred during domestication include a reduction in the length and width of the core, and the surface of the fruit transitioned from being covered by long flexible spines towards a smoother texture. Some of these traits such as the reduction in core width and shape increase the edible starchy portion of the fruit (Chapter 5). The transition from long flexible spines to a smoother skin texture may have been selected for because breadfruit is often peeled before cooking or being preserved by pit fermentation (Cox, 1980; Ragone, 1997), or could be in part a consequence of the morphological changes that contribute to reduced seed production (Hasan and Razak, 1991). The reduction in leaf size exhibited by *A. altilis* relative to *A. camansi* is also different than what has been observed in the domestication of *A. heterophyllus*, where cultivated varieties have larger leaves than wild trees (Khan et al., 2010).

After the initial domestication of *A. altilis*, the early cultivars accompanied pioneers as they colonized the Pacific islands (Zerega et al., 2004, 2006). These early domesticates from Papua New Guinea travelled east into the rest of Melanesia and then into Polynesia and Micronesia (Zerega et al., 2004). During the course of this migration, the process of domestication continued and several distinct trends in breadfruit morphology occurred. The most well-documented of these changes is the further reduction in seed number as this crop moved east (Ragone, 1997). The seeded cultivars found in Melanesia give way to fewer-seeded diploid and true seedless triploid cultivars in western Polynesia, and almost exclusively seedless

triploid cultivars in Eastern Polynesia, indicating that there was a strong selection pressure for seedlessness as peoples migrated east (Ragone, 2001; Zerega et al., 2004, 2006). The overall morphology of cultivars from Melanesia and Western Polynesia are more similar to each other than cultivars from Eastern Polynesia. This may indicate that there was a significant event in breadfruit breeding that occurred somewhere in Polynesia that affected its overall morphology, perhaps the development of triploid cultivars (Ragone, 2001). Unlike other crops such as *A. heterophyllus* where increased fruit size was selected for (Khan et al., 2010), the size of breadfruit declined as it moved out of Melanesia and into western Polynesia before being moved into eastern Polynesia where the fruit are similar in size to Melanesian cultivars. However, while the fruit size does not appear to have been heavily selected for in Polynesia, this is compensated for by fewer seeds and reduction in the length and width of the core, increasing the proportion of starchy pulp in each fruit. The reduction in yellow pigmentation in the flesh of Polynesian cultivars may indicate a preference for white starch which is common in some other staple crops such as rice (Sweeney et al., 2007). The transition from spiny skin towards a smoother surface that occurred during domestication appears to have been further selected for as the crop moved east into Polynesia, perhaps to facilitate cooking and preservation techniques, which generally involve peeling the fruit (Cox, 1980; Ragone, 1997). While there are some trends in leaf characteristics, such as a reduction in the number of lobes and a reduction in the amount and length of trichomes, leaf morphology appears to have changed less than fruit characteristics suggesting there was less selective pressure for leaf traits.

The history of breadfruit domestication is further complicated when it was introduced into Micronesia where the closely related endemic species *A. mariannensis* Trécul originates (Fosberg, 1960; Ragone, 1997). These two species are sexually compatible and based on

morphological characteristics and AFLP markers, many Micronesian cultivars are interspecific hybrids between the two (Fosberg, 1960; Zerega et al., 2005). Early generation hybrids provide insights into the early stages of the domestication process. The fruit from these early hybrids resemble those of *A. mariannensis* to a large extent. However, the leaves are morphologically more similar to *A. altilis*, with many leaf traits being intermediate between the two. These early hybrids likely provided early settlers with agronomic or nutritional benefits as they were perpetuated and developed into a highly domesticated crop. Hybrids are known to be more tolerant of saline soils and do well on low lying atolls (Ragone, 1997), contain significantly higher levels of iron and some other minerals (Chapter 6), and contain higher concentrations of nutritionally important carotenoids (Chapter 7). These traits may have provided the necessary advantages to early breeders to develop these hybrids into the large-fruited, seedless cultivars that exist today. However, the domesticated hybrid cultivars are morphologically more similar to *A. altilis* than the early hybrids (Figure 3-5A). This may be a consequence of subsequent backcrossing with *A. altilis*, the selection for similar characteristics, or a combination of the two. Regardless, some of the domesticated hybrid cultivars produce seedless fruit that weigh over 3.5 kg each, making them some of the largest-fruited cultivars in the NTBG collection. This hybridization and subsequent domestication has resulted in an overall morphologically distinct set of cultivars within Micronesia (Figure 3-5C).

While the differences in morphological characteristics among regions reveal a pattern in the ongoing domestication pattern of breadfruit, it is important to recognize the high level of diversity that has been preserved within each geographical region. Over 2000 vernacular cultivar names have been recorded across Oceania (Ragone, 1995), and even within single island groups a large number of cultivars are maintained. For example, 130 cultivar names have been recorded

from Pohnpei (Fownes and Raynor, 1991), over 40 from Samoa (Ragone et al., 2004), and 132 from Vanuatu (Walter, 1989). Describing and contrasting the overall morphology of such a large number of cultivars present significant challenges. Multivariate statistics such as discriminant analysis provide powerful tools to assess the overall morphological similarity of germplasm collections that can remain undetected using univariate methods (Carter, 1987; Erskine et al., 1989; Flores et al., 1997; Veronesi and Falcinelli, 1988). These methods have been used to identify regions with morphologically distinct cultivars to help guide conservation efforts in several other crops. Discriminant analysis of breadfruit descriptors indicates that cultivars originating from Melanesia, Western Polynesia, Eastern Polynesia and Micronesia are morphologically distinct from one another (Figure 3-5c). However, due to the morphological diversity maintained within each region, discriminant analysis using these traits misclassified 23.3% of the cultivars. While this approach provides some insight into the morphological relationship among these regions and indicates that germplasm conservation efforts will need to include them all, it is impossible to reliably determine the region of origin of unknown cultivars using this model.

Cultivars are traditionally named and identified based on their morphological characteristics such as skin texture or leaf shape (Ragone, 1991; Ragone et al., 2004). The traditional system of nomenclature varies among indigenous peoples and there is no unified method of identification or classification. Additionally, while thousands of cultivar names have been documented, the actual number of morphologically or genetically distinct cultivars is unknown as it is possible to have multiple names for what is actually a single cultivar or two morphologically distinct cultivars that are known by a single name (Ragone, 1995, 2007). These issues were the impetus for the development of a universal cultivar identification key. A multi-

access key was selected over the more traditional dichotomous key because it allows more flexibility for the user as it enables them to use the characteristics that are available to them at any given time, and is highly interactive. The software has the ability to function by eliminating all trees that do not match the selection criteria, or by ranking the cultivars based on the percent of selected traits that match, thus identifying other similar cultivars. As the user narrows down potential matches, a series of detailed photos and other relevant information can be accessed to help in identification. A prototype of this identification key is currently being field tested and will soon be publically available on the Breadfruit Institute's website (http://www.ntbg.org/breadfruit/). This identification key will aid in the identification of cultivars, help identify cultivars with novel traits for germplasm conservation, and help clear the ambiguity of vernacular cultivar names.

Conservation of breadfruit diversity has been identified as a high priority indicated by its inclusion in the International Treaty on Plant Genetic Resources for Food and Agriculture and its prioritization by The Global Crop Diversity Trust (FAO, 2009b; http://www.croptrust.org/). The current study provides the most detailed and comprehensive morphological comparison of breadfruit germplasm conducted to date, providing new insights into the morphological changes that occurred during the domestication of breadfruit and a basis to evaluate the variation that exists among species and geographical regions. Multivariate statistics and the development of a universal cultivar identification key described herein can be used to help identify known and previously undescribed cultivars and regions that exhibit unique morphological traits to guide the conservation effort. Together these data provide the foundation required to develop a methodical approach to breadfruit conservation.

Table 3-1: Morphological descriptors developed for the evaluation of breadfruit (*Artocarpus*, Moraceae) morphology.

Quantitative traits	
Fruit Weight	Weight of a whole fruit
Fruit Length	Length of the fruit measured from the proximal to the distal end
Fruit width at middle	Diameter of the fruit half way between the proximal and distal end
Fruit width at top	Diameter of the fruit near the distal end
Fruit width at bottom	Diameter of the fruit at near the proximal end
Core length	Length of the core measured from where it enters the fruit to where it ends
Core diameter	Diameter of the core at its widest point
Scabbing between sections	Amount of scabbing found between fruit sections 0:none, 1: slight, 2: moderate, 3: heavy
Scabbing around center	Amount of scabbing around center of fruit sections 0:none, 1: slight, 2: moderate, 3: heavy
Flesh colour	Color of the fruit pulp; 1:white, 2:creamy, 3:light yellow, 4:yellow
Amount of latex	Amount of latex on cut surface of mature fruit; 0:none, 1:light, 2: heavy
Peduncle length	length of peduncle
Number of seeds	The number of seeds that show on the surface of a longitudinally halved fruit
Seed length	The length of a mature seed
Seed diameter	The diameter of a mature seed
Male inflorescence length	Length of a mature male inflorescence
Male inflorescence width	The diameter of a mature male inflorescence at its widest point
Leaf length	The length of a mature leaf lamina not including the petiole
Leaf width	The width of a mature leaf lamina at its widest point
Width to length ratio	Leaf width divided by its length
Leaf margin	Description of leaf margin; 1:smooth, 2:slightly wavy, 3:moderatly wavy/sinuate, 4: very wavy/undulate
Distance to widest point	Distance from leaf base to the widest point of the leaf
Number of lobes	Number of lobes on an individual leaf
Lobe spacing	Lobe spacing; 1:close, 2:close, overlapping, 3:wide, 4:wide, overlapping
Distance to 1st lobe	Distance from leaf base to the first lobe
Length of lobes	Average distance from midrib to the end of lobes on the leaf
Sinus depth	Distance from the deepest part of the leaf sinus to the midrib
Distance to first sinus	Distance from the base of the leaf to the center of the first sinus
Dissection ratio	The average sinus depth divided by the average length of the lobes
Petiole length	Distance from the basal end of the petiole to the beginning of the leaf lamina
Petiole diameter	Diameter of the petiole
Upper leaf hair amount	Amount of trichomes on the adaxial side of the leaf veins using a 0-5 scale
Upper leaf hair length	Length of trichomes on adaxial vein using a scale of 1-3
Lower leaf hair amount	Amount of trichomes on the abaxial side of the leaf veins using a 0-5 scale
Lower leaf hair length	Length of trichomes on abaxial vein using a scale of 1-3

Table 3-1 continued: Morphological descriptors developed for the evaluation of breadfruit (*Artocarpus*, Moraceae) morphology.

Qualitative traits	
Fruit shape	Shape of mature fruit; 1:round, 2: broad ovoid, 3:oval, 4:oblong, 5:ellipsoid, 6:heart-shape, 7:irregular
Skin texture	Skin texture at maturity; 1:smooth, 2:smooth with irregular raised sections, 3: sandpapery with persistent stigma dots, 4: flat pebbly, 5:round pebbly, 6:spiky raised centers, 7:pointed flexible spines
Skin color	Skin color at maturity; 1:dark green, 2:light green, 3:yellow green, 4:yellow, 5:brown/green, 6:pink/brick
Scabbing location	Scab location on mature fruit; 0:no scabbing, 1:between sections, 2:around center ofsectioons
Color between sections	Color of scabbing between fruit sections; 1:green, 2:brownish
Color around center	Color of scabbing around center of fruit sections; 1:green, 2:brownish
Peduncle collar	Morphology of fruit collar; 0:none, 1:raised swollen sections, 2:flat with elongated sections
Peduncle insertion	Morphology of peduncle insertion point; 1:open, 2:depressed, 3:tightly clasped
Color of latex	Color of latexon the cut surface of a mature fruit; 1:green, 2:rusty reddish brown, 3: other
Seed color	Color of a mature seed coat; 1: off white, 2:light brown, 3:dark brown
Seed shape	Shape of mature seeds; 1:round, 2:ellipsoid, 3:elongated, 4:oblong, 5:reniform (kidney)
Shape of leaf base	Shape of the distal end of the leaf lamina; 1:flat, 2:rounded, 3:acute
Shape of leaf apex	Shape of the proximal end of the leaf lamina; 1:rounded, 2: diamond shape
Blade color	Color of leaf lamina; 1:yellow/green, 2:green, 3:dark green
Vein color	Color of leaf veins; 1:green, 2:yellow/green, 3:yellow
Leaf surface	Apprearance of leaf lamina; 1:glossy, 2:dull
Leaf flexibility	Flexibility of leaf lamina; 0:none, breaks when folded in hand, 2:flexible, bends without breaking
Velcro	Morphology of trichomes; 0:none or straight trichomes, 1:curled trichomes that act like velcro
Upper hair direction	Orientation of trichomes on adaxial leaf veins; 1:upright, 2:appressed
Upper hair color	Color of trichomes on adaxial leaf veins; 1:white, 2:reddish-white, 3:red
Lower hair direction	Orientation of trichomes on abaxial leaf veins; 1:upright, 2:appressed
Lower hair color	Color of trichomes on abaxial leaf veins; 1:white, 2:reddish-white, 3:red

Table 3-2: Average value and standard error of quantitative fruit, seed, flower and leaf descriptors of 222 accessions of breadfruit conserved in the National Tropical Botanical Garden's breadfruit germplasm repository grouped by species (*Artocarpus camansi, A. altilis, A. mariannensis,* early generation *A. altilis* × *A. mariannensis* hybrids, and late generation domesticated *A. altilis* × *A. mariannensis* hybrids) and by their region of origin (Melanesia, Western Polynesia, Eastern Polynesia, and Micronesia; *A. altilis* and domesticated *A. altilis* × *A. mariannensis* hybrids). Means followed by different letters are significantly different than the others in each grouping system using Students means separation with Tukey's adjustment with a type 1 error rate of 0.05. *A.a = Artocarpus altilis, A.c = Artocarpus camansi, A.m = Artocarpus mariannensis* , *A.a* x *A.m* = domesticated *A. altilis* × *A. mariannensis* hybrids, and *A.a* x *A.m** = early generation *A. altilis* × *A. mariannensis* hybrids.

Descriptor	Grouped by Species										Grouped by Region of Origin							
	A.c		A.a		A.a x A.m		A.a x A.m*		A.m		Melanesia		W. Polynesia		E. Polynesia		Micronesia	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Fruit Weight (g)	1317a	106.3	1482a	15.1	1457a	32.0	459b	57.1	444b	130.2	1593a	37.7	1378c	37	1497ab	22.6	1461bc	28.9
Fruit Length (cm)	18.2ab	0.58	16.8b	0.08	19.1[a]	0.18	14.1c	0.3	13.3c	0.71	17.5a	0.21	16.9a	0.20	17.0a	0.12	18.0a	0.16
Fruit width at middle (cm)	14.1ab	0.39	14.0a	0.06	13.4b	0.12	8.4c	0.21	8.4c	0.48	14.6a	0.14	13.8b	0.13	13.9b	0.08	13.6b	0.10
Fruit width at top (cm)	8.1ab	0.45	8.9a	0.06	8.0b	0.12	5.6c	0.25	4.7c	0.51	9.5a	0.13	9.0ab	0.15	8.7b	0.09	8.2c	0.12
Fruit width at bottom (cm)	10.0a	0.52	8.7a	0.07	8.1b	0.14	7.1c	0.29	6.0c	0.59	9.1a	0.13	8.6b	0.14	8.6b	0.08	8.2b	0.1
Core length (cm)	13.5a	0.51	10.4c	0.07	12.0b	0.15	10.1c	0.27	9.3c	0.62	11.3a	0.18	10.9a	0.18	10.3a	0.11	11.0a	0.14
Core diameter (cm)	5.3a	0.15	4.2b	0.02	3.6c	0.05	2.9d	0.08	2.8d	0.19	4.9a	0.05	4.4b	0.05	4.0c	0.03	3.7d	0.04
Scabbing between sections	1.3	0.129	1.13	0.018	0.78	0.039	0.32	0.072	0	0	0.9b	0.05	1.0b	0.04	1.2a	0.03	1.0b	0.04
Scabbing around center	1.30b	0.129	1.77a	0.018	1.54b	0.039	1.43b	0.072	1.52ab	0.154	1.5a	0.04	1.6a	0.04	1.8a	0.03	1.7a	0.03
Flesh colour	2.6bc	0.14	2.4c	0.02	2.6b	0.04	3.1a	0.08	3.2a	0.16	2.8a	0.05	2.6b	0.05	2.2c	0.03	2.5b	0.04
Amount of latex	.53b	0.114	1.18a	0.016	.82b	0.035	.89b	0.064	.95ab	0.137	1.37a	0.038	1.35a	0.037	1.08b	0.023	.90c	0.030
Peduncle length (cm)	2.0b	1.95	6.3ab	0.24	6.0ab	0.35	4.3ab	3.09	9.9a	1.38	3.6ab	1.27	4.6b	0.57	6.7a	0.31	6.3a	0.32
Number of seeds	34.5a	0.92	1.6b	0.13	0.4c	0.27	1.7bc	0.49	3.0bc	1.06	4.0a	0.12	3.2a	0.10	0.5b	0.07	0.2b	0.09
Seed length (cm)	2.7ab	0.1	2.8b	0.02	2.9ab	0.04	2.9a	0.04	3.0ab	0.15	2.7b	0.05	2.9a	0.04	2.7b	0.03	3.0a	0.04
Seed diameter (cm)	2.2b	0.1	2.3b	0.02	2.5a	0.04	2.5a	0.04	2.4ab	0.14	2.2b	0.05	2.5b	0.04	2.2c	0.02	2.6a	0.04
Male inflorescence length (cm)	23.8a	1.12	20.5b	0.19	22.5a	0.37	16.6c	0.48	17.9bc	0.94	19.8b	0.44	18.7b	0.45	21.6a	0.28	22.5a	0.35
Male inflorescence width (cm)	3.1a	0.13	3.2a	0.02	3.1a	0.04	2.7b	0.05	3.2a	0.11	3.0b	0.05	3.0b	0.05	3.3a	0.03	3.1b	0.04
Leaf length (cm)	56.5a	1.4	41.0c	0.23	44.8b	0.48	41.2c	0.55	28.8d	1.25	40.4bc	0.52	39.7c	0.53	41.7b	0.32	43.6a	0.41
Leaf width (cm)	40.5a	1.14	33.5c	0.18	31.2d	0.39	35.6b	0.45	18.0e	1.02	30.2c	0.43	30.6c	0.44	31.9b	0.26	34.1a	0.34
Width to length ratio	.72d	0.011	.76c	0.001	.79b	0.004	.81a	0.004	.62e	0.01	.745c	0.0042	.774ab	0.0044	.767b	0.0027	0.780a	0.0034
Leaf margin	2.2b	11	2.6a	0.02	2.4b	0.04	2.3b	0.04	1.8c	0.09	2.41a	0.039	2.59a	0.040	2.63a	0.024	2.54a	0.031
Distance to widest point (cm)	32.2a	1.21	24.3c	0.2	26.3b	0.42	23.8c	0.48	17.3d	1.09	24.5a	0.48	22.4b	0.49	24.8a	0.3	25.7a	0.38
Number of lobes	7.9a	0.28	7.1b	0.05	6.3c	0.10	6.4c	0.11	1.2d	0.25	8.2a	0.10	7.7b	0.10	6.7c	0.06	6.0d	0.08
Lobe spacing	2.48b	0.145	2.29b	0.023	2.99a	0.05	2.98a	0.057	1.98b	0.129	2.03c	0.056	2.33b	0.058	2.47b	0.035	2.63a	0.045
Distance to 1st lobe (cm)	24.1a	0.64	13.3c	0.1	14.3b	0.22	12.7c	0.25	11.9c	0.57	12.7a	0.24	12.9a	0.24	13.1a	0.15	13.0a	0.19
Length of lobes (cm)	28.7a	0.78	20.7c	0.13	23.0b	0.27	21.1c	0.31	9.6d	0.7	19.0b	0.27	19.0b	0.28	21.9a	0.17	22.2a	0.22
Sinus depth (cm)	9.5a	0.36	4.9c	0.06	5.0c	0.12	6.5b	0.14	3.3d	0.32	5.5b	0.13	6.1a	0.13	4.5c	0.08	4.4c	0.10
Distance to first sinus (cm)	10.8a	0.5	5.9d	0.08	7.0c	0.17	8.5b	0.2	6.5cd	0.45	5.6b	0.17	5.9ab	0.17	6.2a	0.1	6.3a	0.13
Dissection ratio	.67c	0.023	.74b	0.004	.78a	0.008	.69c	0.009	.43d	0.02	.72b	0.008	.67c	0.008	.79a	0.005	.74b	0.007
Petiole length (cm)	5.1a	0.18	4.1d	0.03	4.5b	0.06	4.4bc	0.08	3.8cd	0.23	4.3a	0.07	4.1a	0.07	4.0a	0.04	4.3a	0.05
Petiole diameter (cm)	1.28a	0.05	0.97c	0.008	1.05b	0.017	1.00bc	0.019	0.66d	0.045	0.89b	0.020	0.91b	0.021	1.02a	0.012	1.05a	0.016
Upper leaf hair amount	3.30a	0.166	2.18b	0.027	1.52d	0.057	1.77c	0.065	0.48e	0.149	2.21a	0.064	2.16a	0.065	1.95a	0.039	1.99a	0.051
Upper leaf hair length	1.25ab	0.1	1.35a	0.016	1.07b	0.034	1.31a	0.039	0.28c	0.09	1.43a	0.035	1.27a	0.036	1.25a	0.022	1.24a	0.028
Lower leaf hair amount	2.15ab	0.176	1.77b	0.028	1.29c	0.06	2.49a	0.069	1.60bc	0.158	1.98b	0.061	2.27a	0.062	1.46c	0.038	1.44c	0.049
Lower leaf hair length	0.98b	0.136	1.31b	0.022	1.17b	0.047	2.08a	0.053	1.20b	0.122	1.54a	0.043	1.20c	0.048	1.09c	0.029	1.34b	0.037

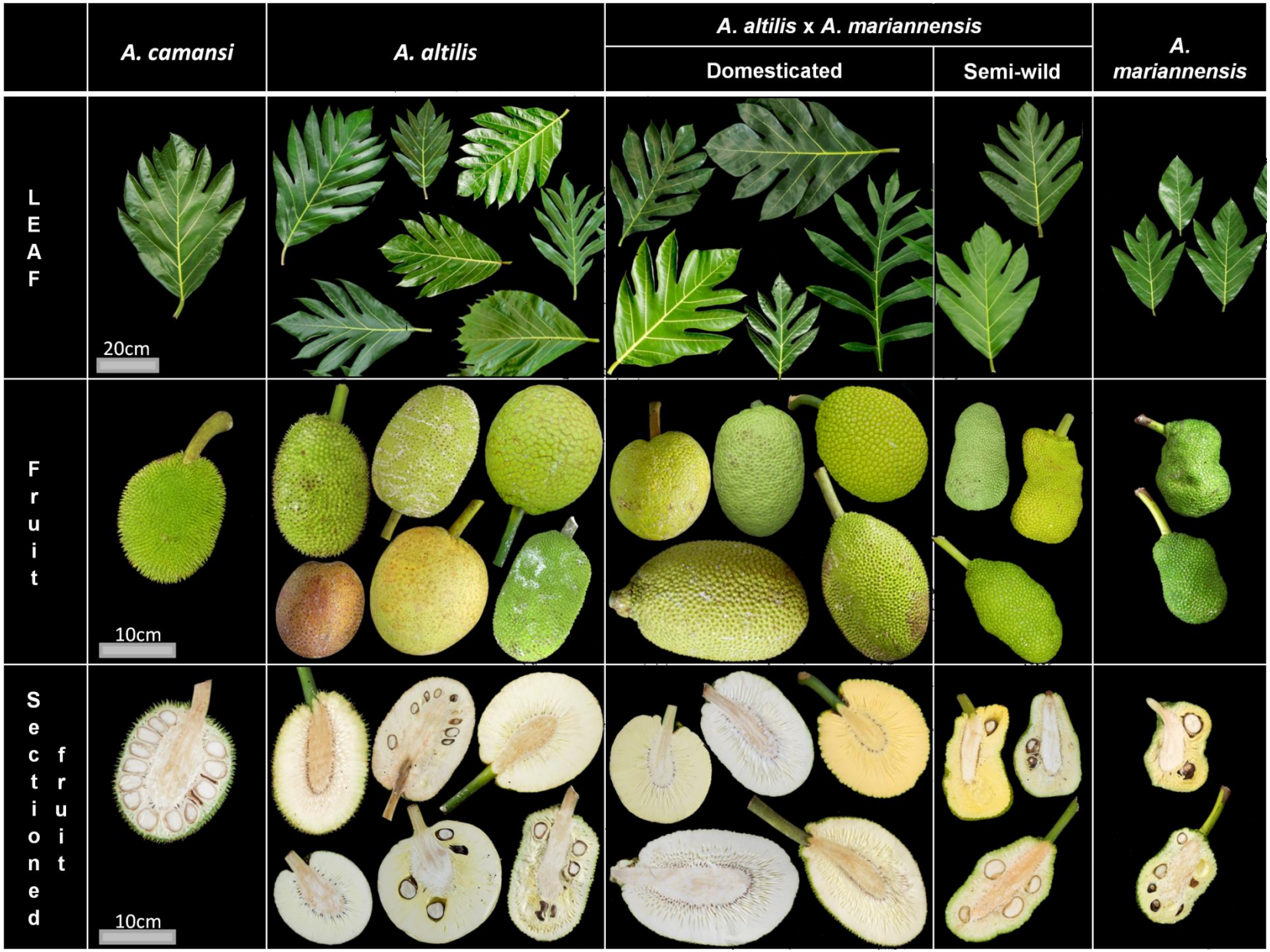

A. camansi
A. altilis
A. altilis x A. mariannensis
Domesticated
Semi-wild
A. mariannensis
LEAF
Fruit
Sectioned fruit
20cm
10cm
10cm

Figure 3-1: Representative leaves, whole fruit, and halved fruit from *Artocarpus camansi*, *A. altilis*, *A. mariannensis*, early generation *A. altilis* × *A. mariannensis* hybrids, and late generation domesticated *A. altilis* × *A. mariannensis* hybrids conserved in the National Tropical Botanical Garden's breadfruit germplasm repository.

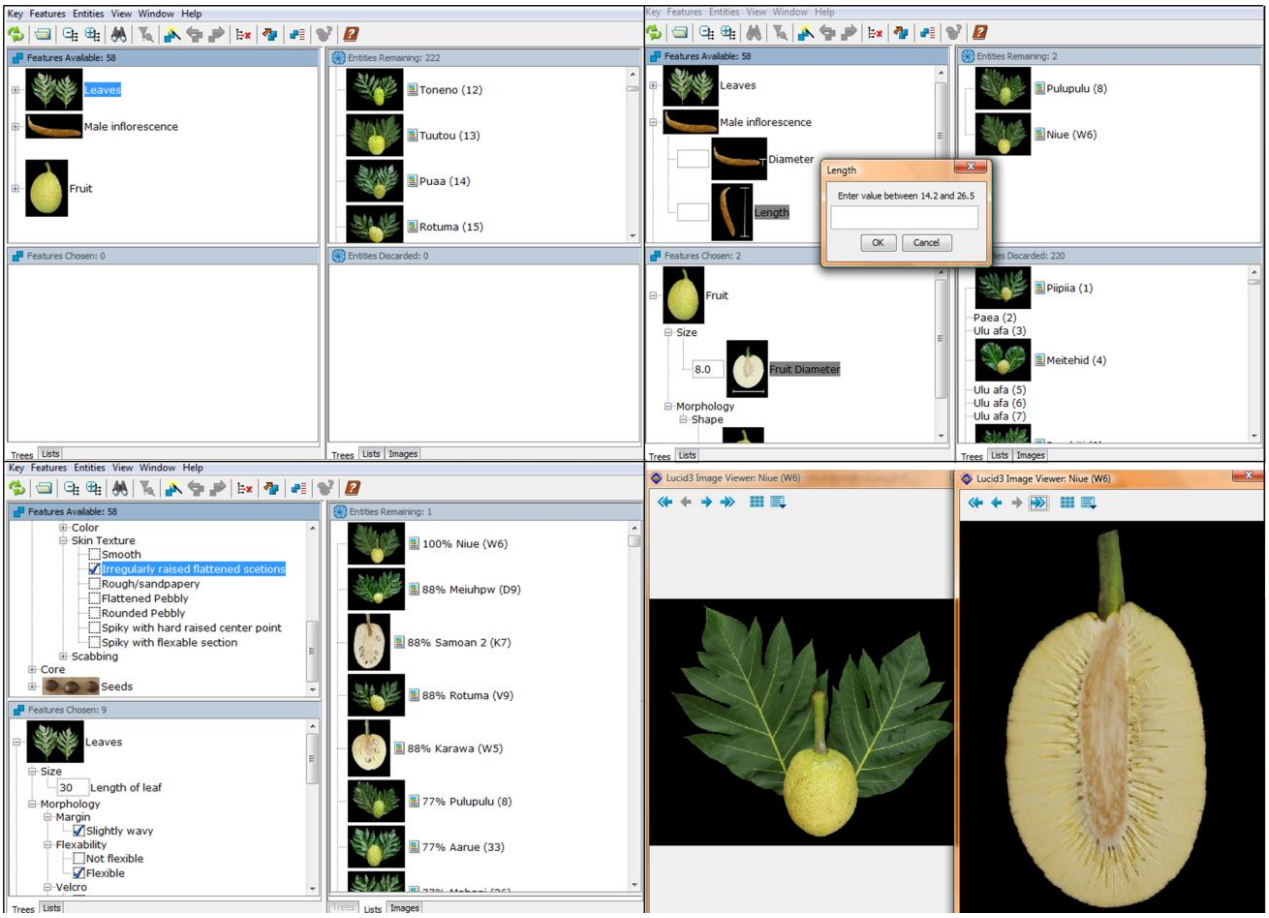


Figure 3-2: Screen shots from the prototype version of a multi-access breadfruit cultivar identification key on a Lucid 3.3 platform; top left, The initial start-up screen showing the general layout, top right, as the user is entering data set on the filtered sorting mode that eliminates all cultivars that do not match with only 2 possibilities remaining, bottom left, after several descriptors have been entered using the ranked sorting mode which shows all cultivars in the database sorted by the number of matching descriptors, and bottom right, images of a cultivar in the database available for the user to compare to their unknown cultivar.


Descriptor
Definition
Grouped by species
A.camansi
A.altilis
A.altilis x A.mariannensis
Developed
Early
A.mariannensis
Grouped by region of origin
Melanesia
W. Polynesia
E. Polynesia
Micronesia
Fruit Shape
Irregular
Heart
Ellipsoid
Oblongs
Oblong
Oval
Round
Skin Texture
Flexible spikes
Sharp spikes
Rounded pebbly
Flat pebbly
Sandpapery
Irregular raised
Smooth
Skin Color
Pink/oragen/brick
Brown Green
Yellow
Yellow green
Light green
Dark green
Scabbing Location
Both
Around Center
Between sections
No scabbing
Color between sections
Brown
Green
None
Color Around Center
Brown
Green
None
Peduncle collar
Flat, elongated
Raised, swollen
None
Peduncle insertion
Tightly clasped
Depressed
Open
Color of latex
Other
Rusty brown
Green
None
Seed Color
Dark brown
Light brown
Off white
Seed Shape
Reniform (kidney)
Oblong
Elongate
Ellipsoid
Round

Figure 3-3: Distribution of qualitative morphological fruit and seed descriptors among 222 accessions of breadfruit conserved in the National Tropical Botanical Garden's breadfruit germplasm repository grouped by species (*Artocarpus camansi*, *A. altilis*, *A. mariannensis*, early generation *A. altilis* × *A. mariannensis* hybrids, and late generation domesticated *A. altilis* × *A. mariannensis* hybrids) and by the region of origin (Melanesia, Western Polynesia, Eastern Polynesia, and Micronesia; *A. altilis* and domesticated *A. altilis* × *A. mariannensis* hybrids).


Descriptor
Definition
Grouped by species
A.camansi
A.altilis
A.altilis x A.mariannensis
Developed
Early
A.mariannensis
Grouped by region of origin
Melanesia
W. Polynesia
E. Polynesia
Micronesia
Shape of Leaf Base
Flat
Cuneate
Rounded
Tailed
Acute
Shape of Leaf Apex
Diamond
Rounded
Blade color
Dark Green
Green
Yellow-green
Vein Color
Yellow-green
Yello-green
Green
Leaf Surface
Dull
Glossy
Leaf Flexibility
Flexible
None
Velcro
Yes
No
Upper Hair Direction
Appressed
Upright
None present
Upper Hair Color
Reddish-white
Reddish-white
White
None present
Lower Hair Direction
Appressed
Upright
None present
Lower Hair Color
Reddish-white
Reddish-white
White
None present

Figure 3-4: Distribution of qualitative morphological leaf descriptors among 222 accessions of breadfruit conserved in the National Tropical Botanical Garden's breadfruit germplasm repository grouped by species (*Artocarpus camansi*, *A. altilis*, *A. mariannensis*, early generation *A. altilis* × *A. mariannensis* hybrids, and late generation domesticated *A. altilis* × *A. mariannensis* hybrids) and by the region of origin (Melanesia, Western Polynesia, Eastern Polynesia, and Micronesia; *A. altilis* and domesticated *A. altilis* × *A. mariannensis* hybrids).

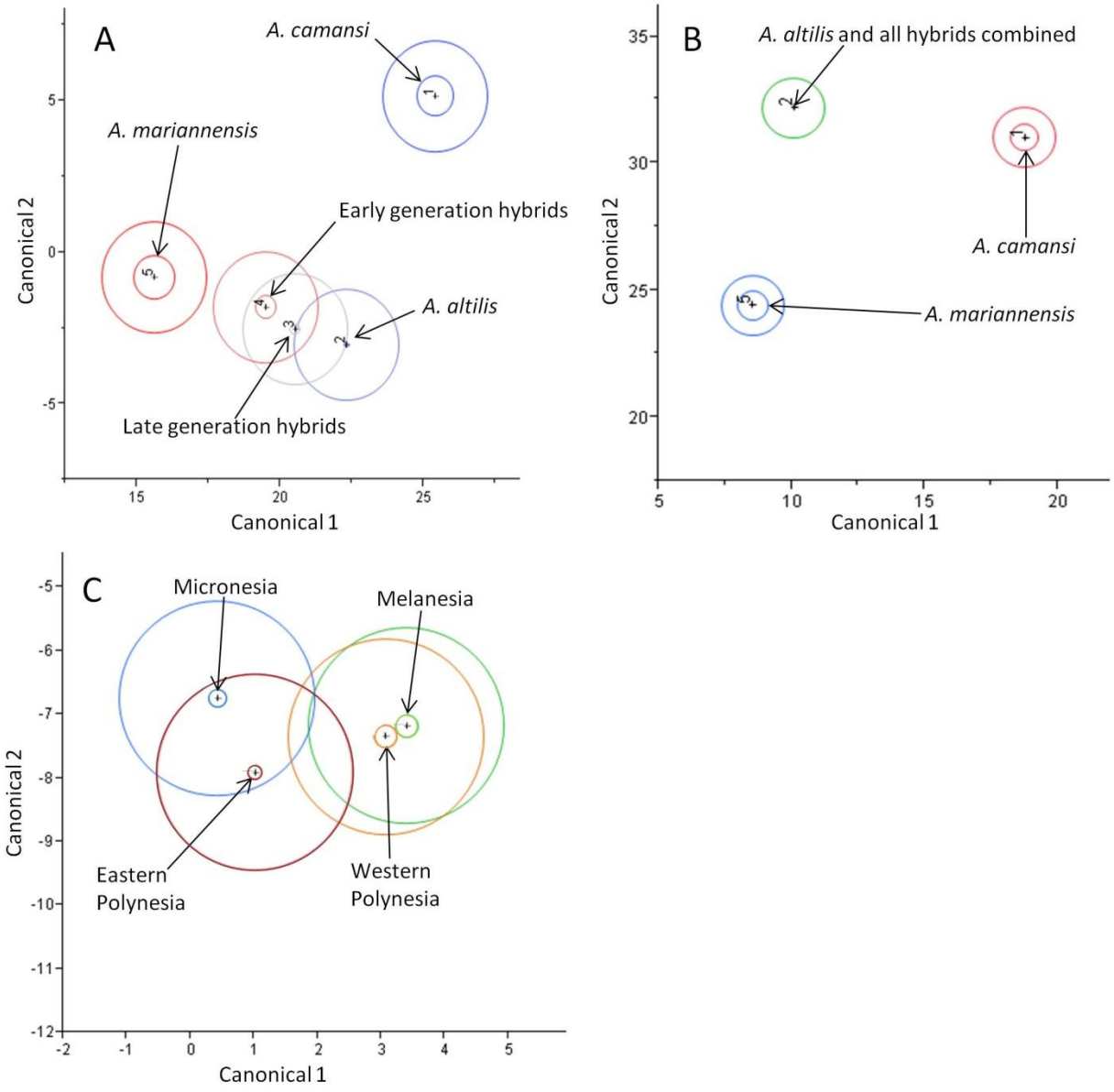


Figure 3-5: Linear discriminant analysis of 222 accessions of breadfruit conserved in the National Tropical Botanical Garden's breadfruit germplasm repository grouped by A: species (*Artocarpus camansi*, *A. altilis*, *A. mariannensis*, early generation *A. altilis* × *A. mariannensis* hybrids, and late generation domesticated *A. altilis* × *A. mariannensis* hybrids), B, species with *A. altilis* and *A. altilis* × *A. mariannensis* hybrids combined, and C, the region of origin (Melanesia, Western Polynesia, Eastern Polynesia, and Micronesia), using 29 quantitative morphological fruit and leaf descriptors. The inner circle represents the mean of each group at 95% confidence, and the outer circle represents the area that would include 50% of the population.

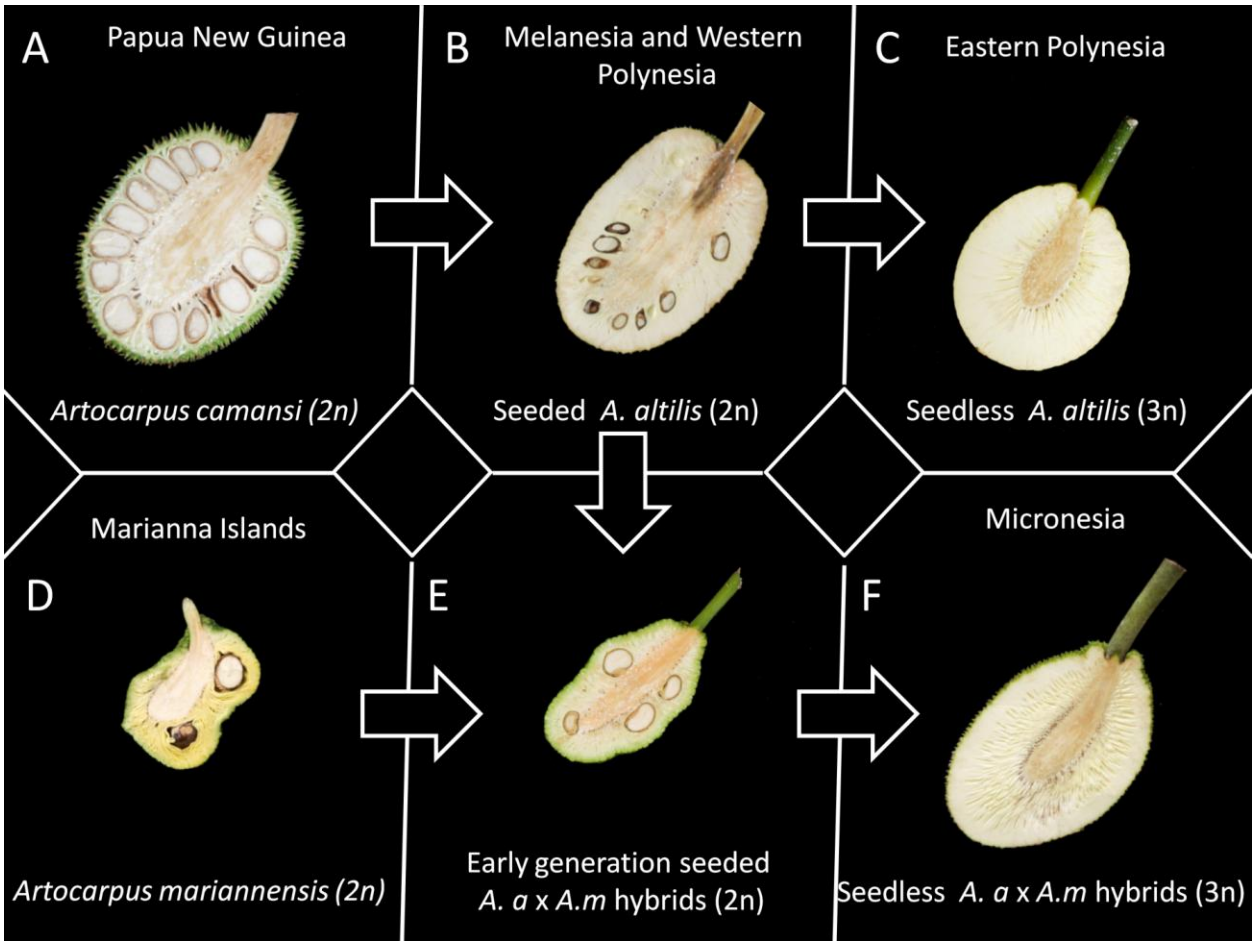


Figure 3-6: A flow chart depicting the domestication of breadfruit starting with A) the wild ancestor *Artocarpus camansi* transitioning into B) Seeded diploid *A. altilis* and its final development into C) seedless triploid *A. altilis*, D) the closely related wild species *A. mariannensis* which can hybridize to produce E) early generation interspecific *A. altilis* × *A. mariannensis* hybrids that represent the basis for the development of F) domesticated *A. altilis* × *A. mariannensis* hybrids.

Chapter 4: Diversity of Breadfruit (*Artocarpus*, Moraceae) Seasonality: A Resource for Year-Round Nutrition[3]

Introduction

Breadfruit, *Artopcarpus altilis* (Parkinson) Fosberg, is an important staple crop originating from the South Pacific Islands (Ragone, 1997). Today, breadfruit is cultivated in over 90 countries throughout the wet tropics but is considered an underutilized crop in most locations. The tree is prized for its high yield of starchy fruit, its relative ease of cultivation, and its prominent role in traditional Oceanic agro-forestry systems (Ragone, 1997; Raynor and Fownes, 1991). Most cultivars of breadfruit are highly seasonal and many have a main fruiting season followed by a second smaller season later in the year (Atchley and Cox, 1985). Predicting the fruiting season is important for the utilization of the crop since the fruit is highly perishable and availability can be limited to a surplus of fruit during the fruiting season(s) followed by a shortage during the off season(s). In order to realize the full potential of breadfruit as a staple food crop to enhance food security, facilitate commercial product development, and further develop an export market, these challenges need to be addressed.

On Pohnpei, the cultivation of phenotypically diverse cultivars of breadfruit with variable fruiting patterns has resulted in an extended season (Fownes and Raynor, 1991), and year round production has been documented (Atchley and Cox, 1985; Redfern, 2007). Pohnpei provides an

[3] A version of this chapter has been accepted for publication and is currently in press: Jones, A. M.P., Murch, S.J., and Ragone, D. Diversity of Breadfruit (*Artocarpus altilis*, Moraceae) Seasonality: A Resource for Year-Round Nutrition. Economic Botany

excellent illustration of how the breadfruit season can be extended by maintaining a rich diversity of cultivars with complementary seasonality profiles. However, to date seasonality studies in breadfruit have been limited to relatively few cultivars (Raynor and Fownes, 1991; Redfern, 2007). Furthermore, it is not clear how the seasonality profile of an individual cultivar will be affected if it is planted in a new location. In order to better predict if and how the seasonality of breadfruit will change when planted in new locations it is critical to gain an understanding of what factors contribute to the phenology of the species. Environmental factors that have been postulated as potential signals that plants could use to synchronize their development in the tropics where photoperiod is essentially constant include precipitation, changes in the timing of sunrise/sunset, and shifts in light intensity/quality that result from changes in the declination of the sun (Borchert et al., 2005; Yeang, 2007).

With the exception of some regions in Oceania, such as Pohnpei, very few breadfruit growing regions currently possess sufficient cultivar diversity to achieve year-round production (Atchley and Cox, 1985; Redfern, 2007). The current study was conducted to evaluate and describe the variation in the seasonality of a genetically diverse group of breadfruit trees growing in a single location. Information obtained from this study will facilitate a methodical approach for cultivar introductions targeted to extend the breadfruit season throughout the wet tropics and deepen our understanding of the regulation of flower and fruit development in the species.

Materials and Methods

Plant Material

A total of 219 accessions of breadfruit (*Artocarpus*, Moraceae) originating from 17 Pacific island groups, the Seychelles, the Philippines and Indonesia were used in this study. The trees evaluated include 156 accessions of *Artocarpus altilis*, 55 accessions of *A. altilis* × *A. mariannensis* hybrids, 5 accessions of breadfruit's putative ancestral species breadnut (*Artocarpus camansi* Blanco), and 3 accessions of dugdug (*Artocarpus mariannensis* Trécul) (Zerega et al., 2004). All of the trees used in the currect study were planted between 1978 and 1991 and are maintained in the Breadfruit Institute's germplasm bank in Kahanu Garden, a division of the National Tropical Botanical Garden, in Hana, Maui, Hawaii (Figure 4-1). The garden is located at 20°47'57.07"N, 156°02'18.42"W at an elevation of 15 m with a mean maximum temperature of 27.1°C, mean minimum temperature of 19.7°C and mean annual precipitation of 2051mm (Western Regional Climate Center; http://wrcc.dri.edu/). The trees are spaced approximately 10 m apart and are maintained using a low input system with minimal pruning/training.

Data Collection

Data were collected at approximately two week intervals from 1996 to 2005 based on the criteria described in Table 4-1. During the compilation of the fruiting data, full size, mature, and ripe fruit were amalgamated into a single category to eliminate subjective differences in data collection by several individuals over the study period. The data were collated into a single Excel-based database containing profiles for each accession representing the proportion of years

that the accessions produced male flowers and fruit each month. Seasonality profiles were grouped into ten seasonality types based on k-means cluster analysis with a maximum of 50 iterations using Genesis 1.7.5 (Institute for Genomics and Bioinformatics; Graz, Austria). Temperature and precipitation data for Hana, Maui were obtained from the Western Regional Climate Center (WRCC; http://wrcc.dri.edu/). A general linear model (GLM) was used to conduct correlation analyses between the annual and monthly fruiting data (percentage of trees with fruit) and the climate parameters using JMP®8.0.2 (SAS Institute Inc., NC, USA).

Results and Discussion

The seasonality of breadfruit is often reported as a discrete period of time during the year. While this form of presentation is useful to describe the peak production period of the crop, it does not convey the annual variability that is known to exist. The annual variation in fruit production for all of the accessions in this study is shown in Figure 4-2. While the primary fruiting season generally occurred between the months of August to January each year, the degree of seasonality varied considerably among years. For example, during 1998 and 2005, the period of time fruit was absent from the trees was relatively short compared to 1999-2002. Detailed examinations of the environmental parameters revealed no correlations between the fruiting period and annual or monthly precipitation or variations in temperature. However, it is possible that subtle differences in precipitation or temperature during a critical period of flower/fruit development that influence fruit production were undetected.

Breadfruit is a monoecious species, producing both male and female inflorescences on the same tree as depicted in Figure 4-1. The development and anthesis of male flowers precedes the emergence of female flowers, thus encouraging cross pollination (Brantjes, 1981). When data

for all years are combined, male flower production peaked approximately 3-4 months prior to the peak fruiting season (Figure 4-3). Likewise, the lowest level of male flower production was about 3-4 months earlier than that of the fruit. The yearly variation observed in the fruiting season was paralleled by male flower production, suggesting that the regulation of male and female flower production are linked.

A closer look at a single cultivar, 'Ma'afala' (Tree ID: 55), shows that annual variability can also be observed in an individual accession (Figure 4-4). 'Ma'afala' consistently fruited from August to October and usually had fruit in July (89%), November (89%) and December (78%) over the course of the study. During the "off season", from approximately January-May for this cultivar, fruit were present 20-40% of the years in the study. This indicates that while it may be concluded that 'Ma'afala' produces fruit from July to December, there is some year to year variability.

The accessions in the NTBG germplasm bank were collected from vast geographical distances over a period of several years and represent different genetic lineages (Zerega et al., 2005). K-means cluster analysis was used to find commonalities in the fruiting patterns of the 219 trees and allowed the accessions to be categorized into 10 seasonality groups (Figure 4-5). Group 1 (32 accessions) consists of trees that have an average fruiting season that extends roughly from September to December with a peak period in November. The low fruit production season for this group occurs around April-June. Group 2 (23 accessions) is comprised of trees with a fruiting season that also extends from approximately September to December but the likelihood of finding fruit during the peak season is slightly less than those in Group 1. Trees in Group 3 exhibit a similar seasonality profile as Groups 1 & 2, but during the peak season the cultivars only had fruit about 65% of the years in this study. Accessions that clustered into Group

4 (23 accessions) exhibited a slightly longer fruiting season than the previous groups, beginning earlier in the fall and ending around the same time of the year. The off season in this group is generally short and even during this period fruit was present about 30% of the years in this study. The fruiting season of trees in group 5 (26 accessions) is similar to Group 1, but occurs approximately 1 month later, peaking in December. Trees in Group 6 (20 accessions) have an early fruiting season extending from August to November and a relatively long off season, lasting from February to June. Group 7 (24 accessions) trees also have a fruiting season that extends approximately from August to November but the off season is much less pronounced in this group; the probability of finding fruit on the trees during the off season was about 40%. Group 8 (13 accessions) is relatively non-seasonal, producing fruit for most of the year. Even during the lowest season in June, trees in Group 8 had fruit 60% of the years Accessions in Group 9 (11 accessions) are also relatively non-seasonal but these cultivars tend to bear fruit less consistently from year to year than those in Group 8. Finally, cultivars in Group 10 (14 accessions) are typified by their highly inconsistent fruiting pattern. The majority of trees in this group were ʾulu afaʻ types collected from Tokelau, and appear to be early generation hybrids between *A. altilis* and *A. mariannensis* grown from seed. Although there is a period when the trees in Group 10 are more likely to have fruit, this is highly inconsistent from year to year.

Within the NTBG breadfruit germplasm collection there is a great amount of phenotypic diversity (see cultivar catalog: http://ntbg.org/breadfruit/database/search/). While the majority of cultivars in this collection are *A. altilis*, many originating from Micronesia appear to be interspecific hybrids between *A. altilis* and *A. mariannensis* (Ragone, 1997; Zerega et al., 2005). Hybrid cultivars were proportionally over-represented in the less seasonal Group 8, accounting for almost one half of this group while they represent only 28% of the total population. Due to

the increased proportion of non-seasonal accessions, hybrid cultivars may represent a valuable genetic resource for the advancement of year round breadfruit production and warrant a more thorough evaluation. *Artocapus camansi*, the putative ancestral progenitor of *A. altilis* generally exhibited a relatively late season and produced fruit most frequently from November to March. They also tend to exhibit a distinct off season from June to August. *Artocarpus mariannensis* most frequently produced fruit from August to December, and had a less pronounced off season than *A. camansi.*

Overall, the seasonality profiles of accessions originating from different locations were similar; however there are some notable differences (Figure 4-6). For example, the average seasonality profile of cultivars from Fiji and Vanuatu had a relatively well defined high and low season while the average profile of cultivars originating from places such as Palau, Kiribati, Samoa, and the Federated States of Micronesia (Chuuk, Pohnpei and Yap) tended to have a less pronounced off season. However, it must be noted that the NTBG collection does not represent the complete diversity of cultivars present in any given island group and there may be cultivars that fill the seasonality gaps in places such as Fiji and Vanuatu. In addition to differences in the degree of seasonality, the timing of the onset of fruit production also differs among cultivars from various island groups.

In order to determine the applicability of using breadfruit cultivars with compatible seasonality patterns, it is important to understand how the profiles will be affected when planted in new locations. Trees from the NTBG germplasm bank have been asexually propagated and shared with collections in other locations where some additional seasonality data have been gathered. Several cultivars from this collection have been introduced to Kiribati and seasonality data for Afara, Momolega, Puupuu, Roihaa and Yuley in this location are available (Redfern,

2007). The resulting fruiting patterns for the five cultivars are similar between the two locations except that the season occurs about 2-3 months earlier in Kiribati than in Hawaii. These data can be extrapolated to suggest that while there may be a temporal shift in the fruiting season when cultivars are grown in different locations the direction and magnitude of the change will be consistent among cultivars. If so, trees that display complementary seasons in Hawaii would maintain their compatibility when cultivated in alternate locations despite a shift in the onset of fruiting.

The onset of the breadfruit season is consistent enough that it is used as a seasonal reference point in some Oceanic cultures (Sakiyama, 1998). This reliability suggests that there is likely an environmental factor with an annual cycle being used by the plants to synchronize their developmental processes. One potential environmental change that could be used by plants to synchronize annual developmental cycles is precipitation (van Schaik et al., 1993). In the case of breadfruit, rainfall patterns do not appear to correlate with the fruiting season (Quartermain, 2007). The data collected in this study were compared with reports from various locations representing the local breadfruit season at different latitudes North and South of the equator (Figure 4-7). Interestingly, the period of fruit production in breadfruit tends to begin around the date the sun reaches zenith prior to the summer months, a date that varies by latitude. Some notable exceptions to this pattern include parts of Papua New Guinea, Kiribati, Australia, and Hawaii. Based on the observations in this study, male flower initiation would be expected to occur 3-4 months earlier, when the declination of the sun is lower. Changes in the declination of the sun represent one of the most consistent annual cycles in the tropics and provide a possible environmental signal that plants could use to synchronize developmental events. Between the tropics of Cancer and Capricorn the sun reaches zenith twice each year; along the equator this

occurs during the equinoxes, as you move north or south the dates move closer together and eventually converge towards the summer solstice. When the sun is at zenith the solar rays are perpendicular to the atmosphere and light intensity is greatest at this time of year. Sun declination also has qualitative effects on light, causing subtle shifts in the spectrum (Awadalla and Alnaser, 1993). Several possible mechanisms exist that could facilitate synchronized flower production in breadfruit using the declination of the Sun. For example, a critical level of irradiance or a specific spectral shift in the light received by the plant could provide a signal to cue floral initiation. Since the angle of declination of the Sun is lower in the winter than in the summer at most tropical latitudes, this could be used as a signal to differentiate the two dates and synchronize fruit production to a specific time of year.

Together, these data provide a predictive tool for the introduction of breadfruit cultivars to new locations. For example, 'Ma'afala,' a member of seasonality Group 7, displays an earlier seasonality profile than most cultivars (Figures 4-3 & 4-5). If this cultivar were planted in Trinidad, (11˚77"N) where the normal breadfruit season is roughly June to September (Roberts-Nkrumah, 2007), we expect that it would produce fruit from March to July. This would effectively extend the breadfruit season in this location by 3 months. Further extensions could be made by strategically introducing new cultivars with complementary seasonality profiles, ultimately enabling year round production. By applying these data, informed decisions can be made regarding new cultivar introductions to extend the breadfruit season in countries with little baseline seasonality data or with known gaps in fruit production.

Conclusions

The Food and Agriculture Organization of the United Nations (FAO) recently announced that the number of hungry people in the world has exceeded 1 billion for the first time in human history. More than 80% of the world's hungry live in tropical and subtropical regions, including 180 million people in Sub-Saharan Africa and 53 million in Latin America and the Caribbean. Facing soaring food, fuel, and fertilizer costs, smallholder farmers in the tropics need sustainable, low input, nutritious crops. Breadfruit has been grown by the indigenous peoples of Oceania for more than 3,000 years and is widely cultivated in the Caribbean and other tropical regions. The current data provide the first detailed comparison of different cultivars grown under consistent conditions in a single environment and demonstrate the range of diversity within the collection. Planting a combination of cultivars with complementary seasons can provide a sustainable food resource for many parts of the world.

Table 4-1: Protocol for collection of seasonality data for breadfruit cultivars by visual estimates in Kahanu Garden, 1996 – 2005.

Category	Description
New male flowers	Male flowers of any size, small and just emerging from the sheath to full size, but still green.
Mature male flowers	Ones that have turned yellow because the anthers have opened
New fruit	Small fruit that have recently emerged from the leaf sheath, they are often prickly and the stigmas still 78yophili and remain green
Less than mature fruit	A wide range of fruit sizes from bigger than the new fruit, 1/2 size, up to almost full sized
Full sized fruit	Fruit that have reached their maximum size, but have not yet started to mature
Mature fruit	Fruit with distinctive characteristics of maturing, such as latex 78yophili on the skin and slight changes in skin color and texture
Ripe fruit	Fruit that are soft and ripe that remain on the tree

Figure 4-1: Flower and fruit development in breadfruit (*Artocarpus altilis*): A) A mature fruit bearing breadfruit tree (Maopo; Y1) growing in the National Tropical Botanical Garden's Kahanu Garden, Hana, Hawaii. B) A young male inflorescence and a developing female inflorescence yet to emerge from the enclosing stipules. C) A mature male inflorescence getting ready to senesce and a developing female inflorescence. D) The fruit from three different cultivars of breadfruit. Photos courtesy of Jim Wiseman (A, B, and D) and Diane Ragone (C).

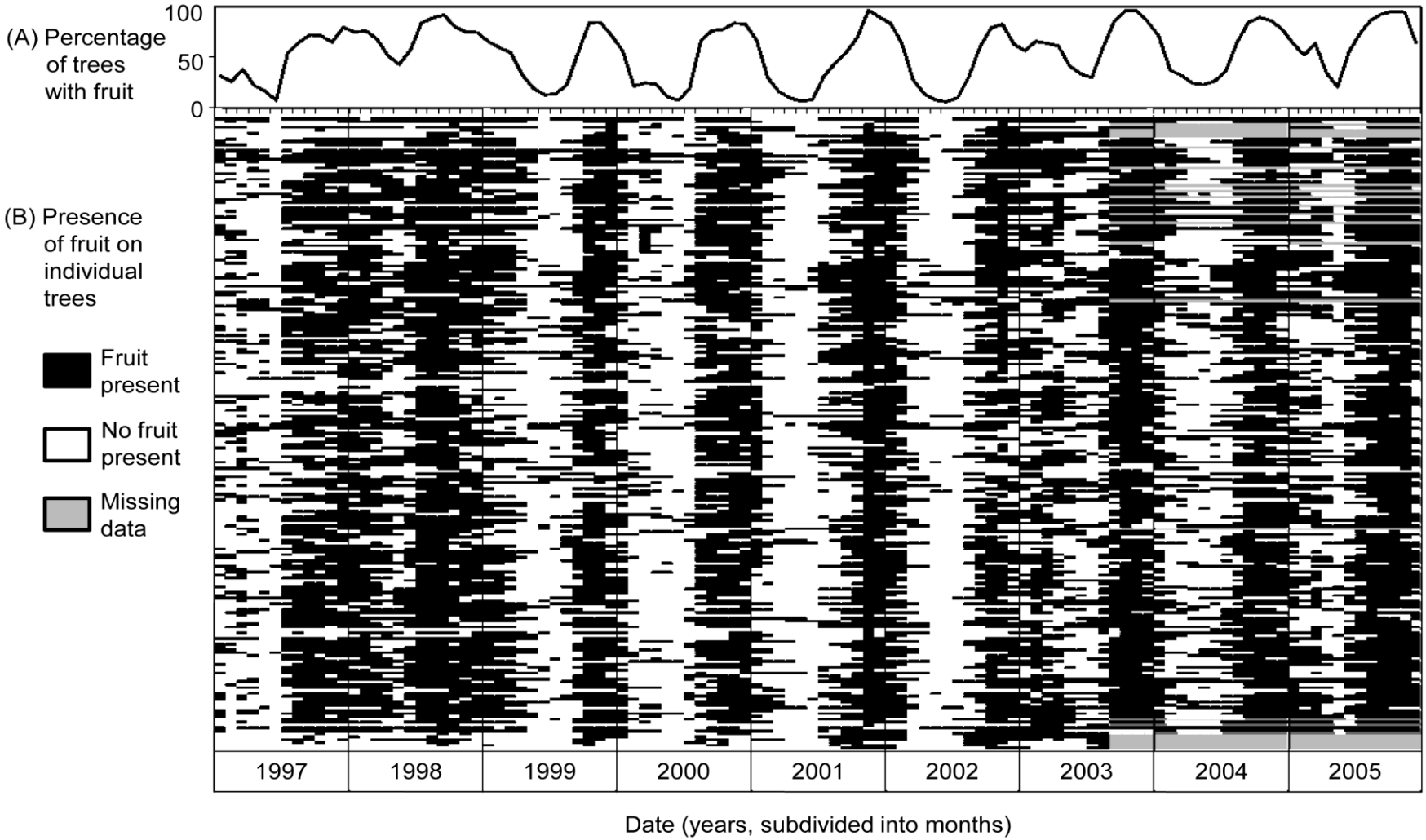


Figure 4-2: Seasonality of fruit production in 219 breadfruit accessions originating from 34 Pacific islands, the Seychelles, the Philippines and Indonesia now growing in the National Tropical Botanical Garden's (NTBG) Kahanu Garden, Hana, Hawaii, were evaluated. A) Proportion of trees in the collection that had full size, mature or ripe fruit present over the course of the study. B) Raw data showing when each accession had full size, mature or ripe fruit present over the course of the study; each row represents an individual accession, a black box indicates a month in which the accession contained fruit.

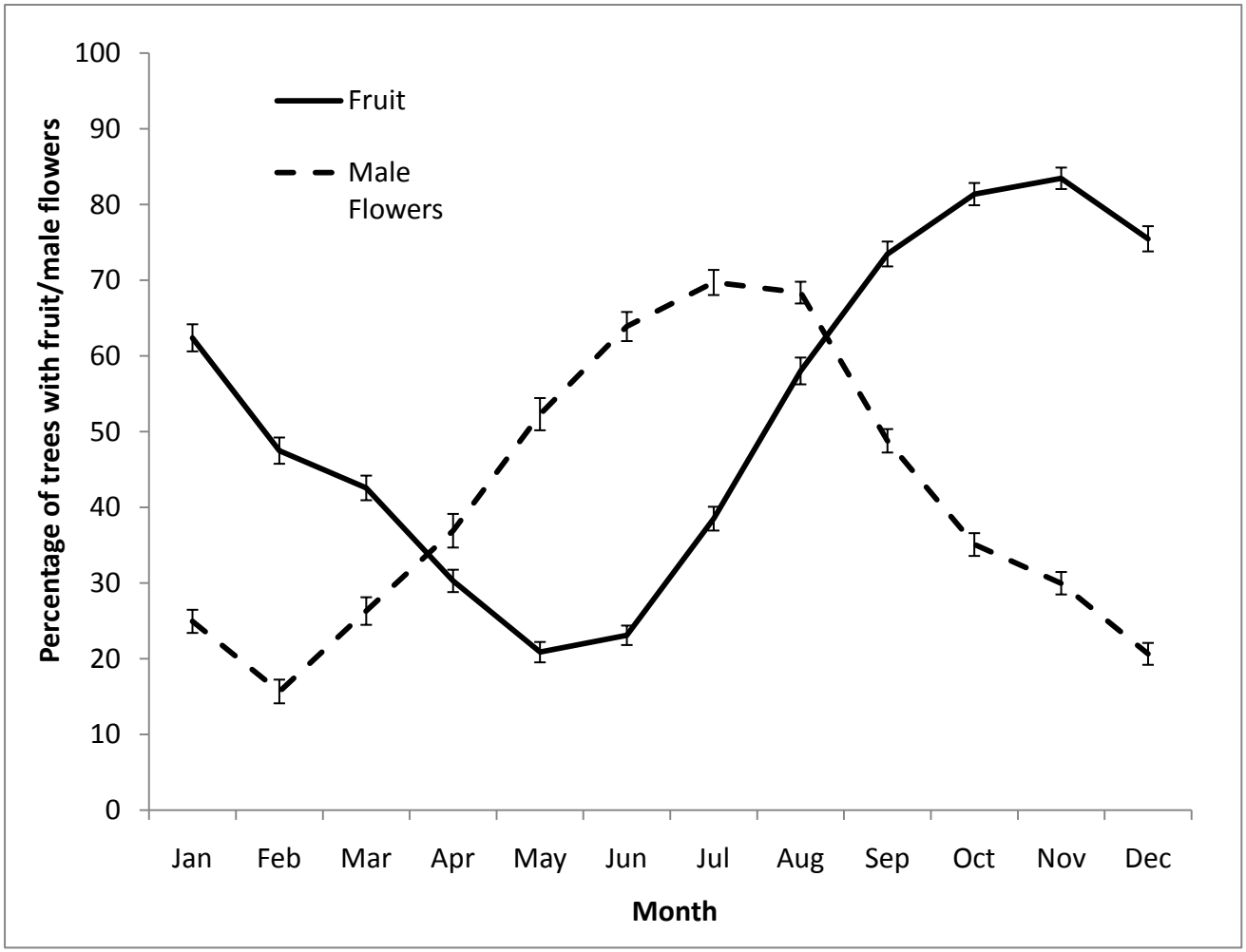


Figure 4-3: Average percentage of 219 breadfruit accessions growing in the National Tropical Botanical Garden breadfruit collection in Kahanu Garden, Hana, Hawaii bearing male flowers and fruit (full size, mature and ripe combined) between 1997-2005; bars represent the standard error of the mean.

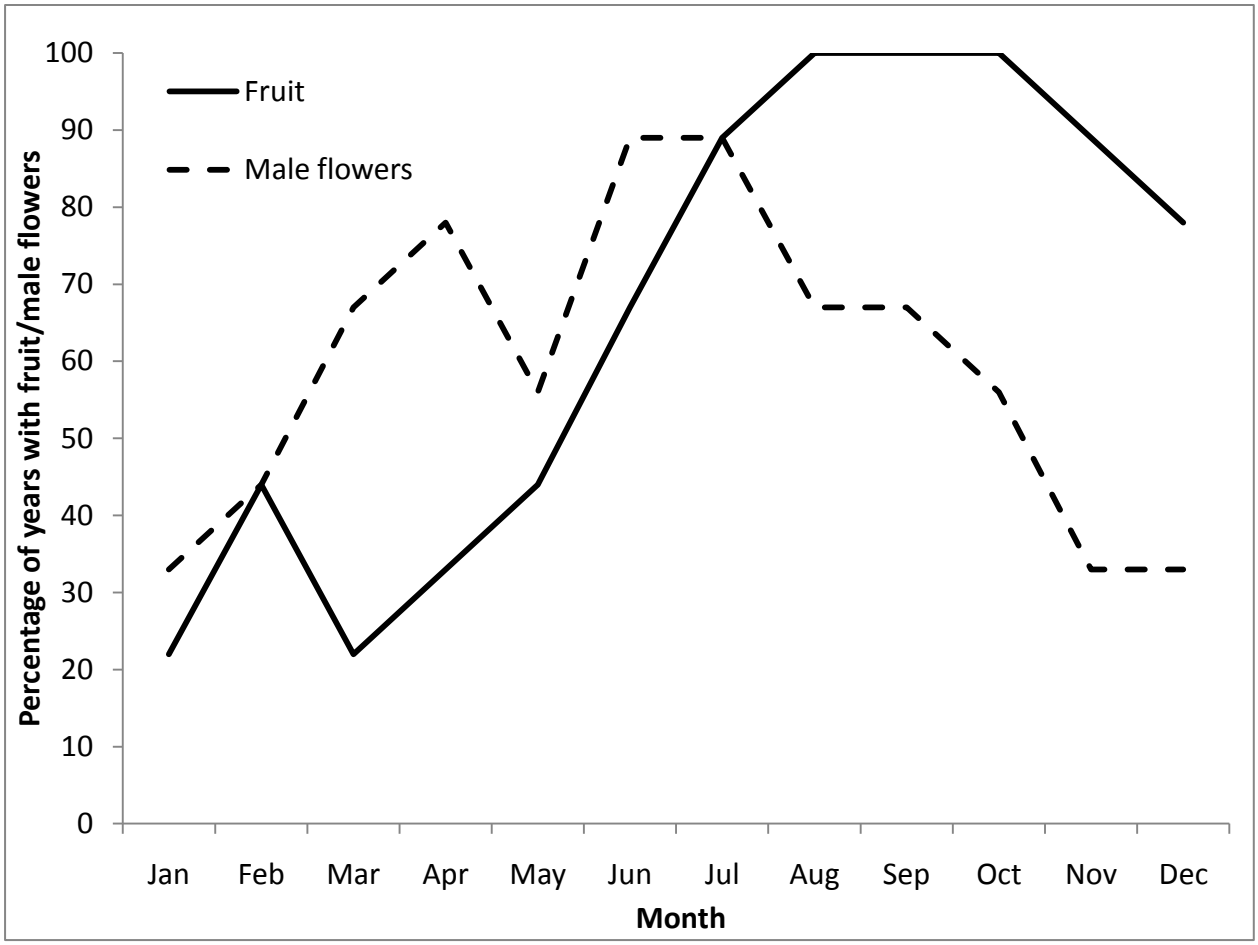
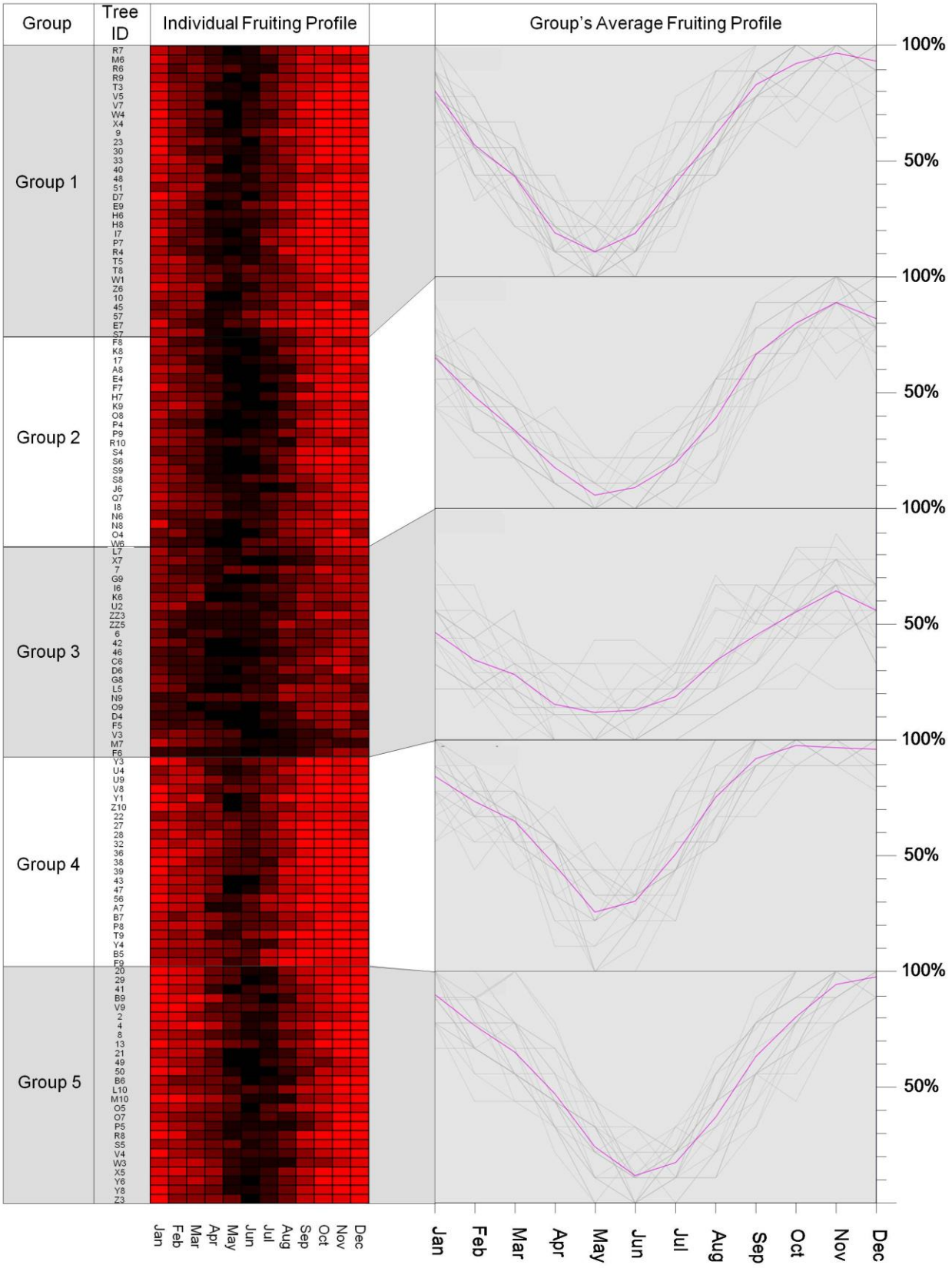


Figure 4-4: The average seasonality profile of 'Ma'afala' from 1997-2005; percentage of years with fruit/male flowers represents the percentage of years encompassed by the study that the accession produced full size, mature or ripe fruit and male flowers, respectively.


Group
Tree ID
Individual Fruiting Profile
Group's Average Fruiting Profile
Group 1
R7 M6 R6 R9 T3 V5 V7 W4 X4 9 23 30 33 40 48 51 D7 E9 H6 H8 I7 P7 R4 T5 T8 W1 Z6 10 45 57 E7 SZ
Group 2
F8 K8 17 A8 E4 F7 H7 K9 O8 P4 P9 R10 S4 S6 S9 S8 J6 Q7 I8 N6 N8 O4 W6
Group 3
L7 X7 7 G9 I6 K6 U2 ZZ3 ZZ5 6 42 46 C6 D6 G8 L5 N9 O9 D4 F5 V3 M7 F6
Group 4
Y3 U4 U9 V8 Y1 Z10 22 27 28 32 36 38 39 43 47 56 A7 B7 P8 T9 Y4 B5 F9
Group 5
20 29 41 B9 V9 2 4 8 13 21 49 50 B6 L10 M10 O5 O7 P5 R8 S5 V4 W3 X5 Y6 Y8 Z3
100%
50%
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

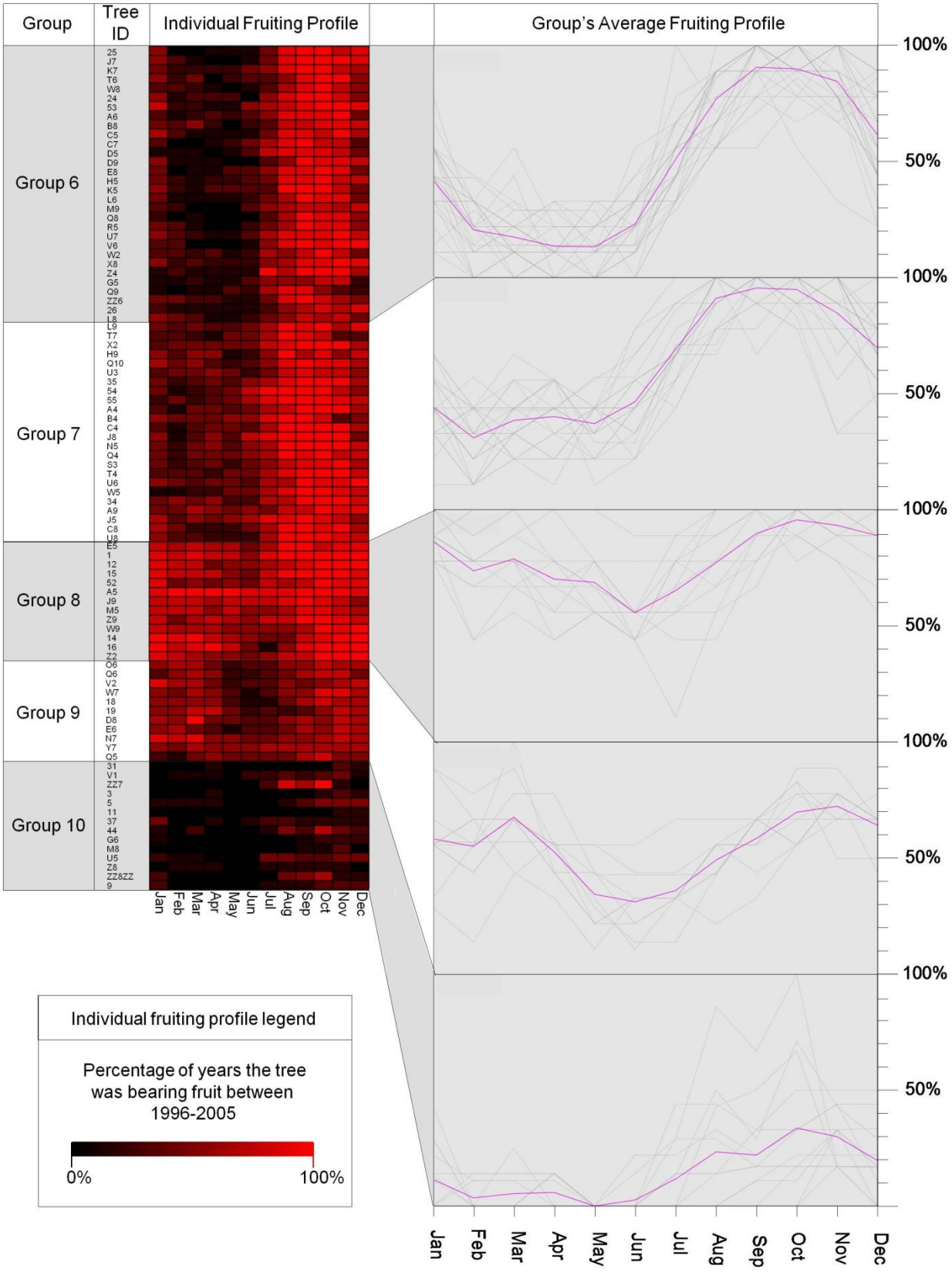

Group
Tree ID
Individual Fruiting Profile
Group's Average Fruiting Profile
Group 6
25
J7
K7
T6
W8
24
53
A6
B8
C5
C7
D5
D9
E8
H5
K5
L6
M9
Q8
R5
U7
V6
W2
X8
Z4
G5
Q9
ZZ6
26
L8
Group 7
L9
T7
X2
H9
Q10
U3
35
54
55
A4
B4
C4
J8
N5
Q4
S3
T4
U6
W5
34
A9
J5
O8
U8
Group 8
E5
1
12
15
52
A5
J9
M5
Z9
W9
14
16
Z2
Group 9
O6
Q6
V2
W7
18
19
D8
E6
N7
Y7
Q5
Group 10
31
V1
ZZ7
3
5
11
37
44
G6
M8
U5
Z8
ZZ8ZZ
9
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
100%
50%
Individual fruiting profile legend
Percentage of years the tree was bearing fruit between 1996-2005
0%
100%

Figure 4-5: Seasonality profiles of 219 breadfruit accessions growing in the National Tropical Botanical Garden breadfruit collection in Kahanu Garden, Hana, Hawaii sorted into 10 groups based on k-means cluster analysis. Tree ID refers to the NTBG grid number assigned to each tree; see Appendix 2 for more detailed information on individual accessions. Individual fruiting profiles represents heat maps depicting the average percentage of years (from 1996-2005) each accession was bearing fruit in a given month. Each row represents an individual breadfruit accession. Group's average fruiting profile depicts graphs displaying the average percentage of accessions in fruit (full size, mature or ripe) for each group over the course of a year.

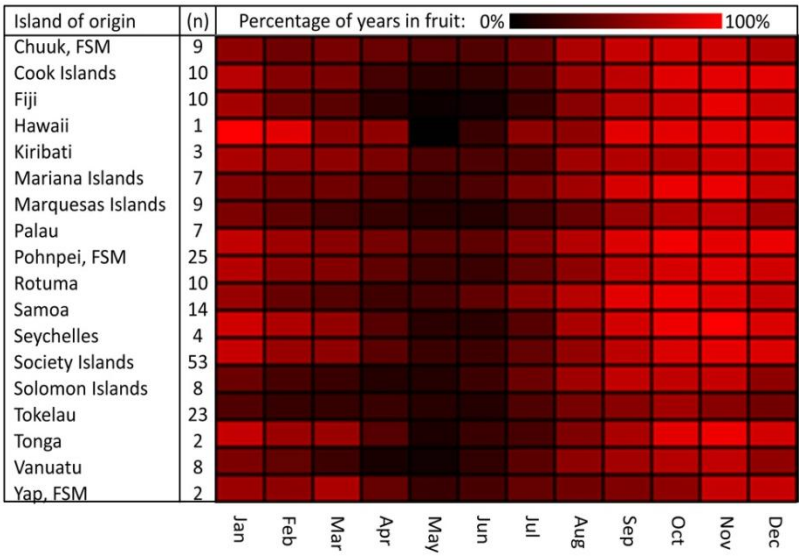


Figure 4-6: Average seasonality profiles of breadfruit accessions collected from various locations growing in the National Tropical Botanical Garden breadfruit collection at Kahanu Garden, Hana, Hawaii. Each row represents the average (from 1996-2005) probability of all accessions originating from a single location (n = number of trees from that location evaluated in the study) to produce fruit (full size, mature or ripe) during each month.

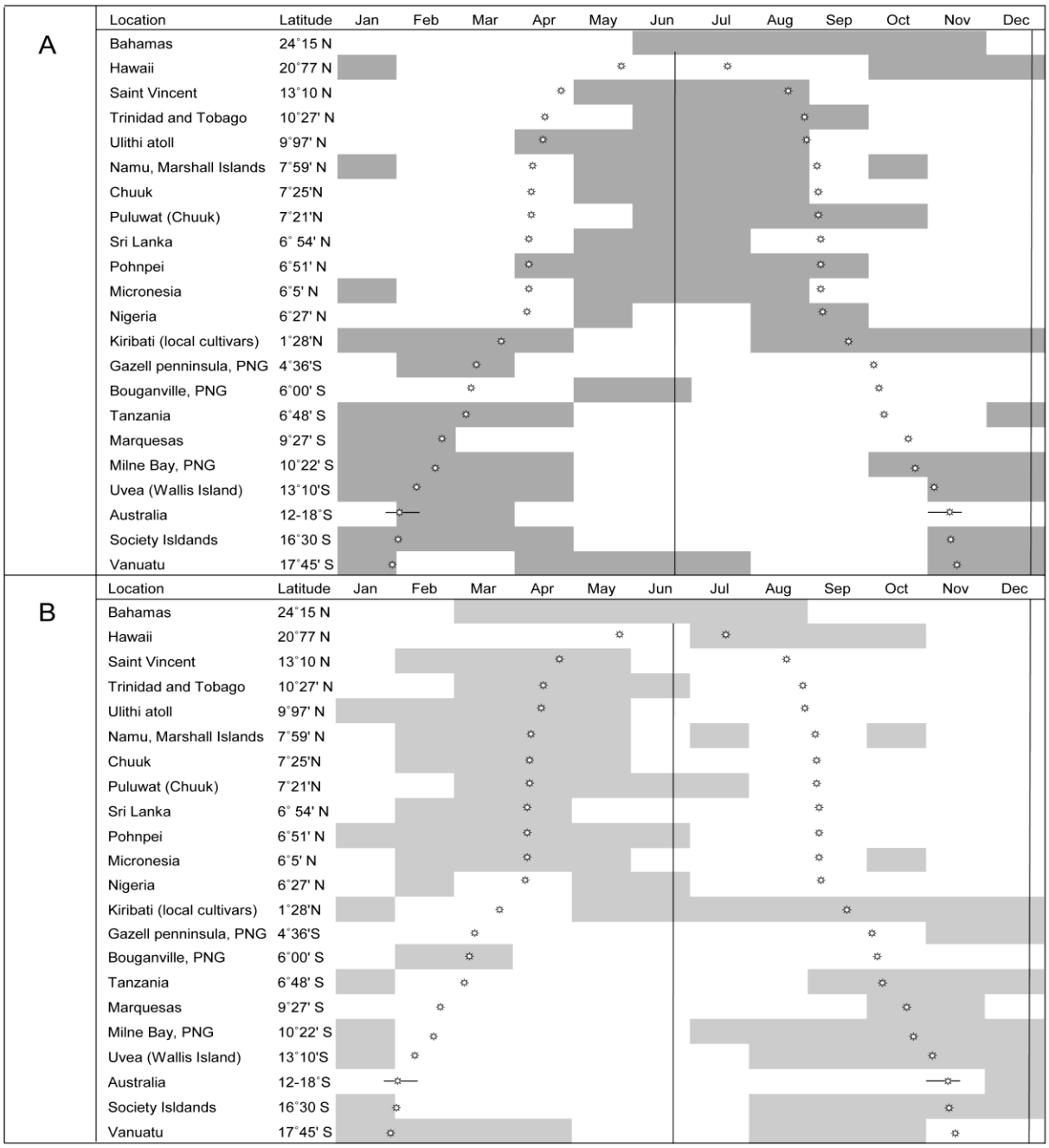


Figure 4-7: Seasonality of breadfruit (*Artocarpus altilis*) reported from regions around the world. A) Main fruiting season reported from various locations, B) Estimated period that male flowers would be produced based on the 4-month difference observed in this study. ☼ = approximate date that the sun reaches its zenith at that latitude. The vertical line indicates when the sun reaches its lowest angle of declination (summer and winter solstices). Seasonality data compiled from (Addison, 2006; Goebel, 2007; Kirch, 1978; Maerere and Mgembe, 2007; Medagoda, 2007; Michio et al., 2003; Morton, 1987; Navarro et al., 2007; Omobuwajo, 2003; Pollock, 1994; Quartermain, 2007; Redfern, 2007; Roberts-Nkrumah, 1997, 2007; Sakiyama, 1998).

Chapter 5: High Yield and Protein Cultivars of Breadfruit (*Artocarpus altilis*): Developing an Underutilized Crop for Food Security and Novel Foods[4]

Introduction

Recent reports estimate that the number of undernourished people in the world has reached approximately 1.02 billion (FAO, 2009a). The majority of the world's undernourished population lives in developing countries, mostly in tropical and subtropical climates where an estimated 80% of the hungry are found. Malnutrition and poverty in these regions is in large part linked to the agricultural sector, making agricultural development an obvious focal point of the development agenda (World Bank, 2007). While rice, corn, and wheat represent the most common staple crops, these require substantial external inputs and modern technologies to obtain adequate yields (Godfray et al., 2010), and many cultivars that are highly optimized to well-fed northern climates are not suitable for the tropics. Enhanced food security in developing and tropical countries could be achieved through the use of underutilized species that are adapted to local climates and technologies.

Breadfruit, *Artocarpus altilis*, has provided Pacific islanders with a versatile staple food for thousands of years (Ragone, 1997; Zerega et al., 2004, 2006). The potential of this crop to

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provide an abundant source of food for the rest of the tropics was recognized by European colonialists as early as the 1700s (Banks, 1962). This tropical tree produces an abundance of starchy fruit with relatively little time investment or external inputs (Bowers, 1981; Ragone and Raynor, 2009). Conservative calculations predict that orchard production of this tree at a density of 100 trees/ha would produce 20 t/ha, approximately 6 t/ha dry matter, of edible fruit yield (Bowers, 1981), higher than the global average yields of 4.0 t/ha for corn, 4.1 t/ha for rice, or 2.6 t/ha for wheat (Calpe, 2007; FAO, 2009c, 2009d). As a tree, breadfruit can also be an important component of agroforestry systems that helps prevent soil erosion and land degradation and are well suited to the tropics (Elevitch and Wilkinson, 2000; Ragone and Raynor, 2009; Raynor and Fownes, 1991). Despite the potential for high yields, low maintenance, agroforestry, and secondary products, breadfruit remains an underutilized species around the world (Ragone, 1997). Some of the issues inhibiting the use of breadfruit are: (a) the fruit is seasonal with a very short shelf life, (b) there are difficulties in propagating and distributing trees (Murch et al., 2007; Shi et al 2007) and (c) there are challenges with introducing a new food item into local culinary traditions (Ragone, 1997; Worrell et al., 2002). Recent efforts have made large numbers of breadfruit trees available for commercial distribution for the first time (Shi et al., 2007; Murch et al., 2007; www.breadfruit.org; www.globalbreadfruit.org) and the development of new technologies to harvest, process and produce food from breadfruit is warranted.

One promising approach to overcome some of these challenges and develop export markets for breadfruit is by processing the fruit into flour (Arcelay and Graham, 1984; George et al., 2007; Wootton and Tumaalii, 1984). Breadfruit flour can be stored for months at room temperature for long periods with little loss in quality (Sharon and Usha, 2006) and can be incorporated into a variety of local recipes including stiff porridges (Mayaki et al., 2003), infant

formulas (Esparagoza and Tangonan, 1993), extruded products (McHugh et al., 2007), bread (Ayodele and Oginni, 2002; Esuoso and Bamiro, 1995; Nochera and Caldwell, 1992), cake (Ayodele and Oginni, 2002), pancakes (Ayodele and Oginni, 2002) and biscuits (Nnam and Nwokocha, 2003; Olaoye et al., 2007; Omobuwajo, 2003). However, a great number of breadfruit cultivars exist and very little information is available regarding their potential for flour production. Once ideal cultivars are identified, modern tissue culture techniques will enable rapid mass-propagation and dissemination of trees (Murch et al., 2007, 2008).

The current study was conducted to evaluate a diverse collection of 94 breadfruit cultivars and two related species for several key attributes that are important for flour production. The characteristics that were evaluated include protein content, fruit moisture content, presence/number of seeds, and several morphological characteristics of the fruit. These data provide baseline information needed to identify cultivars with potential for the development of a breadfruit flour industry.

Materials and Methods

The Breadfruit Germplasm Repository at Kahanu Garden of the NTBG

The Breadfruit Germplasm Repository at Kahanu Garden is the largest and most diverse collection of breadfruit in the world, representing 222 accessions collected from 34 Pacific islands, the Philippines, Indonesia, and the Seychelles (Ragone, 1997). The trees in the breadfruit germplasm repository were planted between 1978 and 2004 at 20°47’57.07”N, 156°02’18.42”W. The garden is situated at an elevation of 15 m, with a mean maximum temperature of 27.1°C, mean minimum temperature of 19.7°C, and a mean annual precipitation

of 2051mm (Western Regional Climate Center; http://wrcc.dri.edu/). The collection was fertilized with 6-6-6 commercial fertilizer at a rate of 0.91 kg/tree in February 2007 but not during the fruit collection period of current study. The trees are growing on Hana Very Stone Silt Clay Loam soil (http://websoilsurvey.nrcs.usda.gov), which is derived from volcanic ash, is generally well draining, slightly to moderately acidic soil that contains about 8% organic matter in the surface horizon. Root-restrictive obstructions occur at a depth of about 1.5m. Under the soil surface layer is a base of deep lava. Soil nutrient analysis of the location can be found in Chapter 6.

Breadfruit Sample Collection

A subset of 94 breadfruit cultivars (*Artocarpus altilis; Artocarpus altilis* × *Artocarpus mariannensis*), three breadnut accessions (*Artocarpus camansi*), and one accession of dugdug (*Artocarpus mariannensis*) were collected according to the illustrated sampling scheme (Figure 5-1). Three fruit were collected from each tree in the first year of harvest between November, 2008 and March, 2009. In a second harvest year (2009-2010), an additional three fruit were collected from each of nine cultivars to evaluate inter-seasonal variability. The fruit were collected at the mature, but not yet ripe, stage of development when the fruit were still firm and starchy (Worrell et al., 1998). After the fruit were picked, the peduncles were removed and the fruit inverted for about 1 h to allow the latex to drain/coagulate. The fruit were weighed, peeled with a household vegetable peeler, quartered longitudinally, and the seeds (when present) were removed (Figures 5-1 & 5-2). One half of each fruit harvested was cut into 5-10 cm pieces, frozen and shipped to the University of British Columbia Okanagan where it was stored at -86°C. The remaining half of each of three fruit per cultivar per year was sliced into 2 mm

sections using a mandolin vegetable slicer (Benriner, Philadelphia, PA) and dried on screen trays in a dark drying room at 22-47°C and 19-47% RH (Figure 5-2). The dehydrated slices of fruit were ground into flour using the finest setting of a commercial coffee grinder (Bunn® model G3, Aurora, ON) creating a representative bulk flour sample for each cultivar. The entire breadfruit sampling procedure was replicated in the second season for nine cultivars and a further eight cultivars were also harvested using the same techniques in the second season to determine the effects of baking and boiling on the protein contents. For cooked breadfruit studies, one half of each fruit from each cultivar was boiled until the flesh could be easily pierced with a fork, while the other half was roasted in an oven at 180˚C until the flesh was tender following typical local food preparation protocols. The average time to cook the fruit by boiling was 34.9 minutes (SE ± 3.71) and 45.4 minutes when roasted (SE ±3.50). The range of boiling times was from 15-50 minutes and the roasting time ranged from 28-63 minutes depending on the individual characteristics of the cultivar.

Fruit Morphological Characteristics

Fruit morphological characteristics were determined in two sequential studies. A large-scale descriptive study was conducted for the entire collection over a 10-year period from 1996-2005 with biweekly quantitative and qualitative observations of the trees (see Chapter 3). These descriptor studies assessed weight, fruit colour, skin texture, and other identifying characteristics for each cultivar in 10 fruits per accessioned cultivar in the NTBG collections. Detailed data of the descriptive aspects of this study are available in an online database (http://ntbg.org/breadfruit/database/search/varlist/). The gross fruit measurements of 10 fruit per cultivar were taken by cutting each fruit in half longitudinally and measuring the fruit length

from the point of peduncle insertion to the distal end of the fruit, the fruit width measured at the widest part of the fruit, the length and width of the receptacle/core of the fruit, and the number of seeds that can be seen on the cut surface of the fruit (Figure 5-2). In addition, the current studies determined the edible yield of each cultivar by fully dissecting the fruit and collecting data for the total weight of the fruit, the weight of the peel, core and seeds, the total number of seeds, and the weight of the edible portion for three fruit per cultivar per harvest (Figure 5-1).

Dry Matter Quantification

The moisture content of the fresh frozen fruit was determined in a 5-10 g wedge-shaped sample representing tissue from throughout each of the three replicate fruit. Samples were weighed in a 15 ml centrifuge tube (Corning Inc., Corning, NY), lyophilized overnight (Freezone 4.5 Freeze Drier, Labconco, Kansas City, MO) and reweighed to determine the moisture content removed through freeze drying. The moisture contents of the bulk flour samples were determined in triplicate using the American Association of Cereal Chemists (AACC) method 44-19 (AACC, 2000). Analytical dry matter was determined in 2 g subsamples of each replicate fruit sample by drying in an Isotemp programmable muffle furnace (Fisher Scientific, Ottawa, ON) at 135°C in a pre-weighed 65 mm aluminum weighing dish (Fisher Scientific, Ottawa, ON). After 2 h, the trays were removed and weighed to calculate the percent moisture lost through heat drying.

Protein Quantification

Protein Extraction

Approximately 10 mg samples of three replicate samples of lyophilized fresh frozen breadfruit or the individual bulk breadfruit flours were weighed in 1.5 ml microcentrifuge tubes (Fisher Scientific, Ottawa, ON). A 0.5 ml aliquot of protein extraction buffer (20 mM HEPES, 150 mM NaCl, 0.3% Tween 20) was added to each sample before they were vortexed (Vortex Genie; Fisher Scientific, Waltham, MA) to suspend the material and sonicated for 15 minutes at room temperature. The tubes were then centrifuged for 10 minutes at 13000 rpm (Galaxy 16DH; VWR, Westchester, PA). The supernatant was used for quantification of the protein content in each individual replicate sample by standardized colourometric protein quantification assays. In a preliminary study, the quantification response of three common colourmetric protein assays, the modified Lowry protein assay (Lowry et al., 1951), the Pierce 660nm protein assay (Antharavally et al., 2009), and the bicinchoninic acid protein assay (Smith et al., 1985), were compared to determine the most reproducible and appropriate assay for the quantification of protein in breadfruit.

Modified Lowry Protein Assay

A modified Lowry assay was conducted on eight cultivars in triplicate using standardized Lowry's reagent. In brief, 40 µl aliquots of three protein extracts per cultivar or each BSA protein standard (0, 0.125, 0.25, 0.5, 1.0, 1.5, 2.0 mg/ml; Fisher Scientific, Ottawa, ON) were incubated in individual wells of a 96 well microplate (BD Scientific, Mississauga, ON) with 200 µl of the modified Lowry reagent (Fisher Scientific, Ottawa, ON). Microplates were covered and mixed for 30 seconds followed by an incubation period of 10 minutes at room temperature.

After the incubation period, 20 µl of 1X Folin-Ciocalteu Reagent (Fisher Scientific, Ottawa, ON) were added to each well with a multi-channel pipettor. The microplate was then covered, mixed for 30 seconds, and incubated at room temperature for 30 minutes. The absorbance of each well at 750 nm was measured using a Synergy HT microplate reader (Biotek, Winooski, VT) and the protein content determined for each replicate sample by comparison to the standard curve run concurrently on each plate.

Pierce 660 nm Protein Assay

The second orthogonal method for protein determination evaluated for suitability for breadfruit samples was the 660 nm protein assay. Three 10 µl aliquots of each protein extract of eight cultivars and BSA protein standard (0, 0.125, 0.25, 0.5, 1.0, 1.5, 2.0 mg/ml; Thermo Fisher Scientific, Ottawa, ON) were transferred into individual wells of a 96 well microplate (BD Scientific, Mississauga, ON). To each well, 150 µl of the protein assay reagent (Fisher Scientific, Ottawa, ON) were added. The plates were mixed at medium speed, incubated at room temperature, and the absorbance at 660 nm was measured using a Synergy HT microplate reader (Biotek, Winooski, VT). The protein contents of the breadfruit samples were quantified based on the standard curve run concurrently on each plate.

Bicinchoninic Acid (BCA) Protein Assay

The BCA assay was initially evaluated in samples from the same eight cultivars for comparison to the modified Lowry and the 660 nm bioassays to select the best method for subsequent analysis. Aliquots of 25 µl from the supernatant of each protein extract and each bovine serum albumin (BSA) protein standard solution (0, 0.125, 0.25, 0.5, 1.0, 1.5, 2.0 mg/ml; Fisher Scientific, Ottawa, ON) were added to 1 ml of the BCA working reagent (1:50 reagent

A:reagent B; Fisher Scientific, Ottawa, ON) in separate 1.5 ml micro-centrifuge tubes (Fisher, Ottawa, ON). The tubes were vortexed (Vortex Genie; Fisher, Walltham, MA) and incubated at 42˚C for 45 minutes in a hot water bath. The tubes were then removed and three 200 µl aliquots from each sample were transferred into a 96 well microplate (BD biosciences, Mississauga, ON). The absorbance of each well was measured at a wavelength of 562 nm using a Synergy HT microplate reader (Biotek, Winooski, VT). On the basis of the preliminary studies, the BCA assay was selected for the detailed comparisons and individual samples of three replicate fruit per cultivar and an additional bulk flour sample from each of the breadfruit flours were analyzed in triplicate using a completely randomized design. The protein content was determined by comparison to a standard curve of varying concentrations of BSA run concurrently on each microplate. A representative breadfruit cultivar was selected as an additional positive control and was quantified on every microplate to detect potential variability and to calculate the %RSD (relative standard deviation) of the assay. The %RSD of repeated extracts of a single sample was 3.5, and the %RSD of repeated extractions of the same breadfruit sample run at different times on different microplates (n=44) was 7.8%.

Statistical Analysis

All statistical analyses were conducted using JMP® 8.0.2 (SAS institute, Cary, NC); see appendix 1-2 for details of analyses. The comparison of orthogonal methods for protein determination in breadfruit was evaluated using a general linear model (GLM) to conduct an analysis of variance (ANOVA) and subsequent Stuident's means separation to determine if the three well established, standardized protein quantification methods produced similar results. On the basis of this preliminary experiment, protein contents were determined for all three fruit of the 94 cultivars in triplicate by BCA quantification (282 samples x 3 analyses per sample = 846

analyses). The BCA protein assay was also used to quantify the protein content of breadfruit flour in triplicate samples. A general linear model was used to conduct ANOVAs to determine if there was significant variation in the protein content among breadfruit cultivars for fresh fruit as well as flour. Similarly, the effects of baking or boiling, and the year-to-year variability in protein content in nine individual cultivars were evaluated using a general linear model to conduct ANOVAs followed by Student's means separations. Statistical differences in fruit morphological characteristics were determined using ANOVAs of the field observations followed by a Student's means separation. All statistical analyses were conducted at a type one error rate of 0.05.

Results

Comparison of Protein Assays

Preliminary experiments were conducted to develop and validate the method for quantification of protein in eight cultivars using triplicate fresh- frozen- freeze dried samples (Figure 5-3). The BCA protein assay was the most reproducible of the three with an average coefficient of variation of 3.5%, compared to 15.2% and 13.6% for the modified Lowry and Pierce 660 nm protein assays, respectively. The average protein content of the eight samples was 3.34% based on the results of the BCA assay, 8.19% using the modified Lowry protein assay, and 0.43% using the Pierce 660nm protein assay. Despite the differences in the estimated protein content determined by the three assays, there was general agreement in the ranking of samples based on their estimated protein content. Protein content of breadfruit pulp reported in the literature using the Kjeldahl method range from 2.2-5.9% on a dry weight basis (Esuoso and Bamiro, 1995; Graham and Negron de Bravo, 1981; Huang et al., 2000; Nochera and Caldwell,

1992; Oladunjoye et al., 2010; Ragone and Cavaletto, 2006; Ravindran and Sivakanesan, 1995). The data demonstrating higher reproducibility and closer agreement with previous studies were used as the criteria for selection of the BCA assay to compare the protein content of the remaining breadfruit samples.

Comparison of Breadfruit Cultivars

Protein Content

The average protein content in the lyophilized fruit of the 94 breadfruit cultivars was 3.9% (SE ± 0.07) on a dry weight basis, or 1.2% (SE ± 0.02) by fresh weight (Table 5-1). Within the population there were significant differences among individual accessions in protein content, ranging from 2.7% to 6.2% by dry weight, or 0.9% to 1.9% by fresh weight. The protein content of fresh-frozen-freeze dried fruit collected in triplicate from the same trees over two seasons was not significantly different for any of the eight cultivars evaluated (see appendix 1). The five cultivars with the highest protein contents by dry weight are "Uto ni viti" from Fiji (6.2% ±0.94), "Ulu elise" from Tokelau (5.4% ± 0.45), "Yellow" from the Seychelles (5.3% ± 0.13), "Mahani" from the Society Islands (5.1 % ± 0.29), and "Meiarephe" from Pohnpei (5.0% ±0.68). There were no significant differences in the protein content between *Artocarpus altilis* and *A. altilis* × *A. mariannensis* hybrids, or seeded and seedless accessions (see appendix 1). The seeds of the three accessions of breadnut (*A. camansi*) had an average protein content of 13.1% (SE ± 0.31) on a dry weight basis. This compared to previous reports of 12.5-19.9% protein by weight in the seed of *A. camansi* (Quijano and Arango, 1979; Negron de Bravo et al., 1983; McIntosh and Manchew, 1993; Ragone, 1997; Adeleke and Abiodun, 2010). The pulp of

the single accession of *A. mariannensis* included in the study had a protein content of 5.5% (SE ± 0.28) by dry weight.

Breadfruit flour contains significantly less protein than fresh fruit with an average protein content across all cultivars of 3.0% (SE ± 0.09) on a dry weight basis (Table 5-1). The protein content of flour ranged from 1.7% - 7.6% depending on the accession from which it was made. The five accessions that were found to have the highest protein content in flour were "Ma'afala" from Samoa (7.6%), "Ulu fiti" from Fiji (5.6 %), "Samoan 1" collected from Fiji (4.5%), and an unidentified cultivar (Grid ID KM3) (4.5%), and "Meinpadahk" from Pohnpei (4.1%). As was observed in the fresh-frozen-freeze dried replicated fruit samples, there was no statistically significant difference in protein content between *Artocarpus altilis* and *A. altilis* × *A. mariannensis* hybrids, or between seeded and seedless accessions.

Fruit that were boiled or baked had significantly lower protein content than the fresh fruit, but were not significantly different from one another (Figure 5-4). The average protein contents of the eight cultivars that were boiled and baked were 2.4% (SE ± 0.32) and 2.8% (SE ± 0.48) respectively, compared to 4.2 % (SE ± 0.46) for the fresh fruit.

Fruit Morphology

There was significant variation in all evaluated fruit morphological characteristics among the 94 cultivars and related accessions (Table 5-1; Figure 5-2). The average fruit weight was 1.60 kg (SE ± 0.055), however, there was more than a seven fold difference between the smallest cultivar, "Ulu afa" with an average fruit size of 0.47 kg (SE ± 0.066), and the largest cultivar, "Lipet", with an average fruit weight of 3.54 kg (SE ± 0.039). The variation in fruit weight was

accompanied by corresponding differences in fruit length and width ranging from 11.9-31.1 cm and 8.8-18.0 cm, respectively. The average size of the core as a percentage of the total fruit was 7.8% (SE ± 0.29), but depending on cultivar ranged from 4.7% (SE ± 0.17) to 17.9% (SE ± 2.36). The peel accounted for 7.8% (SE ± 0.19) of the fruit on average, but ranged from 5.4 (SE ± 1.74) to 15.6% (SE ± 2.03). The majority of cultivars evaluated were seedless, however, for the 25 seeded cultivars, seeds accounted for an average of 4.7% (SE ± 0.48) of the fruit and ranged from 0.8% (SE ± .33) to 10.6% (SE ± 2.11). After the skin, core, and seeds were removed, the remaining portion of the fruit is the starchy pulp which was used to produce the flour. On average, the fruit pulp obtained from an individual fruit weighed 1.35 kg (SE ± 0.051) but depending on cultivar ranged from 0.30 kg (SE ± 0.063) to 3.11 kg (SE ± 0.047). As a proportion of the total fruit weight, the pulp represents an average of 83.0% (SE ± 0.64) of the fruit, but can vary between 60.8 (SE ± 1.80) and 89.6% (SE ± 1.47) depending on the cultivar. The average moisture content of the pulp is 68.0% (SE ± 0.28) and ranges from 60.7 (SE ± 2.38) to 73.7% (SE ± 1.34).

Discussion

Breadfruit has long been recognized for its potential to enhance food security throughout the tropics, yet it remains an underutilized crop in most regions (Banks, 1962; Ragone, 1997). This is in part due to the highly seasonal nature of the crop (Chapter 4) and the highly perishable qualities of the fruit (Worrell et al., 2002) that restrict breadfruit to a short season in a given region. Several authors have identified the potential of processing breadfruit into flour (Arcelay and Graham, 1984; George et al., 2007; Wootton and Tumaalii, 1984) or extracting the starch for industrial purposes to increase its utility (Adebayo and Itiola, 2003; Adebayo et al., 2008;

Adebowale et al., 2005). Breadfruit flour has been successfully used in various recipes including stiff porridges (Mayaki et al., 2003), infant formulas (Esparagoza and Tangonan, 1993), extruded products (McHugh et al., 2007), bread (Ayodele and Oginni, 2002; Esuoso and Bamiro, 1995; Nochera and Caldwell, 1992), cake (Ayodele and Oginni, 2002), pancakes (Ayodele and Oginni, 2002) and biscuits (Nnam and Nwokocha, 2003; Olaoye et al., 2007; Omobuwajo, 2003). The starch extracted from the fruit has similar properties to corn flour and has potential industrial applications such as in the pharmaceutical industry as a binder (Adebayo and Itiola, 2003; Adebayo et al., 2008; Adebowale et al., 2005). However, there are hundreds of known breadfruit cultivars and very little information regarding the differences in the fruit qualities have previously been documented. The current study provides an in-depth comparison of protein content, fruit size, and composition of 94 cultivars of breadfruit to identify those with higher potential for development of food products.

Protein is an important macro-nutrient required in the human diet and is vital for optimal health. Protein-energy malnutrition is generally accompanied by an overall lack of food and leads to various symptoms including wasting and stunted growth (Sukhatme, 2007). On average, breadfruit is lower in protein than wheat, rice, or corn, but compares favourably with many staple foods commonly cultivated in the humid tropics (Dignan et al., 2004). For example, on a dry weight basis breadfruit typically contains more protein than banana (2.8% dw), cassava (2.75%), or sweet potato (3.6%), but less than taro (8.8%) (Dignan et al., 2004). However, protein-rich cultivars identified in the current study compare more favourably and could be used to produce relatively protein-rich products. Flour made from the cultivar “Ma’afala” contained 7.6% protein, which is similar to rice (7.4%), and higher than most of the tropical staples. Additionally, breadfruit flour is gluten free and can be used in a variety of processing techniques

that would normally utilize wheat. It has been estimated that 1 in 133 people in the USA may have gluten allergies (Fasano et al., 2003) and there is a significant need for gluten-free alternative foods which may be an ideal market for the higher protein breadfruit flours. However, further research would be required to optimize large-scale processing and the development of novel food products from traditional recipes.

Another factor that contributes to the final protein content of breadfruit flour is the processing methods utilized. Graham and Negron de Bravo (1981) found that the pulp of the fruit used in their study contained 3.8-4.1% protein while the peels contained 4.6-5.9% protein and the core contained 6.0-7.6% protein. The skin and pulp are also higher in fibre, total ash, and fat content (Graham and Negron de Bravo, 1981). Breadfruit seed is similar in nutritional composition to that of breadnut and has been reported to contain between 15-20% protein by dry weight, is higher in fat than the pulp, and is richer in some minerals (Ragone, 1997). The inclusion or exclusion of the various parts of the fruit would enable the processor to alter the chemical and physical properties of the flour, somewhat analogous to whole wheat vs white flour. Further research is required to determine the effect that these factors will have on the various end products and to determine the optimal processing methods.

The ideal fruit characteristics for flour production will depend to a large extent on the processing methods used. The majority of studies to date have discarded the peel, core, and seeds, producing the flour exclusively from the fruit pulp (Arcelay and Graham, 1984; Ayodele and Oginni, 2002; Esparagoza and Tangonan, 1993; Esuoso and Bamiro, 1995; George et al., 2007; Mayaki et al., 2003; Nnam and Nwokocha, 2003; Nochera and Caldwell, 1992; Olaoye et al., 2007; Omobuwajo, 2003; Oshodi et al., 1999; Ravindran and Sivakanesan, 1995; Sharon and Usha, 2006). For this purpose, a large seedless fruit with a high proportion of fruit pulp would

be highly desirable. The fruit criteria would be much different if the skin, core, and/or seeds were included in the flour, which would increase the levels of several nutrients (Graham and Bravo, 1981). In this case, a fruit with a higher proportion of peel, core and/or seeds may be desirable. In either case, high protein content and low moisture content would be ideal. The specific fruit characteristics of six cultivars selected for various traits are shown in Table 5-2.

A high degree of variability in fruit characteristics was found among the many cultivars of breadfruit conserved in the NTBG breadfruit germplasm repository. Many of these traits, such as protein content, fruit size, and fruit composition, will make cultivar selection an important step in the future development of cultivars for the food industry. It is expected that products may be produced as fresh-frozen fruit and fruit flours. The identification of cultivars with greater potential for food product development is essential to the creation of a breadfruit flour industry, will enhance food security in the tropics, and could develop new healthy alternative food products for other parts of the world. While a number of promising cultivars have been identified in the current study, the ideal type of breadfruit will ultimately depend on the application and will need to be evaluated on a case by case basis.

Table 5-1: Distribution of fruit morphological characteristics and protein content among 94 cultivars of breadfruit (*Artocarpus altilis* and *A. altilis* × *A. mariannensis* hybrids).

	n	Average	Standard deviation		Minimum				Maximum			
					Cultivar	n	Average	SEM[2]	Cultivar	n	Average	SEM[2]
Fruit Length (cm)	94	17.6	3.06		Atu	10	11.9	0.38	Yap	10	31.5	0.64
Fruit Width (cm)	94	14.0	1.67		Ulu elise	10	9.2	0.35	Ulu fiti	10	17.8	0.34
Core length (cm)	94	10.9	2.68		Atu	10	5.9	0.26	Yap	10	24.7	0.68
Core width (cm)	94	4.1	0.69		Mei arephe	10	3.3	0.08	Uto Samoa	10	6.1	0.18
Whole fruit weight (g)	94	1606.9	536.18		Ulu afa	3	477.3	65.98	Lipet	3	3540.7	39.28
Fruit pulp weight (g)	94	1351.4	495.37		Ulu afa	3	302.0	62.92	Lipet	3	3109.3	47.14
Percentage pulp weight	94	83.0	6.17		Ulu elise	3	60.8	1.80	Apu	3	89.6	0.25
Percentage skin weight	94	7.8	1.88		Maopo	3	5.4	1.74	Ulu afa	3	15.6	2.03
Percentage core weight	94	7.8	2.80		Apu	3	4.7	0.17	Ulu afa	3	17.9	2.36
Seed Number[1]	94	4.1	1.97		Puurea	10	0.7	0.70	Samoan	10	8.0	0.87
Percentage seed weight[1]	94	4.7	2.38		Mei kole	3	0.8	0.33	Ulu elise	3	10.6	2.11
Moisture content (%)	94	68.0	2.73		Ulu fiti	3	60.7	2.38	Meinpohnsakar	3	73.7	1.34
Fruit protein content (% dw)	94	3.9	0.88	a	Tuutou ooa	3	2.7	0.09	Uto ni viti	3	6.2	0.94
Fruit protein content (% fw)	94	1.2	0.19	c	Tuutou ooa	3	0.9	0.04	Uto ni viti	3	1.7	0.26
Flour protein content (% dw)	94	3.0	0.90	b	Siviri 3	3	1.7	0.10	Ma'afala	3	7.6	0.24

[1]These values have been calculated using only the seeded cultivars (n=25)

[2]SEM=standard error of the mean

Table 5-2: Fruit morphological characteristics, protein content, species, island of origin, and images of six breadfruit (*Artocarpus altilis* and *A. altilis* x *A. mariannensis* hybrids) cultivars selected for their potential for the food processing industry. Standard errors of the means (SEMs) followed by different letters represent that the cultivars are significantly different for that trait at a p=0.05. Photos © Jim Wiseman.

		Cultivar											
		Ulu fiti		Ma'afala		Lipet		Uto ni viti		Yellow		Mahani	
Image of whole fruit													
Image of half fruit													
[1]Species		*A.a*		*A.a*		*A.a* x *A.m*		*A.a*		*A.a*		*A.a*	
Country of origin		Fiji		Samoa		Pohnpei		Fiji		Seychelles		Society islands	
Accession number		900260001		770519001		910270001		900264001		810289002		800269001	
	n	Average	[2]SEM	Average	[2]SEM	Average	[2]SEM	Average	[2]SEM	Average	[2]SEM	Average	[2]SEM
Fruit Length (cm)	10	17.3	0.46 b	14.1	0.35 c	22.2	0.24 a	22.5	0.48 a	13.6	0.42 c	17.9	0.23 b
Fruit Width (cm)	10	16.1	0.44 bc	11.1	0.26 d	17.6	0.51 a	15.2	0.41 c	12.1	0.31 d	16.6	0.50 ab
Core length (cm)	10	10.9	0.32 c	9.2	0.33 d	14.0	0.27 b	16.2	0.49 a	7.8	0.32 e	9.7	0.22 d
Core width (cm)	10	5.9	0.25 a	3.3	0.08 cd	3.6	0.10 c	5.5	0.23 ab	3.0	0.15 d	5.0	0.31 b
Whole fruit weight (g)	3	1260.3	227.23 cd	1156.7	92.04 d	3540.7	39.28 a	1612.0	63.45 c	1575.0	61.45 c	2499.0	195.39 b
Fruit pulp weight (g fw)	3	908.0	158.02 d	951.0	70.47 d	3109.3	47.14 a	1196.3	57.62 cd	1366.3	55.57 c	2192.7	169.47 b
Fruit pulp weight (g dw)	3	357.1	62.14 c	330.5	24.49 c	899.8	13.64 a	337.4	16.25 c	366.9	14.92 c	684.4	52.90 b
Percentage pulp weight	3	72.3	2.02 c	82.3	1.17 b	87.8	0.35 a	74.2	0.72 c	86.7	0.15 a	87.8	0.67 a
Percentage skin weight	3	8.4	1.20 a	6.0	0.68 b	6.1	0.22 b	7.1	0.88 b	6.7	0.39 b	6.3	0.38 b
Percentage core weight	3	13.5	2.79 a	10.3	1.04 b	6.1	0.40 c	13.5	0.49 a	6.6	0.02 c	5.9	0.90 c
Seed count	10	5.0	0.68 a	0.9	0.18 b	0	0 b	4.1	0.94 a	0	0 b	0	0 b
Percentage seed weight	3	6.1	1.91 a	1.5	0.65 b	0	0 b	5.2	0.17 a	0	0 b	0	0 b
Moisture content (%)	3	60.7	2.38 d	65.3	1.27 cd	71.1	0.74 a	71.8	0.85 a	73.1	2.42 ab	68.8	0.73 bc
Fruit protein content (% dw)	3	4.1	0.19 bc	3.3	0.51 c	4.3	0.27 bc	6.2	0.94 ab	5.3	0.13 a	5.1	0.29 a
Fruit protein content (% fw)	3	1.6	0.07 a	1.1	0.18 c	1.3	0.08 b	1.7	0.26 ab	1.4	0.03 ab	1.6	0.09 a
Flour protein content (% dw)	3	5.6	0.19 b	7.6	0.24 a	3.8	0.18 c	4.3	0.20 c	4.0	0.09 c	3.9	0.27 c

[1]*A.a* = *Artocarpus altilis*, *A.a* x *A.m* = *A. altilis* x *A. mariannensis* hybrid

[2]SEM = Standard error of the mean

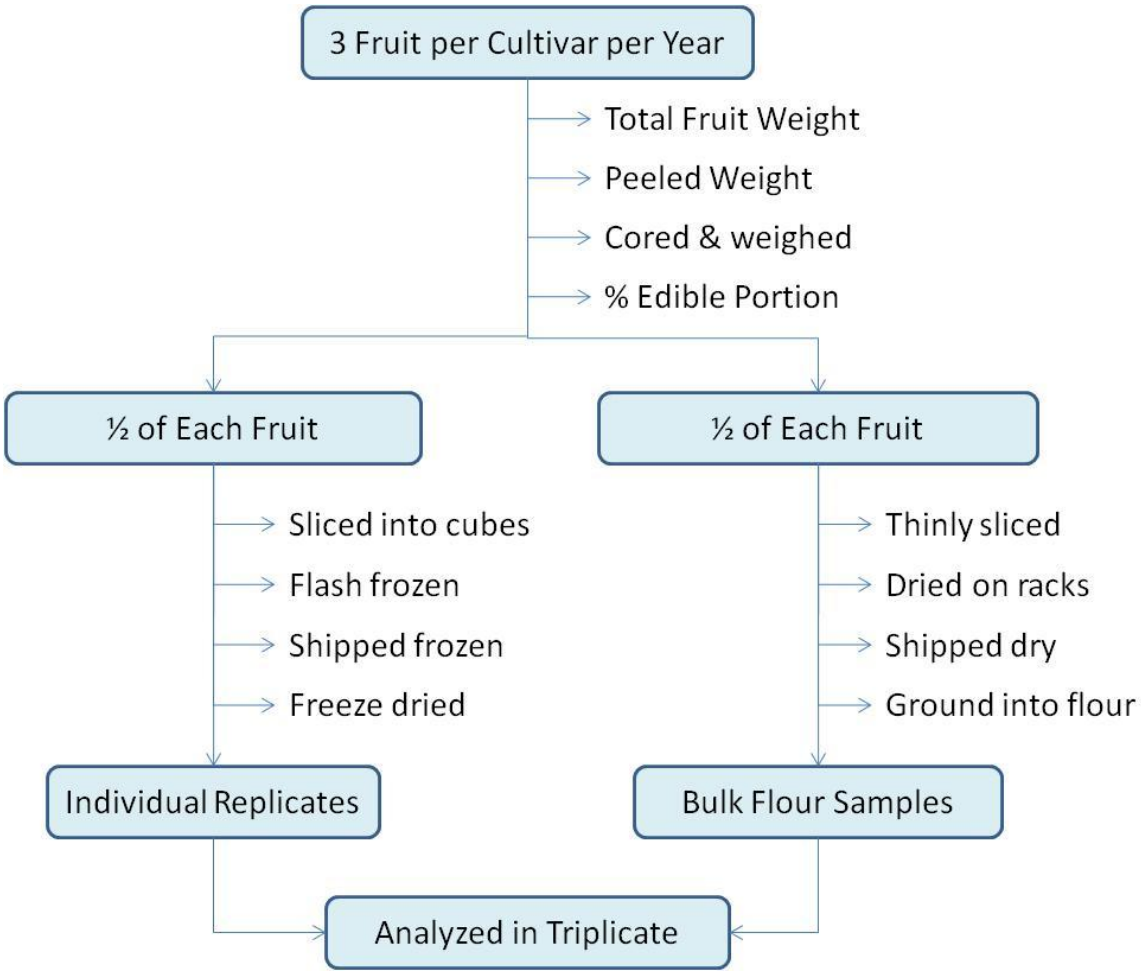

Figure 5-1: Experimantal design of the experiment and harvest scheme for collection of triplicate samples and preparation of bulk flour samples for each of 94 cultivars of breadfruit (*Artocarpus altilis*) and related species.

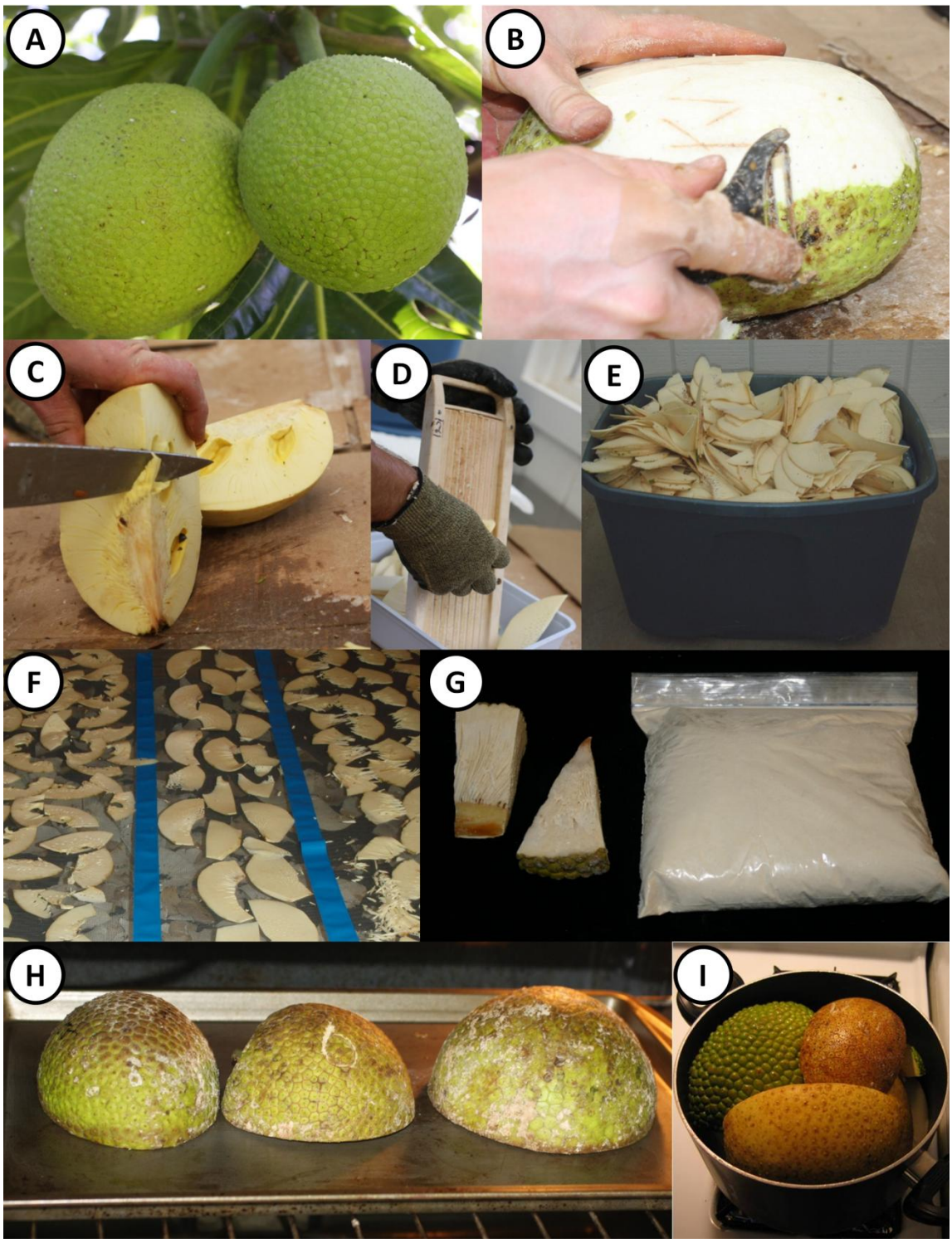

Figure 5-2: Maturation, harvest and processing of breadfruit (*Artocarpus altilis*) and related species. A: Maturing breadfruit (*Artocarpus altilis*) growing on the tree, B: fruit being peeled using a household vegetable peeler, C: the core being removed from a peeled and quartered breadfruit, D: slicing the fruit using a mandolin, E: a container full of sliced breadfruit, F: slices of breadfruit drying on screen trays in a drying room, G: a bag of ground breadfruit flour next to two wedges of fresh fruit, H: halved breadfruit being roasted in an oven, and I: three cultivars of breadfruit being boiled.

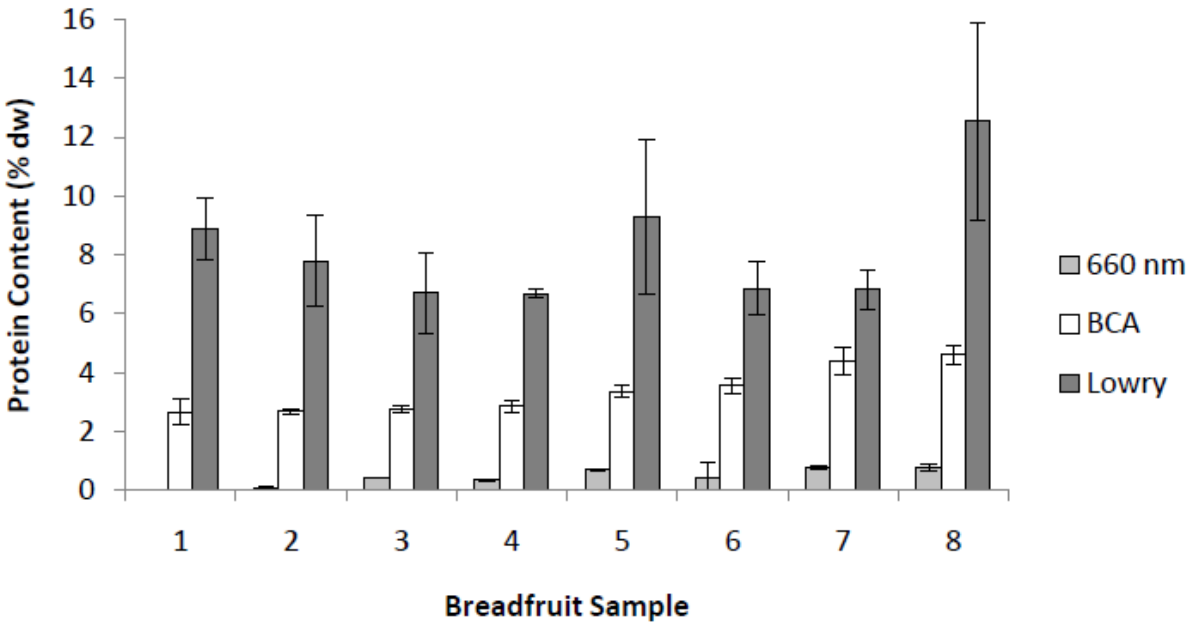

Figure 5-3: Evaluation of protein content of 8 samples of breadfruit (*Artocarpus altilis*) evaluated using three colorometric protein assays, the BCA protein assay, the modified Lowry protein assay, and the Pierce 660 nm protein assay. Bars represent the mean protein estimates of the 8 cultivars, error bars show the standard error of the mean.

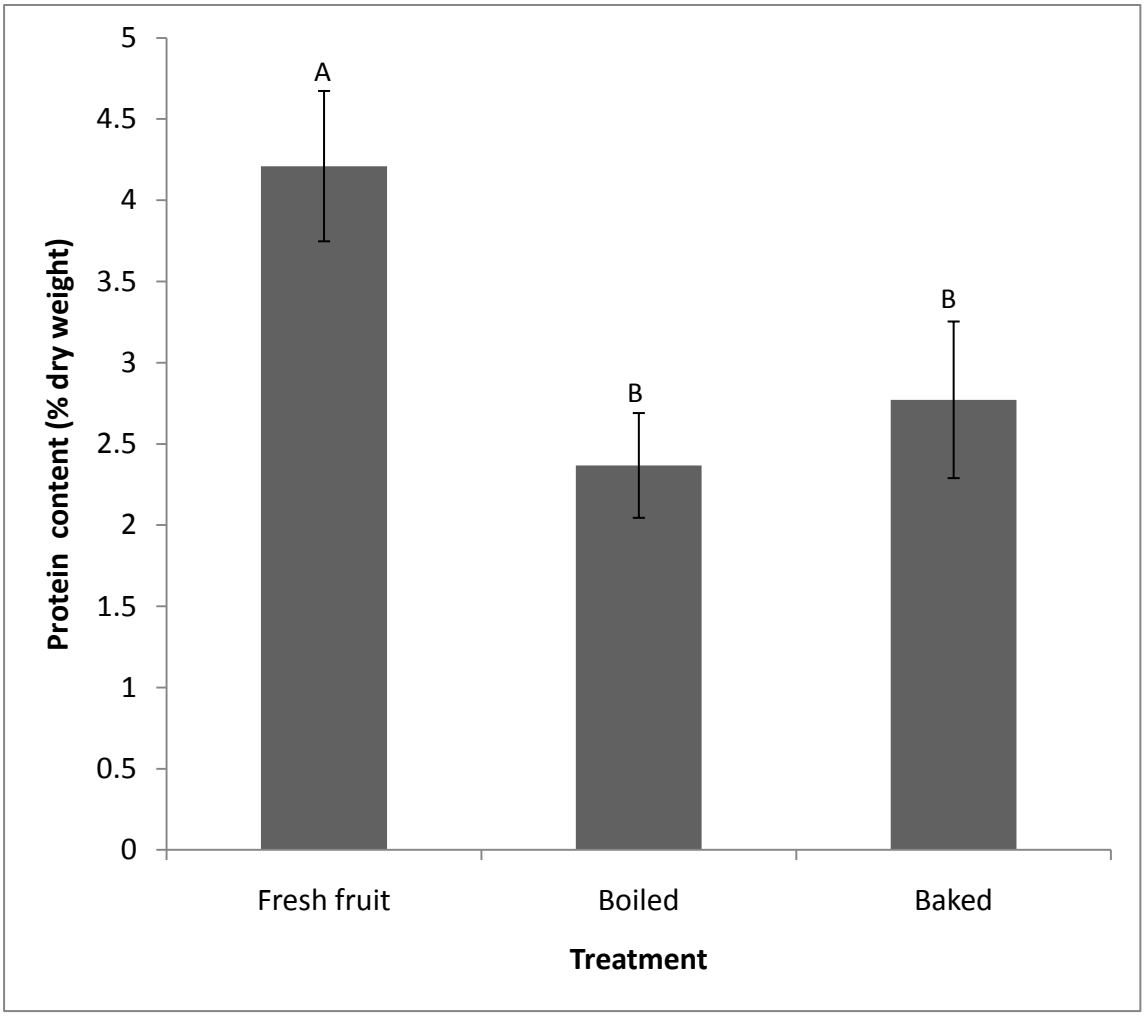

Figure 5-4: The effect of baking and boiling on the protein content of breadfruit, *Artocarpus altilis*. Bars represent the mean protein content of eight cultivars using the BCA protein assay, error bars display the standard error of the mean. Bars with different letters above them are significantly different with a type one error rate of 0.05.

Chapter 6: Breadfruit (*Artocarpus altilis*): An Underutilized Crop with Potential to Address the 'Hidden Hunger' of Micronutrient Deficiency [5]

Introduction

Micronutrient deficiencies are often referred to as "the hidden hunger" and affect more than two billion people worldwide (UNSCN, 2004). While this is a global concern, it is disconcertingly prevalent in developing nations where it contributes to substantially increased rates of mortality and morbidity, especially among women and children (UNSCN, 2004). Diet-related factors such as a lack of variety of nutrient-rich foods coupled with inadequate health care and sanitation, disease, and a lack of education in infant and childcare have resulted in the current estimates of 148 million children under the age of 5 in the developing world suffering from the effects of under-nutrition (CIDA, 2009). Consequences of poor micronutrient nutrition include increased susceptibility to disease, stunted growth, decreased mental capacity and, in extreme cases, death (UNSCN, 2004). Iron deficiency anaemia during pregnancy alone is associated with 115,000 deaths each year, accounting for one fifth of total maternal deaths (CIDA, 2009). The development of food crops rich in mineral micronutrients has enormous potential to reduce the prevalence and impact of micronutrient malnutrition.

[5] A version of this chapter has been submitted for publication and is currently under review: Jones, A.M.P., Regaone, D., Aiona, K, and Murch, S. J. Breadfruit (*Artocarpus altilis*): An underutilized crop with potential to address the 'hidden hunger' of micronutrient deficiency. The Journal of Food Analysis and Composition

Breadfruit, *Artocarpus altilis* (Parkinson) Fosberg, is a high yielding, low input staple crop cultivated throughout the wet tropics (Ragone, 1997). Conservative estimates suggest that yields up to 6 tonnes/hectare (t/ha) dry weight are achievable for breadfruit (Bowers, 1981) as compared to global average yields of 4.1 t/ha for rice, 4 t/ha for corn, and 2.6 t/ha for wheat (Calpe, 2007; FAO, 2009c, 2009d). Previous studies indicate that breadfruit and the closely related breadnut are good sources of copper (Cu), magnesium (Mg), phosphorous (P), and potassium (K), calcium (Ca), iron (Fe), and manganese (Mn) (Adeleke and Abiodun, 2010; Englberger et al., 2003a; Huang et al., 2000; Morton, 1987; Oshodi et al., 1999; Ragone, 1997; Ragone and Cavaletto, 2006; Williams and Badrie, 2005; Wootton and Tumaalii, 1984). However, these studies represent only a small number of breadfruit cultivars from heterogeneous sites throughout the tropics.

The Breadfruit Institute germplasm repository in the Kahanu Garden, part of the National Tropical Botanical Garden (NTBG) in Kauai and Maui, Hawaii, USA holds the world's largest collection of mature, fruit-bearing, breadfruit trees and currently encompasses 270 trees representing more than 120 cultivars collected from 34 Pacific Islands, Indonesia, the Philippines, and the Seychelles. The collection is restricted to a total area of less than 3 ha and is managed using minimal, sustainable, and traditional strategies (Figure 6-1A). The trees produce a large, fleshy fruit (Figure 6-1B) that may be seedless or may contain a few edible, high protein seeds, depending upon the cultivar (Figure 6-1C, D). The related species, breadnut (*Artocarpus camansi*), normally has minimal fruit flesh and numerous edible seeds that are boiled or roasted (Figure 6-1E). Wider distribution of fresh breadfruit and breadnut is limited by very short shelf life and susceptibility to damage in transport. The few seeded and seedless cultivars of breadfruit

have been clonally selected and propagated by the indigenous peoples of Oceania for centuries (Ragone, 1997). These cultivars are ideal for the production of flour (Figure 6-1F, G, H) which can be stored longer than fresh fruit and used in processed foods, infant food, baked goods, or porridges (Ayodele and Oginni, 2002; Esparagoza and Tangonan, 1993; Esuoso and Bamiro, 1995; Nnam and Nwokocha, 2003; Olaoye et al., 2007; Omobuwajo, 2003).

The objectives of the current studies were to conduct a comparison of the mineral content of an extensive collection of breadfruit cultivars growing in a uniform environment. The specific goal was to identify a small group of elite cultivars with higher than average micronutrient contents that would be suitable for large scale propagation and distribution to tropical regions to improve nutrition and food security.

Materials and Methods

The Breadfruit Germplasm Repository at Kahanu Garden of the NTBG

Within the breadfruit collection, the majority of trees are planted in a grid pattern and the trees are labeled alphanumerically with a smaller group of trees planted in a neighboring area in a more sporadic arrangement and labeled numerically (Figure 6-2). The trees used in this study were planted between 1978 and 2004 at 20°47'57.07"N, 156°02'18.42"W at 15 m elevation with a mean maximum temperature of 27.1°C, mean minimum temperature of 19.7°C and a mean annual precipitation of 2051mm (Western Regional Climate Center; http://wrcc.dri.edu/). The collection was fertilized with 6-6-6 commercial fertilizer at 0.91 kg / tree in February 2007 but not fertilized during the 2

year fruit collection period. The soil is classified as Hana Very Stone Silt Clay Loam (http://websoilsurvey.nrcs.usda.gov) derived from volcanic ash. It is generally well draining, slightly – moderately acidic, and contains about 8% organic matter in the surface horizon. Root-restrictive obstructions typically occur at a depth of about 1.5m and the soil surface layer covers deep lava.

Soil Analysis

Soil was sampled at three separate locations within a 3.66m radius and pooled to represent each location indicated by letters in Figure 6-2. The samples were analyzed at the College of Tropical Agriculture & Human Resources' (CTAHR) Agricultural Diagnostic Service Center (Honolulu, HI) for organic matter content, pH, cation exchange capacity, total nitrogen (N), plant micro- and macro-nutrients.

Breadfruit Sample Collection

As part of a larger nutritional study, three fruit were collected from each of the 94 breadfruit cultivars (*Artocarpus altilis* or *Artocarpus altilis* × *Artocarpus 114yophilized114*) and two related species (*Artocarpus mariannensis* and *Artocarpus camansi*) evaluated in this study from November, 2008 to March, 2009. A subset of nine cultivars was collected again in December 2009 to evaluate inter-seasonal variability as described in detail in Chapter 5. Briefly, three fruit from each cultivar were collected at the mature, but not yet ripe stage of development (Worrell et al., 1998), the stem was removed and the fruit left inverted for about 1 h to allow the latex to drain/coagulate. The three replicate fruits were peeled using a household vegetable peeler, cut into quarters,

the seeds and cores removed. One half of each of the three fruits was cut into 5-10 cm cubes, frozen and shipped back to the University of British Columbia where they were stored at -86°C until freeze drying and replicated individual analysis to determine variation among the 94 cultivars. The remaining half of each of the three fruit per cultivar was sliced into approximately 2 mm sections using a mandolin vegetable slicer (Benriner, Philadelphia, PA) and dried in the dark on screen trays at 22-47°C and 19-47% RH before shipping to UBC (Figure 6-2). The dried fruit slices were ground into a bulk flour sample using the finest setting of a commercial coffee grinder (Bunn® model G3, Aurora, ON) for comparison to bulk commercial flour samples of wheat, rice and corn flours.

Cereal Flours

Bulk flour samples of wheat (*Triticum* sp.), rice (*Oryza sativa* L.) and corn (*Zea mays* L.) were obtained from the following commercial sources and were included as reference materials: All Purpose Whole Wheat Flour (Smucker Foods of Canada Co., Markham, ON; Lot number 8 211 548 20:26 3512), Fortified All Purpose White Flour (Smucker Foods of Canada Co., Markham, ON; lot number 9 090 548 10:07 3577), Suraj White Rice Flour (Loblaws Inc., Toronto, ON; Lot number 9051) and Unico Corn Flour (Unico, Concord, ON). A certified analytical standard reference wheat flour was also included to evaluate the accuracy of the analytical methods (National Institute of Standards and Technology (NIST), Gaithersburg, MD; SRM number 1567a).

Dry Matter

Moisture contents of breadfruit, rice, corn and wheat flours were determined in triplicate using the American Association of Cereal Chemists (AACC) method 44-19(AACC, 2000). Briefly, 2 grams of each flour sample were measured in a pre-weighed 65 mm aluminum weighing dish (Fisher Scientific, Ottawa, ON) and dried for 2 h in an Isotemp programmable muffle furnace (Fisher Scientific, Ottawa, ON) at 135°C and the difference in weight was used to calculate the final percent moisture.

Total Ash Analysis

Total ash content was determined as described in AACC method 08-01 (AACC, 2000). Approximately 3-5 g of each breadfruit flour sample and commercial flour sample were weighed in triplicate in pre-weighed porcelain crucibles (Coorstek, Golden, CO) that had been previously dried and stored in desiccators, incinerated in an Isotemp programmable muffle furnace (Fisher Scientific, Ottawa, ON) at 550°C for 24 h, removed from the furnace and re-weighed to determine the total mineral contents.

ICP-OES Sample Preparation

All of the resulting ash samples were dissolved in 3ml of 37% analytical grade HCl (Thermo Fisher Scientific, Waltham, MA). The solutions were quantitatively transferred to 25 ml volumetric flasks and brought to volume using filtered water (Millipore, Billerica, MA). The samples were transferred to 25 ml polypropylene centrifuge tubes (Corning Inc., Corning, NY) until analysis. For analysis, 10 ml aliquots of the samples were transferred into 15 ml polypropylene centrifuge tubes (Corning Inc.,

Corning, NY). An additional 1:19 dilution was prepared from each sample using 0.3 N analytical grade HCl for the quantification of Ca, K and P. Yttrium was added to each sample to achieve a final concentration of 200 ppm as an internal standard.

Mineral Content Determination by ICP-OES

Mineral analysis was conducted by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a modified version of the AACC method 40-75 on a iCAP 6000 series ICP spectrometer (Thermo, Waltham, MA). The following elements were quantified using ICP grade standards: Ca, cobalt (Co), K, Mg, sodium (Na) (Fisher Scientific, Ottawa, ON), Cu (Fluka, Buchs, Switzwerland), Fe (VWR, Westchester, PA), Mn (Accustandard, New Haven, CN), P (Labchem, Pittsburg, PA) and zinc (Zn; Sigma-Aldrich, St. Louis, MO). All standards were prepared in 0.3N analytical grade HCl (Thermo Fisher Scientific, Waltham, MA) prepared with Direct-Q filtered water (Millipore, Billerica, MA) in acid-washed plastic vessels (Nalgene, Rochester, NY). The standard curves were calculated using five concentrations of each element shown in Table 6-1, as well as a blank. Yttrium was added to all standard solutions to a final concentration of 200 ppm as an internal standard. The emission wavelengths used to detect the various elements and other analytical parameters are reported in Table 6-1.

Data Analysis

All statistical analyses were conducted using JMP®8.0.2 (SAS Institute, Cary, NC); see appendix 1-3 for details of analyses. A general linear model was used to conduct a MANOVA with species as the independent variable and mineral contents as

the dependent variables to determine significance of the overall model. Another MANOVA was run using individual elite cultivars and cereals as the independent variable with mineral contents as the independent variables. A series of ANOVAs were conducted to determine if there were significant differences in the concentrations of each mineral among breadfruit species and the commercial bulk flour samples as well as among elite cultivars and the cereal flours. Additional statistical analyses using ANOVAs compared fruits of cultivars growing in two subplots within the plantation that had slightly different soil mineral contents based on a Jackknifed distance outlier test, and to assess the variability between seasons for nine cultivars harvested in two sequential years. When the ANOVAs indicated there were significant differences, a Student's means separation was conducted. All statistical analyses were conducted with a type 1 error rate of 0.05.

Results

Developing Methods for Production of Breadfruit Flours

The first objective of the current study was the development of optimized methods for production of flours from a wide variety of breadfruit cultivars. Field observations and local 118yophilized118118ns indicated that production of flour from fresh fruit required draining the sticky latex from the fruit for at least an hour prior to peeling and slicing to prevent discoloration (Figure 6-1F, G). Each individual fruit was cut in half with one half processed as frozen replicate samples and the other half thinly sliced for bulk flour samples. Several different methods of drying were compared in preliminary experiments. A solar-powered dehydrator was only capable of drying small

quantities of fruit and lacked the capacity to produce large volumes of flour. Slices dried in full sun tended to brown and the lack of covering increased the risk of damage by rainfall and insects. Eventually, a system of drying on wire racks in the dark with adequate ventilation, a dehumidifier and fans was developed. Slices were dried for 48-72 h to a hard, crisp texture suitable for grinding at a temperature ranging from 22-47˚C and a relative humidity of 19-67%. A variety of flour mills were tested for grinding the samples into flour with limited success since the dried fruit is relatively soft and burned or gummed up impact flour mills and other grinder styles. The finely ground flours used in the analysis were produced using a commercial scale coffee grinder (Figure 6-1H).

Site and Soil Characteristics

Soil samples were analyzed at six locations throughout the breadfruit collection representing five collections within the main grid-patterned section of the trees (locations A-E) and one collection from a smaller adjacent area (location H) (Table 6-2). There was no significant difference in the soil pH or % total nitrogen across the collection sites but the smaller area (H) had higher total organic matter in the soil, greater amounts of P, Ca, Mg, Zn, Cu, aluminum (Al), with lower amounts of Co, chromium (Cr), 119yophi (Ni), lead (Pb) and selenium (Se) than were found in soil samples A-E from the collection grid (Figure 6-2; Table 6-2).

Quantification of Minerals in Breadfruit and Cereals

Accuracy and precision of the analyses of mineral contents in breadfruit cultivars and breadnut accessions were determined by replicated validation experiments. The

limits of detection, limits of quantification and linearity of the ICP-OES response were determined with authenticated standards and NIST certified reference materials (Table 6-1). The R^2 values for the quantification range of standards were > 0.999 for all 10 elements (Table 6-1). The mineral content of the NIST wheat Certified Reference Standard was determined to be within 10% of the known values for Ca, Cu, Mg, Mn, Na, P, and Zn. The recovery of Fe was 56.2% and for K, 73.2% of the expected NIST certified reference material potentially indicating that the data may be an underestimation of the total amounts of these two minerals. The data were not corrected and were consistent across repeated preparations of identical biological samples (RSD = 6.3% Fe, and 3.0% K for NIST samples, n=5) (Table 6-1).

Moisture, Ash and Mineral Content of Breadfruit

Studies were undertaken to determine the capacity for flour produced from breadfruit as a source of mineral nutrition comparable to cereal flours. There were no significant differences in the moisture content between breadfruit flours (Table 6-3). Breadfruit flour contained significantly more total ash than whole wheat flour, rice flour, refined wheat, or corn flour (Table 6-3). Breadfruit flour also had significantly more Ca, Fe, K, and Mg, and was comparable in P to the unfortified refined wheat, rice and corn flour (Table 6-3). Breadfruit flour was higher in Co than wheat and corn, but similar to rice flour, and lower in Mn and Zn than the cereal flours with the exception of corn. When breadfruit flour from 94 different cultivars was compared to whole wheat flour (Table 6-4), the breadfruit flour produced was significantly higher in Ca and K, comparable in Co and Cu, and significantly lower in Fe, Mg, Mn, P, and Zn. Flour

produced from the cultivar ‘Meion’, contained the highest amount of iron, equivalent to whole wheat flour and approaching the amount found in fortified wheat flour. The highest amounts of potassium were in the cultivar of *A. altilis* ‘Tuutou’ (16212 µg/g DW) and the hybrid *A. altilis* × *A. mariannensis* cultivar ‘Meinpohnsakar’ (15345 µg/g DW). Significantly more calcium (1491 µg/g DW), magnesium (2281 µg/g DW) copper (5.6 µg/g DW), and cobalt (31 ng/g DW) were found in the *A. altilis* cultivar ‘Ulu Fiti’ than any of the other cultivars, wheat, corn or rice flours. The cultivar ‘Ulu Fiti’ also had significantly more manganese (3.8 µg/g DW) than the other breadfruit varieties but less than the *A. mariannensis* (5.3 µg/g DW), refined wheat (5.7 µg/g DW), rice (18.2 µg/g DW) or whole wheat (31.8 µg/g DW) flours. Similarly, significantly more phosphorous was quantified in ‘Ulu Fiti’ (2379 µg/g DW) than in the other breadfruits, but less than was found in *A. camansi* (3017 µg/g DW) or whole wheat flour (4802 µg/g DW).

The hybrid cultivars (*Artocarpus altilis* × *Artocarpus 121yophilized121*) had significantly higher moisture content in fresh fruit than non-hybrid cultivars (*Artocarpus altilis*). Among all breadfruit cultivars (*A. altilis* and hybrids inclusive) the concentration of minerals varied by factors of 5.3 (Ca), 8.7 (Co), 5.1 (Cu), 5.5 (Fe), 2.1 (K), 3.6 (Mg), 4.2 (Mn), 96.7 (Na), 2.8 (P), and 7.1 (Zn). A single 100g serving of fresh fruit from ‘Ulu fiti’, would provide about 5.9% (Ca), 24.3% (Cu), 6.9% (Fe), 16% (K), 28.9% (Mg), 8.4% (Mn), 13.4% (P) and 5.3% (Zn) of Health Canada’s Recommended Dietary Allowance (RDA)/Adequate Intake (AI) for an adult female between 19-30 years old (Table 6-5; http://www.hc-sc.gc.ca/).

Seasonal Variation and Soil Nutrition

As a group, no significant differences were found in the mineral content in a subset of nine individual cultivars of *A. altilis,* and *A. altilis* × *A. mariannensis* hybrids collected over two seasons. The significant differences in the soil characteristics between the main grid (A-E) and the adjacent area (H) were reflected in a significant difference in the P and Cu content of the fruit between the two locations but there were no other significant differences (see appendix 1).

Discussion

Feeding an increasingly hungry world requires complex solutions that address food availability, education, politics, economics and environmental issues. However, it has become clear that underutilized crops such as breadfruit can have a significant and positive impact on food security (FAO, 2009b). The International Treaty on Plant Genetic Resources for Food and Agriculture has identified breadfruit among its priority crops for development (FAO, 2009b). Recently, the development and seasonality of fruit for the entire NTBG breadfruit collection (Chapter 4) and methods for mass propagation and distribution of breadfruit trees from this collection (Murch et al., 2007, 2008) have been described. The current work was designed to identify and characterize specific cultivars that provide a good source of mineral-dense staple food for undernourished communities.

Breadfruit can be grown across the tropics from South East Asia to the Caribbean and tropical Africa (Ragone and Taylor, 2007). Within the Breadfruit Institute's collection at the NTBG, there are 24 cultivars that often produce fruit throughout all 12

months of the calendar year (Chapter 4). Individual fruits can weigh anywhere from 100g to over 4.5 kg (unpublished data) and it has been estimated that an average mature tree can produce 150 – 200 kg or more of fresh fruit per year (Ragone, 1997). The current data indicate that one cultivar of breadfruit in particular, "Meion", contains concentrations of iron similar to whole wheat flour, almost as much as fortified wheat flour, and more than four times what is found in unfortified refined wheat, rice or corn flour. An individual fruit from 'Meion' weighs an average of 1.45 ± 0.101 kg and fruits from approximately September to February in Hawaii (Chapter 4). For a more balanced source of mineral nutrition, the cultivar 'Ulu Fiti' contains fairly high levels of iron, more than twice what is found in refined wheat, rice, or corn, and is exceptionally rich in Ca, Co, Cu, Mg, Mn, P, and Zn. An individual 'Ulu Fiti' fruit has an average weight of 1.07 ± 0.039 kg and has a 6-month fruiting season between June and December in Hawaii (Chapter 4). The production of these breadfruit cultivars as functional foods in developing countries could have an enormous impact. Iron is one of the most widespread and devastating nutrient deficiencies in the world and adequate amounts are essential for women and children's health (CIDA, 2009; UNSCN, 2004). It has been estimated that adequate iron nutrition would reduce the 130,000 maternal deaths per year by 20% (CIDA, 2009). As well, anaemia in adults causes a loss of approximately 2% of the total GDP in developing countries; increasing iron nutrition could produce a significant economic outcome (CIDA, 2009). Calcium and magnesium deficiencies have been associated with pre-eclampsia, the leading cause of maternal and perinatal mortality, as well as hypertension and a variety of other health problems (Jain et al., 2009; Sibai et al., 2005). Zinc deficiencies result in weakened immune systems, diarrhea and delayed

development of the central nervous system in children (CIDA, 2009). These data indicate that a single fruit from 'Ulu Fiti' made into a curry, baked, boiled, or fried could contribute much of the required mineral nutrition for two adult women or a woman and two children.

Unlike cereals which are often grown as a monocultures, breadfruit is a key component in traditional agroforestry based cropping systems (Ragone, 1997; Ragone and Raynor, 2009; Raynor and Fownes, 1991). Breadfruit is a low input crop growing as a large tree that produces food for many decades with relatively little maintenance, care, fertilization or pest control (Ragone, 1997; Ragone and Taylor, 2007; Ragone and Raynor, 2009). A survey of 54 farms on Pohnpei found that this agricultural system supports a high degree of agricultural diversity, cultivating an average of 26 useful species including breadfruit, coconut (*Cocos nucifera*), mango (*Mangifera indica*), peppers (*Caspsicum sp.*), and banana (*Musa sp.*) (Raynor and Fownes, 1991). The large-scale distribution of breadfruit has been limited by difficulties in propagation and transportation of trees but solutions to these problems have recently been developed through the use of plant tissue culture (Murch et al., 2008) and larger scale bioreactor production systems (Murch et al., 2007).

The last major introduction of breadfruit outside of Oceania was the voyages of Captain Bligh aboard the ships HMS Bounty and HMS Endeavour to bring breadfruit to the Caribbean. In countries where breadfruit has been introduced to date, local traditions have evolved many recipes and local culinary techniques. In Sri Lanka, curries are popular, in Jamaica breadfruit is eaten boiled, roasted, fried or even in puddings, and in Granada it is the base of "oiled down", the national dish. Only about 600 plants of a

handful of cultivars were carried by Bligh from Tahiti for distribution in the Caribbean (Spary and White, 2004), but hundreds of cultivars bred by the indigenous peoples across Oceania exist that remain to be shared with the rest of the world. The further development of breadfruit to reduce malnutrition requires continuing research to (a) identify the most micro-nutrient dense cultivars, (b) determine the contents of starches, fats, oils and other functional constituents, (c) further develop methods for mass propagation, (d) establish standardized virus screening to prevent distribution of pathogens, (e) develop a database to make data about specific cultivars widely and freely available and (f) create localized educational efforts to develop and disseminate knowledge with respect to agricultural practices, culinary uses, and secondary products. The results of the current study provide another step in the process and demonstrate the potential of the widespread use of breadfruit for food security.

Table 6-1: Analytical parameters used for the quantification of minerals in breadfruit by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

	Element										
	Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn	Yt
Blank (PPM)	0	0	0	0	0	0	0	0	0	0	200
Standard 1 (PPM)	20	1	0.2	5	20	10	1	5	6.6	5	200
Standard 2 (PPM)	50	2	0.4	10	50	20	2	10	16.5	10	200
Standard 3 (PPM)	100	4	0.8	15	100	30	4	20	33	15	200
Standard 4 (PPM)	150	6	1	20	150	40	6	50	66	20	200
Standard 5 (PPM)	200	8	2	30	250	50	8	100	99	30	200
Line of best fit	Linear	Curvilinear	Linear	Linear	Linear	Curvilinear	Linear	Linear	Linear	Linear	N/A
R^2 Value	0.999995	0.999995	0.99996	0.99979	0.9994	0.99997	0.99982	0.99996	0.99992	0.99996	N/A
*Detection limit (PPM)	0.0081	0.0009	0.0021	0.0003	0.0324	0.0003	0.0018	0.0495	0.0009	0.0003	N/A
**Quatitation limit (PPM)	0.027	0.003	0.007	0.001	0.108	0.001	0.006	0.165	0.003	0.001	N/A
***Recovery of NIST	103.9%	N/A	110.8%	56.2%	73.2%	97.7%	90.4%	N/A	108.8%	93.9%	N/A

*Limit of detection represents the mean reading of 10 measurements of the blank plus 3 standard deviations

**Limit of quantification represents the mean reading of 10 measurements of the blank plus 10 standard deviations

***Recovery of NIST represents the average concentration of each element detected as a percentage of the amount reported by NIST reference standard

Table 6-2: Composition and characteristics of the soil at Kahanu Garden Breadfruit Germplasm Repository. See figure 6-2 to view site locations.

Soil Characteristic	Site Location						Average	StdEr	%RS
	A	B	C	D	E	H			
pH	6.1	6.1	6.1	6.2	5.8b	6.2	6.1	0.1	2.4
Organic Matter (%)	9.78	7.66	10.57	11.64	11.48	14.03	10.2	0.7	15.8
Total N (%)	0.8	0.65	0.9	1	1.02	1.13	0.9	0.1	18.8
Minerals									
Phosphorous	40	30	25	21	17	78[a]	26.6	3.6	33.5
Potassium	316	225	378	554	121	499	348.8	66.8	46.9
Calcium	1861	2030	2072	2340	1478	3816[a]	1956.2	129.7	16.2
Magnesium	633	662	747	784	668	1146[a]	698.8	26.0	9.1
Iron	67b	92	90	86	88	97	86.7	4.2	11.9
Manganese	7.9	9.4	16	14	9.9	18	12.5	1.7	32.4
Zinc	2.6	1.5	1.9	2.2	2.4	6.2[a]	2.1	0.2	20.4
Copper	2.6	2.6	2.4	2.5	4.7	6.2[a]	3.0	0.4	33.0
Boron	0.18	0.16	0.15	0.12	0.11	0.13	0.1	0.0	18.6
Aluminum	0.66	0.62	0.68	0.73	0.62	1.1[a]	0.7	0.0	7.0
Arsenic	152.5	137.5	120.6	153.63	74.79b	171.8	135.1	14.0	25.3
Cadmium	6.18	5.78	5.02	6.46	3.04b	7.21	5.6	0.6	25.9
Cobalt	42.27	44.87	43.46	44.36	45.82	29.49[b]	44.2	0.6	3.1
Chromium	49.01	48.51	45.36	49.63	44.76	30.41[b]	47.5	0.9	4.7
Nickel	56.91	66.29	60.37	53.69	49.32	38.0[b]	57.3	2.6	11.3
Lead	100.4	103.7	91.51	119.8	124.9	79.39	108.1	5.7	12.9
Selenium	99.45	105.4	110.8	119.5	121.3	78.35[b]	111.3	3.8	8.3

[a] Values that were significantly higher than average as determined by a Jackknifed distance outlier test.

[b] Values that were significantly lower than average as determined by a Jackknifed distance outlier test.

Table 6-3: Average moisture, total ash, and mineral contents of flour made from breadfruit (*Artocarpus altilis*), hybrid breadfruit (*A. altilis* × *A. mariannensis*), fortified white and whole wheat, unfortified wheat, rice and corn flours.

Species	Total Bulk Samples	Total Analytical Replicates	% Dry Matter		% Ash	Mineral Content (µg/g dW)									
			Flour	Fruit		Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
Artocarpus altilis average	84	251	87.1[a]	32.2[b]	3.01[a]	583[b]	0.011[ab]	2.6[bc]	11[c]	11081[a]	1022[c]	1.7[g]	195[b]	1319[e]	2.6[f]
Min	1	3	85.2	25.7	2.19	283	0.0036	1.1	6.2	7577	630	0.9	70	846	1.5
Max	1	3	93.6	39.3	4.34	1491	0.031	5.6	21.2	16212	2281	3.8	843	2379	10.7
A. altilis × *A. mariannensis average*	17	54	88.3[a]	29.9[c]	3.2[a]	559[bc]	0.010[ab]	3.1[d]	15.1[b]	11399[a]	933[c]	1.9[f]	1222[a]	1390[de]	3.1[e]
Min	1	3	85.6	26.3	1.9	294	ND	2.2	10	9083	754	1.1	125	1140	1.8
Max	1	3	91.7	32.8	4.02	987	0.023	4.4	34	15345	1228	3	6767	2117	5.5
A. camansi	2	6	NA	39.8[a]	2.87[a]	658[b]	0.007[bc]	6.4[a]	14.1[b]	10876[a]	1337[b]	2.9[e]	75[b]	3017[b]	6.9[d]
A. mariannensis	1	3	NA	34.9[b]	3.14[a]	1188[a]	0.014[a]	5.0[b]	17.5[b]	10702[a]	1783[a]	5.3[d]	1288[a]	1583[cd]	3.8[ef]
Whole wheat flour	1	3	92.1[a]	NA	1.57[b]	249[bcd]	0.006[bcd]	4.0[bc]	34.5[a]	2828[b]	1694[ab]	31.8[a]	8[b]	4802[a]	28[a]
[2]Fortified refined wheat flour	1	3	87.5[a]	NA	0.51[c]	145[cd]	0.002[cd]	2.0[ef]	38.5[a]	982[b]	388[d]	5.7[d]	12[b]	1516[cde]	8.5[cd]
Refined wheat flour	1	5	91.6[a]	NA	0.59[c]	198[d]	-	2.3[ef]	7.9[d]	899[b]	342[d]	8.5[c]		1413[de]	10.9[c]
Refined rice flour	1	3	88.7[a]	NA	0.63[c]	61[d]	0.010[ab]	3.0[cde]	5.8[d]	1008[b]	490[d]	18.2[b]	56[b]	1819[c]	18[b]
Corn flour	1	3	89.4[a]	NA	0.42[c]	17[d]	0.00[cd]	1.4[f]	5.9[d]	1108[b]	327[d]	1.1[g]	58[b]	953[f]	4.4[e]

[1] Mean values followed by different letters are significantly different with a type one error rate of 0.05

[2] This flour is fortified with reduced iron to approximately 5.3mg/100g

Table 6-4: Provenance information, dry matter, ash and mineral content of breadfruit accessions representing the top 5% evaluated for each mineral compared to commonly consumed cereal flour.

D number	Cultivar name	Sp[1]	Island of Origin	% Dry Matter		% Ash (dW)	Mineral Content (µg/g dW)									
				Fruit	Flour		Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
770517001	Ma'afala	*A.a*	Samoa	34.7 bcd	86.9 jk	3.21 hi	481.9 fgh	0.018 cd	2.8 ij	21.2 d	12423 ef	1106 ghi	1.8 ijk	169 hij	1305 kl	2.5 l
900237001	Mei puou	*A.a*	Marquesas Is.	29.7 ef	86.8 jk	3.43 ef	579.8 efgh	0.012 efg	3.1 i	11.6 k	14265 bcd	1045 ghi	1.4 kl	108 kl	1501 hijk	2.4 l
790489001	Piipiia	*A.a*	Society Is.	30.1 def	85.6 m	3.25 h	496 fgh	0.020 cde	4.4 cd	14.1 hij	11518 fg	990 ghi	2.3 hi	844 c	1684 efgh	3.3 ijkl
890475002	Sagosago	*A.a*	Samoa	29.8 ef	88.2 g	3.58 d	589 efgh	0.016 de	2.9 ij	16.1 gh	13197 de	1473 cde	2.3 hij	204 gh	1354 kl	3.4 ijkl
910279002	Siviri2	*A.a*	Vanuatu	28.3 ef	90.5 d	3.44 ef	650.5 efg	0.019 cd	3.9 defg	20.5 de	13463 cde	1127 fghi	2.0 ijk	258 f	1634 fghi	5.1 fg
890465001	Teahimatoa	*A.a*	Society Is.	28.5 ef	86.2 l	3.28 gh	922.2 cd	0.008 gh	3.0 ij	7.3 l	11861 efg	1181 fgh	1.8 ijk	137 jkl	1379 jkl	2.6 l
790488001	Toneno	*A.a*	Society Is.	27 f	90.7 d	3.89 c	987.4 bc	0.000 j	4.4 cd	20.7 de	13476 cde	1228 efg	2.3 hij	1864 a	2117 d	4.0 hij
790491001	Tuutou	*A.a*	Society Is.	26.9 f	87.6 h	4.27 a	446.1 ghi	0.012 efg	3.3 ghi	11.8 k	16212 a	1143 fghi	2.8 fgh	132 jkl	1844 ef	2.3 l
890258001	Ulu fiti	*A.a*	Rotuma	39.1 ab	93.6 a	4.35 a	1491 a	0.019 cd	5.6 b	18.3 f	14375 bcd	2281 a	3.8 e	180 hi	2379 c	10.7 c
900260001	Ulu fiti	*A.a*	Fiji	39.3 ab	90.5 d	3.09 j	935.5 cd	0.028 ab	4.0 def	12.9 jk	12507 ef	2131 a	3.0 f	135 jkl	1737 efg	7.0 e
900228001	Unidentified2	*A.a*	Samoa	26.3 f	86.6 k	3.94 bc	1462 a	0.016 de	3.1 hi	12.5 jk	13275 de	1559 bcd	2.7 fgh	144 ijk	1469 hijkl	3.0 jkl
900264001	Uto ni viti	*A.a*	Fiji	26.9 f	88.2 g	3.37 fg	905.1 cd	0.016 de	2.4 jk	11.0 k	13094 def	1583 bc	3.0 f	182 hi	1251 l	4.0 ghij
810289002	Yellow	*A.a*	Seychelles	26.9 f	87.1 ij	4.02 b	687.2 ef	0.031 a	4.9 bc	11.5 k	14962 abc	1136 fghi	1.7 jk	106 l	1881 e	2.9 kl
910272001	Mein-pohnsakar	*A.a* x *A.m*	Pohnpei, FSM	26.3 f	91.3 c	4.02 b	739.6 de	0.015 def	3.3 fghi	18.9 ef	15331 ab	1067 ghi	2.6 fgh	366 d	1643 fgh	5.1 fgh
910268001	Meion	*A.a* x *A.m*	Truk, FSM	27.4 ef	89.4 e	3.53 de	391.5 hi	0.023 bcd	3.2 hi	34.0 b	13010 def	927	2.4 ghi	1193 b	1413 ijkl	5.5 f
890183001	Midolab	*A.a* x *A.m*	Palau	30.4 cdef	91.3 c	3.52 de	546.7 efgh	0.011 efgh	3.8 defgh	15.6 ghi	13211 de	881 i	1.7 kl	224 fg	1840 ef	3.5 ijkl
890182001	Ulu elise	*A.a* X *A.m*	Tokelau	31.8 cde	91.4 c	3.42 f	660.2 ef	0.015 def	3.4 efghi	25.1 c	12189 efg	1058 ghi	3.0 f	315 e	1587 ghij	4.4 fghi
900252002	Dugdug	*A.m*	Mariana Is.	34.9 bc	-	3.14 ij	1188 b	0.014 def	5.0 bc	17.5 fg	10702 g	1783 b	5.3 d	1288 c	1583 ghij	3.8 ijk
910280001	breadnut (seed)	*A.c*	Pohnpei, FSM	39.8 a	-	2.87 k	658 ef	0.007 h	6.4 a	14.1 ij	10876 g	1337 def	2.9 fg	75 l	3017 b	6.9 e
-	Whole wheat	*T.*	-	-	92.1 b	1.57 l	249 ij	0.006 hi	4.0 de	34.5 b	2828 h	1694 bc	31.8 a	8 m	4802 a	28.0 a
-	White flour[2]	*T.*	-	-	87.5 hi	0.51 no	145 jk	0.002 ij	2.0 kl	38.2 a	982 i	388 j	5.7 d	12 m	1516 ghijk	8.5 d
-	White flour (NIST)	*T.*	-	-	91.2 c	0.59 mn	198 jk	- -	2.3 k	7.9 l	899 i	342 j	8.5 c	- -	1413 l	10.9 c
-	Rice flour	*O.s*	-	-	88.7 f	0.63 m	61 jk	0.010 fgh	3.0 ij	5..8 l	1008 i	490 j	18.2 b	56 m	1819 ef	18.0 b
-	Corn flour	*Z.m*	-	-	89.4 e	0.42 o	17 k	ND j	1.4 l	5.9 l	1108 i	327 j	1.1 l	58 m	953 m	4.4 gfhi

[1] *A.a =Artocarpus altilis*, *A.a* X *A.m = A. altilis X A. mariannensis* hybrid, *A.m = A. mariannensis*, *A.c = A. camansi*, *Z.m = Zea mays*, *O.s = Oryza sativa*, *T. = Triticum* species

[2] This flour is fortified with reduced iron to approximately 5.3mg/100g

[3] Means followed by the same letter within each column are not significantly different from each other at p=0.05 based on a students means separation

Table 6-5: Percentage of Health Canada's Recommended Daily Allowance/Adequate Intake for a female between 19-30 years of age in A; flour made from breadfruit flesh and B; fresh fruit, comparing all evaluated cultivars, *Artocarpus altilis* cultivars, and *A. altilis* x. *A. mariannensis* hybrids.

A Element	Mineral content (% recommended dietary intake/30 g dry weight)											
	All cultivars				*Artocarpus altilis*				*A. altilis X A. mariannensis hybrids*			
	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max
Ca	1.7	± 0.06	0.8	4.5	1.7	± 0.07	0.8	4.5	1.7	± 0.12	0.9	3.0
Co	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cu	9.0	± 0.25	3.8	18.6	8.7	± 0.28	3.8	18.6	10.4	± 0.46	7.3	14.7
Fe	2.4	± 0.09	1.2	6.8	2.2	± 0.06	1.2	4.2	3.2	± 0.31	2.0	6.8
K	7.2	± 0.11	4.8	10.3	7.1	± 0.12	4.8	10.3	7.4	± 0.25	5.8	9.8
Mg	9.5	± 0.26	6.1	22.1	9.7	± 0.31	6.1	22.1	9.1	± 0.31	7.3	11.9
Mn	2.8	± 0.10	1.5	6.4	2.7	± 0.11	1.5	6.4	3.3	± 0.21	1.8	5.1
Na	0.6	± 0.11	0.1	7.7	0.3	± 0.02	0.1	1.7	1.8	± 0.48	0.3	7.7
P	5.7	± 0.11	3.6	10.2	5.6	± 0.12	3.6	10.2	6.2	± 0.26	4.9	9.1
Zn	1.0	± 0.05	0.5	4.0	0.9	± 0.06	0.5	4.0	1.2	± 0.10	0.7	2.0
B Element	Mineral content (% recommended dietary intake/100 g fresh fruit)											
	All cultivars				*Artocarpus altilis*				*A. altilis X A. mariannensis hybrids*			
	Mean	SE	Min	Max	Mean	SE	Min	Max	Mean	SE	Min	Max
Ca	1.8	± 0.07	0.8	5.9	1.8	± 0.08	0.8	5.9	1.7	± 0.11	0.8	2.7
Co	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cu	9.5	± 0.27	4.2	24.3	9.3	± 0.32	4.2	24.3	10.3	± 0.39	7.8	13.2
Fe	2.5	± 0.09	1.4	6.2	2.3	± 0.08	1.4	4.9	3.1	± 0.27	2.1	6.2
K	7.5	± 0.10	5.8	12.0	7.6	± 0.12	5.8	12.0	7.3	± 0.18	5.9	8.6
Mg	10.0	± 0.33	6.9	28.9	10.3	± 0.40	6.9	28.9	9.0	± 0.22	7.7	10.9
Mn	2.9	± 0.11	1.7	8.4	2.8	± 0.13	1.7	8.4	3.2	± 0.20	1.7	5.4
Na	0.6	± 0.10	0.2	6.8	0.3	± 0.03	0.2	1.7	1.7	± 0.42	0.3	6.8
P	6.0	± 0.12	4.1	13.4	6.0	± 0.14	4.1	13.4	6.1	± 0.22	5.3	8.2
Zn	1.0	± 0.06	0.5	5.3	1.0	± 0.08	0.5	5.3	1.2	± 0.08	0.7	1.9

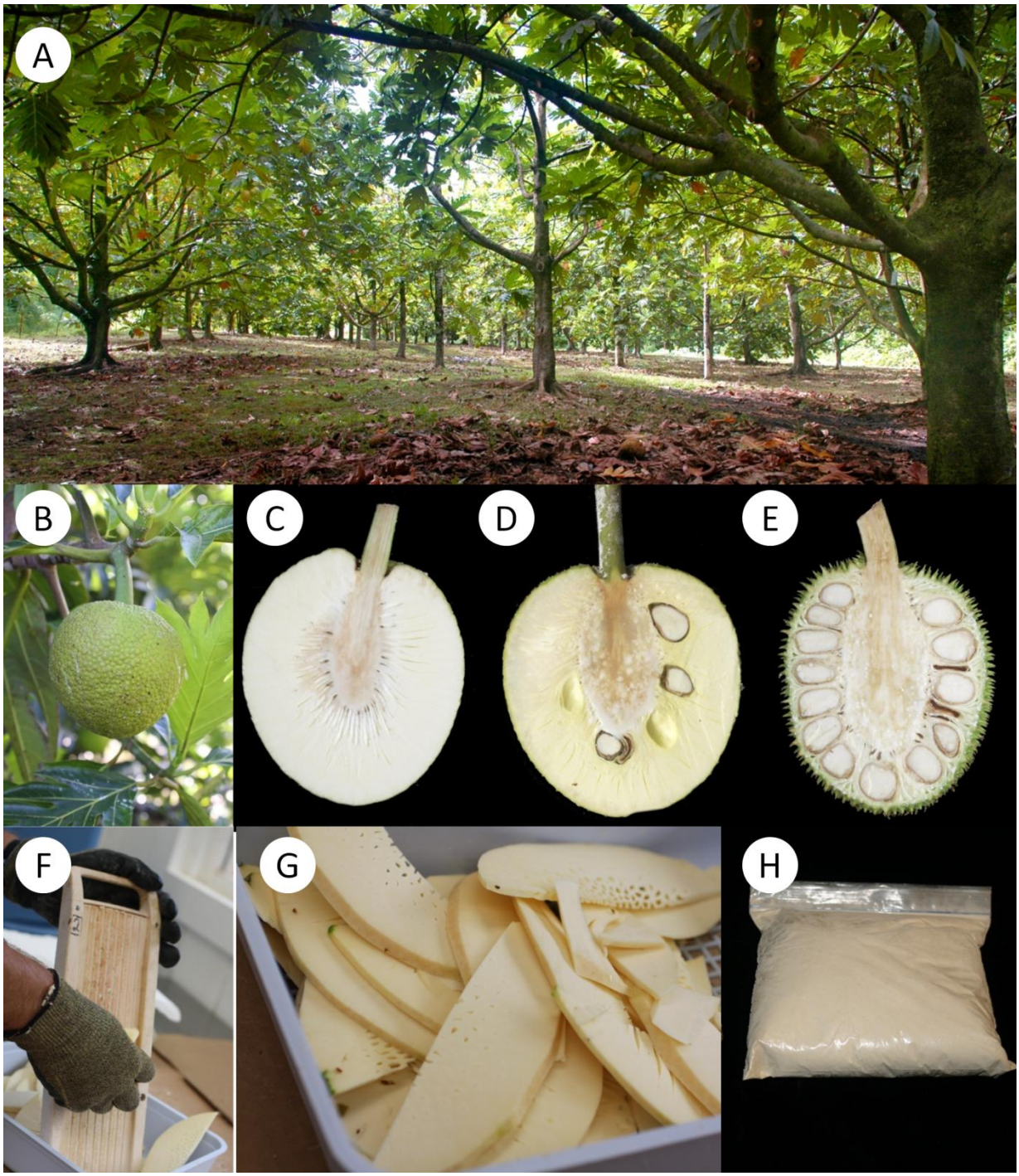

Figure 6-1: The breadfruit collection and processing methods used at Kahanu Garden, Maui. A. Trees are planted in a linear grid pattern on a 2.5 ha site. B. Mature fruit of the cultivar 'Ulu Fiti'. C. Cross section of an *A. altilis* fruit, cultivar 'Aumee' originating from the Society Islands. D. Cross section of an *A. altilis* fruit, cultivar 'Karawa' originating from Fiji. Note: seeds are found in some *A. altilis* fruits. E. Cross section of a fruit of the species *A. camansi* collected from the Society Islands. F. Peeled and seeded fruits were sliced manually on a mandolin-style blade to produce sections for drying. G. Sections that were dried to produce flour. H. Breadfruit flour.

Figure 6-2: Map of the breadfruit collection and soil sampling at Kahanu Garden. Trees are indicated by small circles with alphanumeric designations and larger circles with letters indicate soil sampling zones

Chapter 7: In Search of the Golden ‘Ulu: Evaluating Breadfruit (*Artocarpus*, Moraceae) for Pro-Vitamin A Carotenoid Rich Cultivars.

Introduction

Vitamin A deficiency (VAD) is one of the most common and devastating micro-nutrient deficiencies in the world, especially common in tropical developing nations (WHO, 2009) . This form of malnutrition is estimated to affect one third of pre-school age children worldwide, and between 44-50% of pre-school age children in some regions of Africa and South-East Asia (WHO, 2009). Globally, 15% of pregnant women are estimated to suffer from this condition. The most specific health consequence of vitamin A deficiency is xerophthalmia, but it can also cause anemia and reduced resistance to infection. Vitamin A deficiency is the leading cause of preventable childhood blindness, results in the death of untold millions, and is a major contributing factor to maternal mortality. Addressing VAD, and other micronutrient deficiencies, requires a close look and re-evaluation of our food production systems to ensure that people not only have enough food to eat, but that the food is sufficient to meet nutritional requirements.

Vitamin A is obtained in its native state from animal products, or can be semi-synthesized from pro-vitamin A carotenoids that are present in fruit, vegetables, and some animal products (FAO and WHO, 2004). The portion of the population most at risk of VAD are the poor, who tend to consume very little meat and rely almost exclusively on plants for their nutritional needs (Nestel et al., 2006). This portion of the global population is further stratified

with the poorest people having little access to fresh fruit and vegetables and must rely heavily on staples such as rice and wheat. Neither rice nor wheat naturally produces provitamin A carotenoids, making populations that rely heavily on these staples at particularly high risk of VAD. A number of less common staple foods such as cassava , bananas, sweet potatoes, and breadfruit naturally produce pro-vitamin A carotenoids and have the potential to prevent VAD in many vulnerable populations (Davey et al., 2006; Dignan et al., 2004; Englberger et al., 2003a).

Breadfruit (*Artocarpus altilis* and *A. altilis* × *A. mariannensis* hybrids) is a tropical tree crop that produces large starchy fruit most often eaten as a staple food (Ragone, 1997). Within this crop is great diversity in fruit morphology (Ragone, 2007; Chapter 3), mineral content (Chapter 5; Ragone and Cavaletto, 2006) and carotenoid content (Englberger et al., 2003a, 2003b; Englberger et al., 2007a, 2007b; Ragone and Cavaletto, 2006). The presence and variability in pro-vitamin A carotenoids in breadfruit provide a relatively untapped resource with great potential to help fight VAD in the tropics. However, due to the high variability in pVAC content, identifying and disseminating elite cultivars is critical. To date, only a select number of cultivars have been evaluated for their pro-vitamin carotenoid content and while some carotenoid dense cultivars have been reported (Englberger et al., 2003a, 2003b; Englberger et al., 2007b), more thorough investigations are needed.

The National Tropical Botanical Garden (NTBG) houses the largest and most diverse breadfruit germplasm repository in the world with accessions collected from over 34 Pacific islands, Indonesia, the Philippines, and the Seychelles (Ragone, 2007). The current study was conducted to evaluate the amount of variability that is present in the carotenoid content within this collection, identify elite cultivars with high levels of pro-vitamin A carotenoids for

international distribution and food security in the tropics, and compare species and geographic origins of breadfruit cultivars to guide further screening programs.

Materials and Methods

The NTBG Breadfruit Germplasm Repository

The National Tropical Botanical Garden maintains the largest and most diverse breadfruit germplasm collection in the world with over 325 accessioned trees that originated from across its traditional range growing in a geographically restricted area and has been described in detail in Chapter 3. Soil nutrient data for this collection is described in detail in Chapter 6.

Breadfruit Sample Collection

As part of a larger nutritional study, three fruit were collected from each of the 97 breadfruit accessions evaluated in this study between November, 2008 and March, 2009. An additional set of three fruit from a subset of nine accessions was collected again in December 2009 to evaluate inter-seasonal variability. The fruit were collected at the mature, but not yet ripe stage of development (Worrell et al., 1998), the stem was removed and the fruit left inverted for about 1 h to allow the latex to drain/coagulate. The fruit were peeled using a household vegetable peeler, cut into quarters, and the cores removed. Fruit composition data were collected as described previously (Chapter 3). One half of each fruit was frozen and shipped back to the University of British Columbia where they were stored at -86°C. A subset of eight cultivars was selected for cooking trials to determine the effect of typical preparation methods – boiling and baking – on the carotenoid content. For cooked breadfruit studies, one half of a fruit from each

cultivar was boiled until the flesh could be easily pierced with a fork, while the other half was roasted in an oven at 180˚C until the flesh was tender following typical local food preparation protocols. The average time to cook the fruit by boiling was 34.9 minutes (SE ± 3.71) and 45.4 minutes when roasted (SE ±3.50). The range of boiling times was from 15-50 minutes and the roasting time ranged from 28-63 minutes depending on the individual characteristics of the cultivar. The cooked fruit were frozen and treated in the same manner as the raw fruit.

Sample Preparation and Moisture Content

Subsamples of approximately 9 g of each fruit were weighed and transferred into 15 ml centrifuge tubes (Corning inc., Corning, NY). The tubes were 137yophilized for 24 h until dry (Freezone 4.5 Freeze Drier; Labconco, Kansas City, MO), reweighed and stored at -86^{o}C until analysis. The weight differences between the fresh samples and lyophilized samples were used to determine the moisture content of the fruit and to calculate the carotenoid content on a fresh weight basis.

Extraction of Carotenoids

Carotenoid extractions were conducted as described by Davey et al. (2006). The 137yophilized samples were homogenized to a fine powder in liquid nitrogen using a mortar and pestle. Upon crushing, 74-76 mg of the lyophilized breadfruit material from each sample was transferred into 2 ml microcentrifuge tubes (Fisher Scientific, Ottawa, ON). Five acid washed 3 mm glass beads and 700 µl of the extraction buffer (0.25% BHT (MP Biomedicals, Solon, OH) extraction buffer in 95% ethanol) were added to each sample. The tubes were then vortexed for 5 s on the highest setting (Vortex Genie; Fisher Scientific, Waltham, MA) to agitate

samples. The samples were placed into a hot water bath at 85 °C for 10 m in batches of 24, then immediately placed on crushed ice for 5 m. The samples were then agitated by hand at 120 beats per minute for 30 s, and centrifuged for 15 m at 13,000 rpm (Galaxy 16DH; VWR, Westchester, PA). The supernatants were transferred to 2 ml microcentrifuge tubes (Fisher Scientific, Ottawa, ON) and set aside. The entire extraction process was repeated and the supernatant was combined with the first extraction. The combined extracts were filtered through 25 mm syringe filters with a pore size of 0.2 µm (Fisher Scientific, Ottawa, ON) into new 2 ml microcentrifuge tubes (Fisher Scientific, Ottawa, ON). In initial tests a third extraction of the samples was conducted but contained very low levels of carotenoids and diluted the concentration of the final extract reducing the sensitivity of the analysis. Carotenoid recovery was evaluated by spiking one of the breadfruit samples with 10 µg of β-carotene in triplicate prior to extraction. Differences between the spiked and the original breadfruit samples were compared to determine the percentage of the added β-carotene that was recovered.

Spectrophotometric Analysis of Total Carotenoids

The total carotenoid content in the extracts was estimated using methods described by Davey et al. (2006). Three 200 µl aliquots of each extract were transferred into 96 well microplates (BD biosciences, Mississauga, ON). The absorbance of each aliquot was measured at a wavelength of 450 nm in a Synergy HT microplate reader (Biotek, Winooski, VT). The absorbance of the extracts at 450 nm was used to estimate total carotenoid content using the equation derived from a linear standard curve of predominantly trans β-carotene (Sigma-Aldrich, Oakville, ON) in extraction buffer at concentrations of 0, 0.1, 1, and 10 µg/ml. A standard curve was run on each microplate to ensure that the instrument was producing consistent data.

HPLC Analysis of Individual Carotenoids

The quantification of individual carotenoids by HPLC was conducted as described by Davey et al. (2006). The remaining portions of the extracts used for the estimation of total carotenoids were used for HPLC analysis. Aliquots of 250 µl were transferred into 300 µl HPLC vial inserts (National Scientific, Rockwood, TN), placed in 3 ml HPLC vials (Target DP C4000-2W; National Scientific, Rockwood, TN) and sealed with bonded pre-slit, PTFE silicone septa screw caps (Waters, Milford, MA). The HPLC analysis was conducted using an Alliance 2695 separation system fitted with a temperature-controlled auto-sampler (Waters, Milford, MA), and a 2998 UV-VIS Photodiode array detector (Waters, Milford, MA). The column was maintained at a temperature of 30°C, and the autosampler was kept at 15°C. Separation was achieved using a Nova-Pak C18 4 µm, 3.9 x 150 mm HPLC column (Waters, Milford, MA). The solvent system included mobile phase A (acetonitrile/0.05%TEA/0.1%BHT) and mobile phase B (methanol:grade ethyl acetate 1:1 v:v/0.05% TEA/ 0.1% BHT). All solvents were HPLC grade purchased from Fisher Scientific, Ottawa, ON. The flow rate for the separation was 1.00 ml/min, from minute 1 to 6 the mobile phase was comprised of 95.0% mobile phase A / 5.0% mobile phase B, from minute 6 to 20, 70.0% mobile phase A / 30.0% mobile phase B, from minute 20 to 21, 40.0% mobile phase A/ 60.0% mobile phase B, from minute 21 to 30, 100% mobile phase B, and from minute 30 to 35, 95.0% mobile phase A/ 5.0% mobile phase B. The injectors were purged and a blank consisting of the extraction solvent was run after every 7 injections. Absorbance at a wavelength of 450 nm was used for quantification, however, absorbance in the range of 300-600 nm was collected to compare the absorbance spectrum of individual carotenoids to the standards and literature values to aid in accurate compound identification.

Data were collected and integrated using Waters Empower 2.0 software. The following individual carotenoids were identified based on their retention time and absorbance spectrum compared to authenticated reference standards: lutein, β-carotene (Chromodex, Irvine, CA), β-cryptoxanthan (Sigma-Aldrich, St. Louis, MO) and lycopene (Chromodex, Irvine, CA). Stock solutions of each standard were prepared individually in THF/0.25%BHT at concentrations of 1 mg/ml and stored in the dark at -86°C. The stock solutions were used to prepare working standards ranging from 0.005 μg/ml to 2.0 μg/ml and analyzed in triplicate to prepare calibration curves using the peak area of the absorbance at a wavelength of 450 nm for each carotenoid. Due to the lack of a commercial source of α-carotene standard, this compound was identified based on its retention time relative to β-carotene as described by Davey et al.(2006), and by comparing its absorbance spectrum (λmax at 444 nm and 473 nm) to literature values (Tan and Soderstrom, 1989). To estimate the quantity of α-carotene, the standard curve produced using β-carotene was used with a correction factor of 0.925 based on their relative molar absorptivities (Davey et al., 2006). The peak area of each carotenoid was auto-integrated using apextrack integration analysis on Empower 2.0 (Waters, Milford, MA) and manually refined to present accurate peak areas of each compound. In many samples, a number of unidentified carotenoids were present. Total carotenoid contents of the extracts were estimated by combining the peak area of all peaks and comparing it to the β-carotene standard curve so that it was comparable to the microplate estimate.

Statistical Analysis

All statistical analyses were conducted using JMP® 8.0.2 (SAS Institute, Cary, NC); see appendix 1-4 for details of analyses. A general linear model (GLM) was used to conduct a

regression analysis to compare the relationship between the two analytical methods used for the estimation of total carotenoids. A GLM was used to conduct a MANOVA using species as the independent variable and carotenoid concentrations as the dependent variables. Another MANOVA was conducted using geographic origin as the independent variable and the carotenoid concentrations as the dependent variables. A series of independent ANOVAs were conducted to determine if there were significant differences in total carotenoid content or any individual carotenoid among the folloing groups: species, geographic origin, cooking method, or harvest season. For the comparison of geographical regions, cultivars were grouped into Melanesian, western Polynesian, eastern Polynesian, and Micronesian cultivars as described in Chapter 3. In cases where the ANOVAs indicated significant differences, a Student's means separation was conducted. A subset of elite cultivars representing the ten accessions with the highest pVAC content were selected and an ANOVA and a means separation were conducted to determine the differences in total carotenoid content, individual carotenoid concentrations, and fruit characteristics. All statistical analyses were conducted with a type 1 error rate of 0.05.

Results

Comparison of Analytical Techniques for Estimating Total Carotenoids

The standard curve developed for β-carotene using the spectrophotometric method was highly linear with a coefficient of determination generally 0.999 or better. The relative standard deviation (RSD) of repeated extracts of the same breadfruit sample was 2.2%, indicating that the extraction method using these methods is sufficiently reproducible. The recovery of β-carotene from breadfruit samples spiked with a known amount of the β-carotene reference standard was

85.9%. Based on this method, the average total carotenoid content of all breadfruit cultivars (*Artocarpus altilis* and *A. altilis* × *A. mariannensis)* was estimated to be 169.7 µg/100 g (SE±10.18) of fresh fruit. Among the cultivars there was approximately a17.9 fold difference in total carotenoid content ranging from 56.3 µg/100 g (SE±7.29) to 1008.7 µg/100 g fresh fruit (SE±169.26).

The coefficient of determination of the standard curve for β-carotene used for total carotenoid estimation in the HPLC analysis was 1.000, indicating a high degree of linearity within this range. The RSD of repeated injections of the standard solutions across all concentrations was 2.5% on average. The average estimate of total carotenoid content based on the β-carotene standard curve was 57.2 µg/100 g fresh fruit (SE±5.31), significantly less than the amount estimated using the microplate method. However, linear regression revealed a significant predictive relationship between the two techniques with a coefficient of determination of 0.892 for cultivars of *A. altilis* and 0.64 for hybrid cultivars. Based on HPLC the total carotenoid content varied from none detected to 668.4 µg/100 g fresh fruit (SE±120.46) among cultivars.

Quantification of Individual Carotenoids by HPLC

The four reference standards, lutein, lycopene, β-cryptoxanthan, and β-carotene were well resolved in this system (Figure 7-1). At the concentrations used in this study, the absorbance at 450nm of all four compounds exhibited linear relationships with coefficients of determination of 0.9999, 0.9909, 1.000, and 0.9999, and RSDs of repeated injections of 0.94%, 1.41%, 2.52%, and 1.09%, respectively. The predominant carotenoid in most of the breadfruit samples was lutein, with an average of 45.0 µg/100 g fresh fruit (SE±4.80). The amount of

lutein ranged from 0.5 µg/100 g fresh fruit (SE±0.10) to 580.40 µg/100 g fresh fruit (SE±114.97). The next most abundant carotenoid observed in breadfruit was β-carotene, with an average of 15.3 µg/100 g fresh fruit (SE±0.54). Cultivars ranged from having no detectable β-carotene up to 61.9 µg/100 g fresh fruit (SE±10.42). Lycopene was detected in trace amounts in approximately 14.3% of the samples but was below the limit of quantification in all but one sample. A similar pattern was observed with β-cryptoxanthan, where approximately 7.0% of the samples contained trace amounts but it was only quantifiable in a single cultivar. Many cultivars contained α-carotene, identified by its retention time relative to β-carotene together with its absorbance spectrum (Davey et al., 2006; Tan and Soderstrom, 1989). The average α-carotene content was estimated to be 2.6 µg/100 g fresh fruit (SE±0.27), and varied from no detectible levels to a maximum of 26.9 µg/100 g fresh fruit (SE±3.16). A number of other unidentified carotenoids were present to varying degrees in some samples. On average, the sum of the pro-vitamin A carotenoids (pVACs), β-carotene, α-carotene, and β-cryptoxanthan, represents 32.5% (SE±1.40) of the total carotenoid content, however this, ranged from 0 to 90.1% (SE±6.28) depending on the cultivar. Based on FAO/WHO recommended conversion factors of 1:6 for β-carotene, and 1:12 for the remaining pVACs (FAO and WHO, 2004), breadfruit has an average retinol equivalency of 2.6 µg/100 g fresh fruit (SE±0.11) but varies from zero to 12.5 µg/100 g fresh fruit (SE±1.77) depending on the cultivar.

Comparison of Breadfruit Cultivars and Identification of Elite Accessions

While the total carotenoid content of *A. altilis* × *A. mariannensis* hybrids tended to be greater than *A. altilis* based on both analytical methods, this difference was only statistically significant using the microplate method (Table 7-1). This trend was also observed for lutein, β-

carotene, and retinol equivalents; they are numerically higher in the hybrids but these differences were not statistically significant. The single accession of *Artocarpus mariannensis* included in this study contained more total carotenoids than all but one other cultivar based on the microplate assay, and was among the highest based on HPLC analysis (Table 7-2). This accession is also one of the highest in lutein, β-carotene, and retinol equivalents, and is fairly high in α-carotene. However, because this species was only represented by an individual accession, it is not possible to make a comparison between the species. The *A. mariannensis* included in this study also had a more complex carotenoid profile than *A. altilis* or the hybrids and contained a number of unidentified carotenoids. The seeds of breadnut, *A. camansi*, contain a similar level of lutein as the flesh of *A. altilis* and the hybrids, but only trace amounts of β-carotene or α-carotene were detected.

Total carotenoid content determined by the microplate method shows that there are significant differences among breadfruit cultivars originating from the four geographic regions. Melanesian cultivars contain the highest concentration of total carotenoids, followed by Micronesian, western Polynesian, and eastern Polynesian cultivars (Table 7-1). This trend was similar based on HPLC analysis, but the only statistically significant differences were that Melanesian cultivars contain significantly higher concentrations of total carotenoids, lutein, β-carotene, α-carotene, and retinol equivalents than cultivars from the three other regions. Cultivars from western Polynesia, eastern Polynesia, and Micronesia contain statistically similar concentrations of the measured carotenoids. The ratio of pVAC to total carotenoids was similar among cultivars from all regions.

The 10 cultivars with the highest retinol equivalents have been selected as elite cultivars with potential to contribute significantly to vitamin A requirements (Table 7-2). While these

cultivars were selected based primarily on their β-carotene and α-carotene, the majority of them are generally carotenoid rich and contain among the highest levels of lutein and total carotenoids found in any of the cultivars. Hybrid cultivars represent about 19% of the cultivars evaluated in this study, but they account for three of the ten cultivars identified. Six of the remaining elite cultivars are *A. altilis*, and the final one is *A. mariannensis.* Three of the elite *A. altilis* cultivars are from Melanesia, two are of unknown origin, and could also be from Melanesia, and one is from eastern Polynesia. Of the three hybrid cultivars two originate from Micronesia, one is an early generation hybrid from western Polynesia, and one is from eastern Polynesia. In addition to carotenoid content, a number of important fruit characteristics for these cultivars are included in Table 7-2 to help identify which ones have potential as a staple food crop.

Effect of Cooking on Measured Carotenoid Content

Based on the both the microplate and HPLC techniques, baked and boiled breadfruit contained significantly greater total carotenoid content than raw fruit (Figure 7-2). Based on the microplate assay, raw, baked, or boiled fruit contained 121.4 µg/100 g (SE±12.76), 250.5 µg/100 g (SE±54.04), and 263.7 µg/100 g (SE±40.24) respectively (Figure 7-2). Based on HPLC analysis 25.1 µg/100 g (SE±8.86), 183.8 µg/100 g (SE±57.78), and 178.5 µg/100 g (SE±52.03) was detected from raw, baked and boiled fruit, respectively. The differences in carotenoid content between the raw and cooked breadfruit were also observed in the concentration of individual carotenoids, but cooking affected the various carotenoids differently. Lutein was the most drastically affected with an average of only 17.7 µg/100 g (SE±7.32) in the raw fruit, 154.4 µg/100 g (SE±46.43) in baked fruit, and 152.1 µg/100 g (SE±45.05) in boiled fruit. While the effect was less dramatic on β-carotene, it was still significant and an average of 9.8 µg/100 g

(SE±0.83) was detected in the raw fruit compared to 19.5 µg/100 g (SE±2.70) and 17.7 µg/100 g (SE±2.38) in the baked and boiled fruit, respectively. The effect on α-carotene displayed a similar pattern, but the differences were not significantly different, with an average of 5.0 µg/100 g (SE±2.85) detected in the raw fruit, 9.8 µg/100 g (SE±8.87) in baked fruit, and 8.7 µg/100 g (SE±7.18) in boiled fruit.

Annual Variation of Carotenoids in Breadfruit

There were no significant differences detected in total carotenoid content or any of the individual carotenoids between the two seasons for any of the cultivars included in this portion of the study (See appendix 1).

Discussion

Vitamin A deficiency (VAD) is one of the most widespread micronutrient deficiencies, is the leading cause of preventable childhood blindness, and is responsible for millions of deaths annually (WHO, 2009). The populations at greatest risk of VAD are those that rely heavily on starchy staples that lack pro-vitamin A carotenoids (pVACs) such as rice and wheat, and have relatively low rates of meat, fruit, and vegetable consumption. Promoting increased production and consumption of staple foods that contain pVACs has the potential to reach the most vulnerable segments of society and could improve the vitamin A nutrition of millions of people around the world. Breadfruit is a high yielding staple food crop that grows throughout the tropics. Depending on the cultivar, the starchy fruit ranges from almost white to a rich yellow color due to the presence of carotenoids (Englberger et al., 2003a, 2003b, 2007b; Ragone and Cavaletto, 2006). The predominant carotenoid in breadfruit is lutein, which has significant

health benefits but lacks vitamin A activity (Granado et al., 2003) , however, significant amounts of pVACs have been detected in some cultivars (Englberger et al., 2003a, 2003b, 2007b; Ragone and Cavaletto, 2006). The current study compares the carotenoid profile of a diverse group of breadfruit trees to identify pVAC-rich cultivars, determine the variability in the carotenoid profile among cultivars, and compare species and geographic regions to help guide future efforts to screen for pVAC-rich germplasm.

Large-scale screening efforts for pVAC-rich cultivars of food crops are often restricted by the laborious and expensive methods used for carotenoid analysis (Davey et al., 2006). The initial phase of this project was to determine if a rapid method that measures total carotenoids could be used as a quick screen for pVAC-rich breadfruit (Davey et al., 2006). However, spectrophotometric methods that do not have any prior separation steps cannot distinguish individual carotenoids and are only applicable for pVAC estimates if the carotenoid profile remains consistent among samples (Davey et al., 2006; Tee, 1991). The high degree of variability in the ratio of pVACs to total carotenoids among breadfruit cultivars makes total carotenoid content a poor determinant of its pVAC content. This variability also makes the selection of pVAC-rich cultivars based on the colour of the flesh problematic and unreliable as has been previously recognized (Englberger et al., 2003a). As such, screening breadfruit germplasm for pVAC-rich cultivars cannot be conducted by flesh colour or simple spectrophotometric analysis and requires more advanced methods such as HPLC.

Previous studies have reported breadfruit cultivars with much higher levels of pVACs than what was observed in the current study, up to 868 µg/100 g of β-carotene and 142 µg/100 g of α-carotene (Englberger et al., 2003a). However, these values were observed in the closely related species, *Artocarpus mariannensis*. While *A. mariannensis* is often referred to as

breadfruit, it produces relatively small fruit of about 450 g that are typically eaten when they are ripe and sweet (Ragone and Manner, 2006). The types of breadfruit that are typically eaten as a starchy staple, *A. altilis* and *A. altilis* × *A. mariannensis* hybrids, weigh about 1.5 kg on average and are most often eaten before they ripen when they are still starchy (Ragone, 1997, 2006a; Chapter 3). These types of breadfruit range from almost white to yellow, but are generally less yellow and have lower concentrations of pVACs than *A. mariannensis* (Englberger et al., 2003a, 2003b, 2007b; Ragone and Cavaletto, 2006; Chapter 3). While *A. mariannensis* is a valuable food resource and has potential to improve vitamin A nutrition in the tropics, it should be acknowledged that it is not a true staple food and fills a different niche in the diet.

The current study compares the carotenoid content of more cultivars than have been previously investigated, and identifies a number of cultivars with higher levels of pVACs than have been reported for raw fruit at the mature stage of development (Englberger et al., 2003a, 2003b, 2007b; Ragone and Cavaletto, 2007). One cultivar in particular, "Samoan 1", from Fiji contains an average of 61.9 µg/100 g (SE±10.68) β-carotene, 22.9 µg/100 g (SE±3.05) α-carotene, and 3.3 µg/100 g (SE±1.66) β-cryptoxanthan. While the bioavailability and conversion rate of pVACs into vitamin A varies depending on the plant matrix and how it is prepared, based on FAO recommended conversion rates this is equivalent to approximately 12.5 µg/100 g (SE±1.77) of retinol (FAO and WHO, 2004). Based on typical staple consumption rates of 0.75-1.0kg per day (Englberger et al., 2003a), consuming "Samoan 1" as the primary staple food would provide about 31-42% of the minimum daily requirement of an adult male (FAO and WHO, 2004).

There is a distinct relationship between the geographic origin of breadfruit cultivars and their total carotenoid and pVAC contents. The highest total carotenoid content was found in

cultivars originating from Melanesia, followed by Micronesia and western Polynesia with eastern Polynesian cultivars containing the lowest amount. This matches the differences in flesh colour of cultivars from these regions to a high degree (Chapter 3). It appears that as humans migrated out of Melanesia into Polynesia they selected for white fruit, and subsequently, lower concentrations of carotenoids. Many of the Micronesian cultivars are hybrids between *A. altlis* and *A. mariannensis* (Zerega et al., 2006; Zerega et al., 2004; Zerega et al., 2005), which tend to contain higher levels of total carotenoids than cultivars of *A. altilis*. This pattern was also observed in total carotenoid content and the concentration of individual carotenoids except that the only significant difference was between Melanesian cultivars and those from other regions. Cultivars from the three other geographic regions were not significantly different from one another. These observations suggest that future screening programs to identify pVAC-rich cultivars of breadfruit should focus on cultivars from Melanesia as well as hybrid cultivars from Micronesia.

Several other factors appear to affect the carotenoid content of breadfruit. For example, significantly higher concentrations of carotenoids were detected in fruit that had been boiled or baked than in raw fruit in this study. This phenomenon has been observed in previous studies of breadfruit (Englberger et al., 2003a) and other food items (Dietz and Erdman, 1989). In their natural state in the plant, carotenoids are often protein bound, making them difficult to extract (Dietz and Erdman, 1989). The cooking process may denature the proteins, or otherwise make the carotenoids more amenable to the extraction process, and the increases observed during cooking are likely a result of increased extractability rather than a true increase in the compounds (Dietz and Erdman, 1989). Individual carotenoids in breadfruit were differentially affected by the cooking process, with an increase of nearly 10 fold for lutein and approximately two fold for

β-carotene and α-carotene. A similar difference has previously been reported between raw and boiled *A. mariannensis* fruit in regards to their β-carotene and α-carotene content, but was not observed to this degree in lutein content, which could reflect the different extraction protocols that were employed in these studies (Englberger et al., 2003a). Based on the two-fold increase in pVAC content observed in this study during cooking, consumption of boiled "Samoan 1" as the primary staple food would provide about 62-84% of the minimum pVAC requirement for an adult male (FAO and WHO, 2004). While this cannot replace a well balanced diet, it could make a significant contribution to the nutritional status for millions of people in the tropics.

Another factor that appears to play a major role in the carotenoid content of breadfruit is the ripeness of the fruit (Englberger et al., 2003a, 2003b, 2007b). While breadfruit is most often eaten at the mature, but not yet ripe stage of development (Ragone, 1997; Worrell et al., 1998), reported levels of carotenoids in ripe fruit of *Artocarpus altilis* are much higher than what has been reported for mature fruit or what was observed in the present study (Englberger et al., 2003a, 2003b, 2007a, 2007b; Ragone and Cavaletto, 2006). While further studies are required to determine the chronology and magnitude of carotenoid accumulation in breadfruit, it appears that simply harvesting the fruit at a slightly later stage of development may increase the contribution of breadfruit to vitamin A nutrition, and that educational initiatives to promote this behavior could be beneficial.

The current study identifies a number of relatively pVAC-rich cultivars and provides guidance for future breadfruit screening programs. Melanesia and Micronesia represent two regions with rich genetic resource for pVAC-rich cultivars of breadfruit that have not been thoroughly explored. Further screening programs focusing on these regions may identify cultivars with even greater concentrations of pVACs than those identified in this study. Until

then, however, the cultivars identified here have the potential to provide a much needed source of vitamin A nutrition in the tropics and improve the lives of millions of people.

Table 7-1: Carotenoid composition of 94 accessions of breadfruit grouped by species and geographic origin. Means followed by different letters within a column of each grouping are significantly different at a type 1 error rate of 0.05.

		Individual carotenoid content (µg/100g)			% pVACS[1]	Retinol equivelants[2]	Total Carotenoids (µg/100g)	
Grouped by species		Lutein	β-carotene	α-carotene			HPLC	Microplate
Artocarpus altilis (n=75)	Mean	44.4a	15.0a	2.7a	34.1a	2.6a	55.1a	153.2b
	SE	±5.34	±0.61	±0.70	±1.54	±0.12	±6.18	±10.14
	Min	BQL	BQL	BQL	0	BQL	BQL	56.3
	Max	580.4	61.9	26.9	90.7	12.5	668.4	1008.7
A. altilis x *A. mariannensis* (n=17)	Mean	47.5a	16.2a	2.2a	39.5a	2.8a	65.5a	234.1a
	SE	±10.61	±1.15	±0.58	±3.05	±0.23	±9.48	±29.07
	Min	1.9	9.7	nd	12.2	1.6	11.6	71.6
	Max	168.7	26.4	10.2	83.9	4.6	247.2	965.8
Grouped by region								
Melanesia (n=12)	Mean	112.7a	21.1a	8.12a	33.8a	3.95a	128.5a	276.2a
	SE	±27.19	±2.60	±2.19	±3.86	±0.25	±12.72	±49.99
	Min	2.1	7.2	nd	0	1.2	0	58.3
	Max	580.4	61.9	26.9	64	12.5	668.4	1008.7
Western Polynesia (n=8)	Mean	35.5b	12.5b	0.03b	31.1a	2.5b	47.1b	156.9bc
	SE	±6.94	±0.60	±0.03	±3.81	±0.30	±15.76	±11.98
	Min	11	9.6	nd	13.2	1.6	21.5	116
	Max	87.3	15	0.3	0.6	2.5	99	244.8
Eastern Polynesia (n=41)	Mean	23.3b	13.2b	0.51b	35.0a	2.21b	31.5b	114.8c
	SE	±2.08	±0.42	±0.29	±2.24	±0.15	±7.29	±4.47
	Min	0.5	9.3	nd	0	0	0.3	56.3
	Max	66.7	23.9	10.2	90.7	4.3	80.5	204.6
Micronesia (n=20)	Mean	36.2b	12.5b	0.95b	38.9a	2.07b	49.4b	171.3b
	SE	±5.60	±65.00	±0.35	±2.90	±0.19	±9.90	±12.32
	Min	1.9	9	nd	4.5	1.5	9.9	66.3
	Max	134.7	26.4	5.9	83.9	4.6	160	439

[1] %pVACs refers to the % of total carotenoids with pro-vitamin A activity

[2] Retinol equivalents were calculated using a conversion factor of 1:6 for β-carotene and 1:12 for α-carotene and β-cryptoxanthan

Table 7-2: Provenance information, fruit characteristics, and carotenoid content of 10 elite cultivars of breadfruit (*Artocarpus*, Moraceae) identified for their pro-vitamin A content. Means followed by different letters within each row are significantly different with a type 1 error rate of 0.05.

		Cultivar									
Cultivar name		Samoan 1	Karawa	Unidentified	Dugdug	Sewan	Ulu elise	Unidentified	Huehue	Samoan 3	Faine
NTBG accession number		900234001	900265001	900224001	900252002	890164002	890182001	900226001	790487001	900261001	910269001
NTBG grid ID		N6	C5	U7	A4	T9	X9	P5	27	F7	A9
Species[2]		*A.a.*	*A.a.*	*A.a.*	*A.m*	*A.a* x *A.m*	*A.a* x *A.m*	*A.a.*	*A.a*	*A.a.*	*A.a* x *A.m*
Region		Melanesia	Melanesia	Unknown	Micronesia	Micronesia	W. Polynesia	Unknown	E. Polynesia	Melanesia	Micronesia
Country of origin		Fiji	Fiji	Unknown	Mariana Islands	Chuuk	Tokelau	Unknown	Society Islands	Fiji	Chuuk
Island of origin		Viti Levu	Viti Levu	Unknown	Rota	Moen	Fakaofo	Unknown	Tahaa	Viti Levu	Uman
Flesh colour		light yellow-yellow	light yellow-yellow	creamy- light yellow	light yellow	creamy- light yellow	light yelllow	white-creamy	light yellow	creamy-light yellow	light yellow-yellow
Total carotenoids by microplate (µg/100g)	Mean	1008.4a	647.5b	637.9b	1260.8a	439.0bc	965.8a	329.9bc	204.6c	269.2c	263.9c
	SEM	±169.26	±40.09	±66.59	±151.50	±53.79	±196.31	±4.59	±73.92	±40.56	±33.64
Total carotenoids by HPLC (µg/100g)	Mean	668.4a	370.4bc	271.3cd	516.4ab	160.0def	247.2cde	126.8def	55.1f	157.1def	97.5ef
	SEM	±120.46	±5.43	±37.52	±73.78	±65.86	±64.10	±34.82	±22.31	±25.89	±20.28
Lutein (µg/100g)	Mean	580.4a	307.4b	214.3bc	471.7a	131.9cd	168.7bcd	98.2cd	27.8d	130.6cd	75.6cd
	SEM	114.97	6.07	31.17	66.16	64.74	41.09	31.08	11.03	22.62	18.33
β-carotene (µg/100g)	Mean	61.9a	38.2bcd	38.2bc	42.9b	26.4bcd	24.1cd	24.3cd	23.9cd	23.4cd	21.5d
	SEM	±10.68	±3.00	±5.01	±7.46	±2.80	±1.91	±3.87	±9.58	±4.19	±2.98
α-carotene (µg/100g)	Mean	22.9a	26.9a	18.8ab	1.8c	2.6c	5.7bc	trace	trace	4.7bc	trace
	SEM	±3.05	±3.16	±9.72	±0.95	±1.40	±1.92	-	-	±1.30	-
β-cryptoxanthan (µg/100g)	Mean	3.3a	nd	nd	nd	nd	nd	nd	nd	nd	nd
	SEM	1.66	-	-	-	-	-	-	-	-	-
Lycopene (µg/100g)	Mean	nd	nd	nd	nd	nd	48.7a	nd	nd	nd	nd
	SEM	-	-	-	-	-		-	-	-	-
Retinol Equivilents (µg/100g)[3]	Mean	12.5a	8.3b	7.9b	7.3bc	4.6cd	4.5cd	4.4cd	4.3d	4.2d	3.6d
	SEM	±1.77	±0.49	±0.84	±1.25	±0.47	±0.31	±0.67	±1.62	±0.70	±0.49
% pVAC	Mean	13.8de	17.0bcde	21.1bcd	8.6e	22.5bcd	14.1cde	24.2bc	49.2a	17.1bcde	25.0b
	SEM	±2.60	±0.53	±1.30	±0.52	±6.33	±4.00	±3.29	±0.68	±0.76	±4.91
Fruit weight (g)	Mean	1526.4bcd	1553.2bc	1223.5de	313.4g	979.8ef	715.7f	1706.9b	1647.5bcd	2084.4a	1362.6cd
	SEM	±92.20	±149.44	±96.65	±30.19	±29.69	±74.57	±114.37	±143.64	±206.35	±136.21
Fruit length (cm)	Mean	17.4bcd	16.7cde	15.6e	11.2f	18.4bc	16.4de	18.5b	19.4ab	20.6a	16.4de
	SEM	±0.49	±0.73	±0.47	±0.64	±0.49	±0.76	±0.66	±0.67	±0.78	±0.68
Fruit width (cm)	Mean	14.7ab	14.0ab	13.7b	8.3e	11.8c	10.7d	14.1ab	14.3ab	14.9a	14.0ab
	SEM	±0.39	±0.56	±0.37	±0.33	±0.18	±0.34	±0.25	±0.60	±0.54	±0.43
Seed count[1]	Mean	3.7cd	4.5cd	4.8bc	3.0d	0	3.9cd	6.4ab	0	8.0a	0
	SEM	±0.67	±0.43	±0.74	±0.49	±0.00	±0.55	±0.98	±0.00	±0.87	±0.00

[1] Seed count refers to the average number of seeds showing in a longitudinally halved fruit, not the total number of seeds per fruit

[2] *A.a = Artocarpus altilis, A.m = Artocarpus mariannensis, A.a* x *A.m = Artocarpus altilis × Artocarpus mariannensis* hybrids

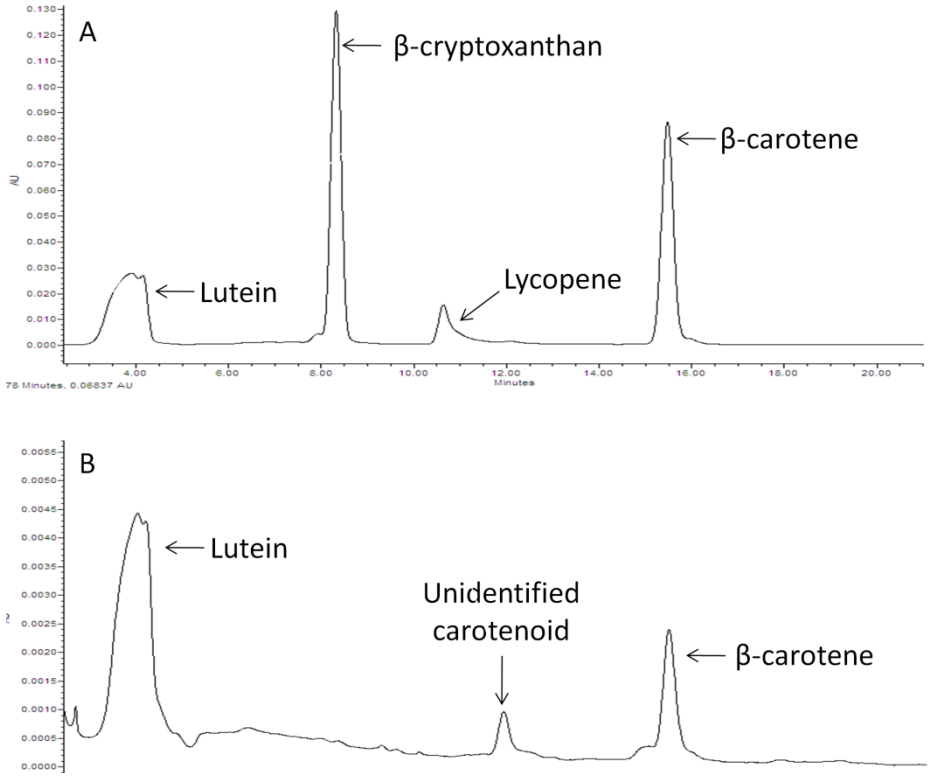

Figure 7-1: HPLC chromatogram showing the absorbance at a wavelength of 450nm for: A, carotenoid standards, and B, an extract of fruit from breadfruit cv. "Yap" (*Artocarpus altilis* × *A. mariannensis*).

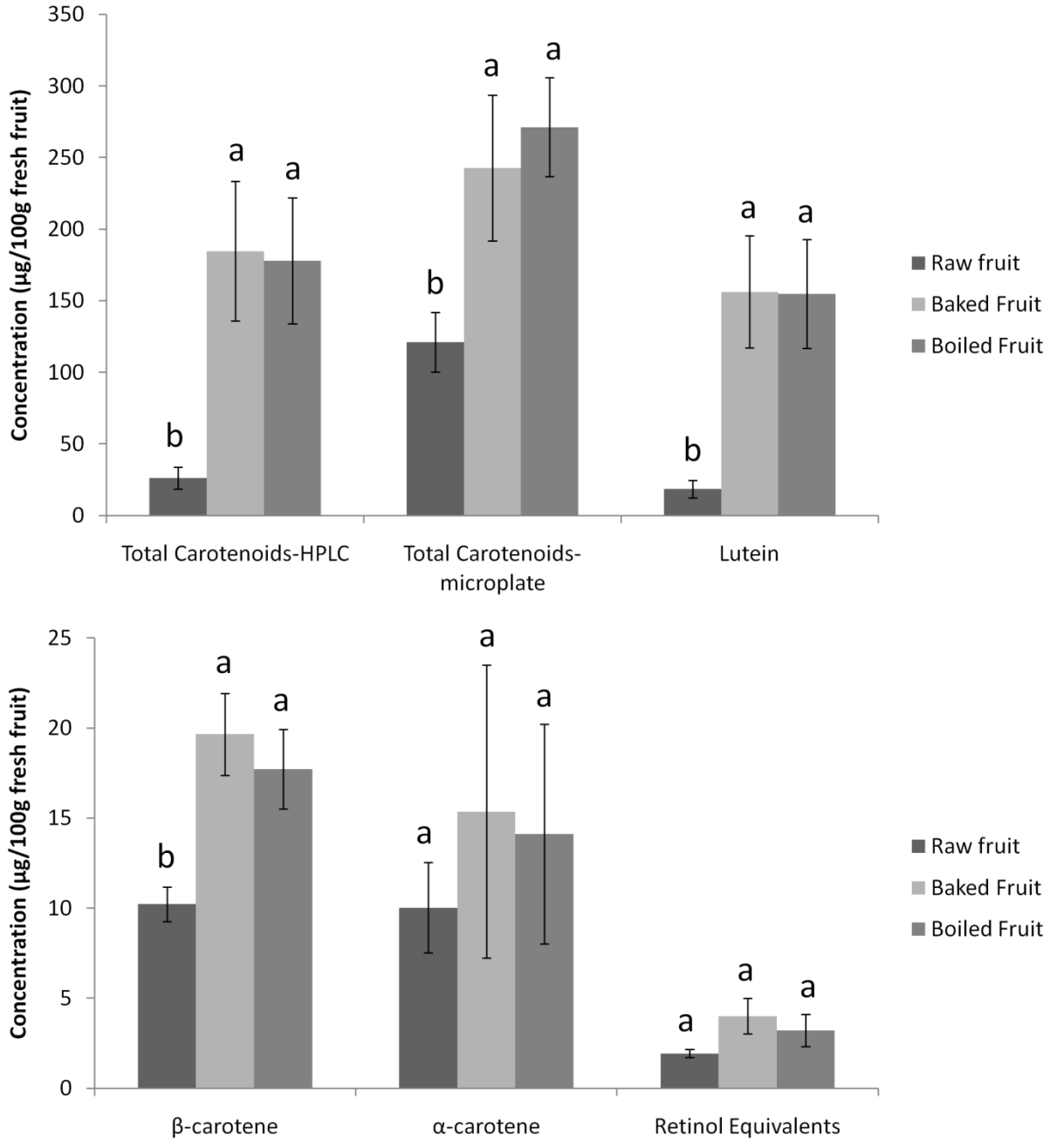

Figure 7-2: The effect of boiling and baking breadfruit on the total carotenoid content determined by HPLC and a microplate assay, lutein, β-carotene, α-carotene, and retinol equivalents. Bars represent the standard error of the mean, bars with different letters above them are significantly different with a type 1 error rate of 0.05.

Chapter 8: Conclusions and Future Directions

The overarching trend that becomes apparent from this set of studies is the sheer amount of diversity that exists within the breadfruit germplasm. Morphological diversity represents the most obvious manifestation of this variability and provides insights into the history of crop domestication as well as providing the basis for informed cultivar selection. The story of breadfruit domestication is intricately linked to human migration patterns and represents thousands of years of human selection (Zerega et al., 2004, 2006). The morphological comparison described in Chapter 3 represents the most comprehensive evaluation and analysis of breadfruit morphology conducted to date and depicts the progressive changes that occurred as was domesticated and bred as humans migrated eastward. These data also describe the morphological traits that were incorporated into the germplasm in Micronesia due to the inter-specific hybridization with a closely related wild relative. Understanding the history of breadfruit domestication and the geographical distribution of this variability provides the necessary framework for identifying regions with unique germplasm to guide conservation efforts, to create the first universal breadfruit cultivar identification key to identify known cultivars, and to provide detailed information to enable informed cultivar selection.

While morphological characteristics are dramatic manifestations of the diversity within the breadfruit germplasm and have obvious ramifications for crop productivity and food security, the differences are more than skin deep and include variability in the seasonality of fruit production. Breadfruit seasonality, along with the short shelf life of the fruit, are major limiting factors hindering the more widespread utilization of the crop as a staple food in the tropics. One way this has been overcome by traditional societies is through the co-cultivation of cultivars with complementary fruiting seasons, resulting in year-round fruit production (Fownes and Raynor,

1991). While this has been observed on some islands, the baseline seasonality data for most cultivars and how/if this will change in new locations needed to achieve year round production throughout the tropics was previously lacking. The in depth comparison of breadfruit seasonality presented in Chapter 4 provides the much needed seasonality data for a diverse group of breadfruit trees and a model to predict the fruiting season by latitude and will enable extended fruit production throughout the tropics.

Another manifestation of the genetic diversity found within the breadfruit germplasm is the variability in the nutritional content of the fruit. A comparison of the protein, mineral, and pro-vitamin A carotenoid content of fresh fruit and flour from a diverse group of breadfruit cultivars are presented in Chapters 5, 6, and 7. Overall, breadfruit contains protein levels similar to several other tropical staples, is a relatively good source of calcium, copper, iron, potassium, and magnesium, lutein, and contains some pro-vitamin A carotenoids. However, there was a significant amount of variability among cultivars in the concentration of all nutrients, often by several orders of magnitude. Some of the nutrient dense cultivars identified in the above Chapters include a cultivar with iron levels almost equal to fortified wheat flour and another with enough pVACs to fulfill over 60% of the minimum daily vitamin A requirement. Identification of nutrient dense elite cultivars provides an approach to improve the nutrition of millions of people throughout the tropics.

Together, this set of studies represents the most detailed and inclusive assessment of the phenotypic diversity within the breadfruit germplasm and emphasizes the importance of cultivar selection for food security. These data provide the necessary information for the identification of elite cultivars for a variety of traits. Elite cultivars may include trees with specific morphological traits such as smooth skin, seedlessness, or large fruit size, they may exhibit a complementary

fruiting season to enable year round production in regions currently limited to a restricted season, or they may represent trees with increased nutritional value to combat specific nutrient deficiencies. Regardless, utilization of elite cultivars that fulfill a specific need will enable efficient utilization of the vast genetic resources found within the breadfruit germplasm and increase food security in the tropics.

In conclusion, the diversity exhibited by breadfruit represents a rich resource to address food insecurity in the tropics and the rest of the world. While the current studies provide a wealth of information to utilize breadfruit, more fundamentally it provides the foundation for further development of this valuable resource. In order to fully realize the potential of breadfruit to provide food security, there is still much research that needs to be done. Some of the future directions that will enable this potential to be fulfilled include:

I. Continue screening breadfruit germplasm for elite cultivars within the breadfruit germplasm to identify, disseminate, and conserve elite cultivars
II. Make identified elite cultivars widely available through international distribution networks
III. Develop value added products from breadfruit to increase demand and develop export markets
IV. Document and preserve traditional breadfruit cultivation and utilization methods
V. Conduct field trials to optimize production systems
VI. Promote the cultivation and consumption of breadfruit and provide educational materials
VII. Expand ex situ germplasm collections and develop strategies for in situ conservation

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Appendix 1: Multivariate analyses of variance (MANOVAs), univarate analyses of variance (ANOVAs) and other supplementary statistics.

1-1 Statistics from chapter 3

1-1-1 MANOVA with species as the independent variable and the quantitative descriptors as the dependent variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	1.197e-10	78.6227	102	3.8965	0.0004*
Pillai's Trace	2.9155581	3.0465	102	9	0.0361*
Hotelling-Lawley	10795954	.	102	-1	.
Roy's Max Root	10795891	952578.63	34	3	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	67636421	1989306.5	34	1	0.0006*

species

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	1.197e-10	78.6227	102	3.8965	0.0004*
Pillai's Trace	2.9155581	3.0465	102	9	0.0361*
Hotelling-Lawley	10795954	.	102	-1	.
Roy's Max Root	10795891	952578.63	34	3	<.0001*

1-1-2 Analysis of variance comparing individual quantitative descriptors among species

Fruit weight

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	14986918	3746729	17.7960	<.0001*
Error	200	42107633	210538		
C. Total	204	57094551			

Fruit Length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	285.9108	71.4777	10.5329	<.0001*
Error	200	1357.2279	6.7861		
C. Total	204	1643.1387			

Fruit width at middle

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	456.0311	114.008	38.8613	<.0001*
Error	200	586.7427	2.934		
C. Total	204	1042.7738			

Fruit width at top

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	104.20241	26.0506	8.4470	<.0001*
Error	193	595.21107	3.0840		
C. Total	197	699.41348			

Fruit width at base

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	58.55350	14.6384	8.9037	<.0001*
Error	193	317.30530	1.6441		
C. Total	197	375.85880			

Length of core

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	102.2352	25.5588	4.6456	0.0013*
Error	200	1100.3485	5.5017		
C. Total	204	1202.5837			

Diameter of core

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	38.18252	9.54563	21.9154	<.0001*
Error	200	87.11352	0.43557		
C. Total	204	125.29605			

Scabbing between sections

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	14.517609	3.62940	11.9758	<.0001*
Error	202	61.218150	0.30306		
C. Total	206	75.735759			

Scabbing around center

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	4.046695	1.01167	4.4867	0.0017*
Error	201	45.322099	0.22548		
C. Total	205	49.368794			

Flesh colour

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	7.797862	1.94947	5.4487	0.0003*
Error	202	72.273042	0.35779		
C. Total	206	80.070904			

Amount of latex

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	4.442531	1.11063	5.6455	0.0003*
Error	202	39.739014	0.19673		
C. Total	206	44.181545			

Length of peduncle

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	43.18409	10.7960	1.2923	0.2834
Error	59	492.88944	8.3541		
C. Total	63	536.07353			

Seed count

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	3653.8026	913.451	81.1884	<.0001*
Error	201	2261.4500	11.251		
C. Total	205	5915.2526			

Seed length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	0.485385	0.121346	0.7448	0.5644
Error	78	12.707518	0.162917		
C. Total	82	13.192903			

Seed diameter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	1.105760	0.276440	1.9542	0.1098
Error	78	11.034083	0.141463		
C. Total	82	12.139843			

Length of male inflorescence

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	525.0002	131.250	3.9091	0.0045*
Error	192	6446.4293	33.575		
C. Total	196	6971.4295			

Diameter of male inflorescence

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	3.623751	0.905938	2.4078	0.0509
Error	192	72.240347	0.376252		
C. Total	196	75.864098			

Leaf length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	2166.012	541.503	11.8483	<.0001*
Error	217	9917.541	45.703		
C. Total	221	12083.554			

Leaf width

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	1906.0171	476.504	15.4533	<.0001*
Error	217	6691.2324	30.835		
C. Total	221	8597.2495			

Width to length ratio

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	0.19542116	0.048855	20.7274	<.0001*
Error	217	0.51147639	0.002357		
C. Total	221	0.70689755			

Leaf margin

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	6.343755	1.58594	6.2477	<.0001*
Error	217	55.083632	0.25384		
C. Total	221	61.427387			

Distance to widest point

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	640.3128	160.078	7.5938	<.0001*
Error	217	4574.4123	21.080		
C. Total	221	5214.7250			

Number of lobes

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	181.87676	45.4692	19.0643	<.0001*
Error	217	517.55495	2.3850		
C. Total	221	699.43171			

Lobe spacing

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	21.83869	5.45967	9.1448	<.0001*
Error	217	129.55415	0.59702		
C. Total	221	151.39284			

Distance to first lobe

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	561.6956	140.424	12.5855	<.0001*
Error	217	2421.2001	11.158		
C. Total	221	2982.8957			

Lobe length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	1045.3298	261.332	19.9671	<.0001*
Error	215	2813.9513	13.088		
C. Total	219	3859.2811			

Sinus depth

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	154.17675	38.5442	10.9822	<.0001*
Error	217	761.60401	3.5097		
C. Total	221	915.78076			

Distance to first sinus

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	238.6190	59.6547	9.8812	<.0001*
Error	217	1310.0703	6.0372		
C. Total	221	1548.6893			

Dissection ratio

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	6463.770	1615.94	10.5494	<.0001*
Error	217	33239.666	153.18		
C. Total	221	39703.436			

Petiole length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	10.20064	2.55016	3.7342	0.0059*
Error	197	134.53401	0.68291		
C. Total	201	144.73465			

Petiole Diameter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	1.0686610	0.267165	9.2892	<.0001*
Error	217	6.2410831	0.028761		
C. Total	221	7.3097441			

Upper leaf hair amount

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	31.89382	7.97346	7.9713	<.0001*
Error	217	217.06005	1.00028		
C. Total	221	248.95387			

Upper leaf hair length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	6.789372	1.69734	4.6383	0.0013*
Error	217	79.409412	0.36594		
C. Total	221	86.198784			

Lower leaf hair amount

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	17.82299	4.45575	3.7772	0.0054*
Error	217	255.98494	1.17965		
C. Total	221	273.80793			

Lower leaf hair length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	4	12.99570	3.24893	4.5293	0.0016*
Error	217	155.65551	0.71731		
C. Total	221	168.65122			

1-1-3 Supplementary statistics for linear discriminant analysis of breadfruit cultivars grouped by species using 29 quantitative descriptors

Eigenvalue	Percent	Cum Percent	Canonical Corr
3.20232408	44.3843	44.3843	0.87294699
2.36992155	32.8472	77.2314	0.83860431
0.92538603	12.8259	90.0573	0.69327025
0.71736641	9.9427	100.0000	0.64630726
5.0029e-15	0.0000	100.0000	0
1.1804e-15	0.0000	100.0000	0
6.3998e-16	0.0000	100.0000	0
5.4213e-16	0.0000	100.0000	0
3.8006e-16	0.0000	100.0000	0
3.4547e-16	0.0000	100.0000	0
2.2757e-16	0.0000	100.0000	0
1.8926e-16	0.0000	100.0000	0
1.4146e-16	0.0000	100.0000	0
6.9422e-17	0.0000	100.0000	0
3.1277e-17	0.0000	100.0000	0
2.0347e-17	0.0000	100.0000	0
2.9612e-18	0.0000	100.0000	0
-2.398e-17	0.0000	100.0000	0
-5.67e-17	0.0000	100.0000	0
-1.126e-16	0.0000	100.0000	0
-1.715e-16	0.0000	100.0000	0
-2.724e-16	0.0000	100.0000	0
-3.147e-16	0.0000	100.0000	0
-5.767e-16	0.0000	100.0000	0
-7.906e-16	0.0000	100.0000	0
-9.786e-16	0.0000	100.0000	0
-2.525e-15	0.0000	100.0000	0
-7.327e-15	0.0000	100.0000	0

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0213555	9.4822	112	649.96	<.0001*
Pillai's Trace	2.3636303	8.5634	112	664	<.0001*
Hotelling-Lawley	7.2149981	10.4098	112	545.07	<.0001*
Roy's Max Root	3.2023241	18.9852	28	166	<.0001*

1-1-4 Statistical information for linear discriminant analysis of breadfruit cultivars grouped by species with Artocarpus altilis and A. altilis x A. mariannensis hybrids combined using 29 quantitative descriptorts

Eigenvalue	Percent	Cum Percent	Canonical Corr
2.80180273	71.7396	71.7396	0.85846776
1.10371236	28.2604	100.0000	0.72432717
2.7805e-15	0.0000	100.0000	0
1.1789e-15	0.0000	100.0000	0
9.0114e-16	0.0000	100.0000	0
5.9497e-16	0.0000	100.0000	0
3.2022e-16	0.0000	100.0000	0
2.4747e-16	0.0000	100.0000	0
1.7012e-16	0.0000	100.0000	0
1.4338e-16	0.0000	100.0000	0
8.4498e-17	0.0000	100.0000	0
4.21e-17	0.0000	100.0000	0
2.2986e-17	0.0000	100.0000	0
2.0009e-17	0.0000	100.0000	0
7.0728e-18	0.0000	100.0000	0
-1.018e-18	0.0000	100.0000	0
-4.39e-18	0.0000	100.0000	0
-1.098e-17	0.0000	100.0000	0
-2.073e-17	0.0000	100.0000	0
-2.654e-17	0.0000	100.0000	0
-4.104e-17	0.0000	100.0000	0
-6.924e-17	0.0000	100.0000	0
-1.425e-16	0.0000	100.0000	0
-2.074e-16	0.0000	100.0000	0
-3.595e-16	0.0000	100.0000	0
-7.722e-16	0.0000	100.0000	0
-1.511e-15	0.0000	100.0000	0
-7.297e-15	0.0000	100.0000	0

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.1250328	10.7725	56	330	<.0001*
Pillai's Trace	1.2616167	10.1297	56	332	<.0001*
Hotelling-Lawley	3.9055151	11.4432	56	303.63	<.0001*
Roy's Max Root	2.8018027	16.6107	28	166	<.0001*

1-1-5 MANOVA with region of origin as the independent variable and the quantitative morphological descriptors as the dependent variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.1346327	2.7578	116	487.37	<.0001*
Pillai's Trace	1.4003224	2.3218	116	500	<.0001*
Hotelling-Lawley	3.2359351	3.3641	116	405.33	<.0001*
Roy's Max Root	2.2740018	9.8017	29	125	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	149.93792	630.7733	29	122	<.0001*

region split 2

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.1346327	2.7578	116	487.37	<.0001*
Pillai's Trace	1.4003224	2.3218	116	500	<.0001*
Hotelling-Lawley	3.2359351	3.3641	116	405.33	<.0001*
Roy's Max Root	2.2740018	9.8017	29	125	<.0001*

1-1-6 ANOVAs of individual quantitative descriptors among breadfruit cultivars grouped by geographic origin (Melanesia, western Polynesia, Eastern Polynesia, and Micronesia)

Fruit weight

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	4958212	1652737	6.0663	0.0006*
Error	186	50675024	272446		
C. Total	189	55633236			

Fruit Length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	63.3847	21.1282	2.5714	0.0556
Error	186	1528.3151	8.2167		
C. Total	189	1591.6998			

Fruit width at middle

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	111.4859	37.1620	7.5610	<.0001*
Error	186	914.1816	4.9150		
C. Total	189	1025.6675			

Fruit width at top

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	63.01216	21.0041	6.0728	0.0006*
Error	179	619.11223	3.4587		
C. Total	182	682.12439			

Fruit width at base

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	24.25230	8.08410	4.2930	0.0059*
Error	179	337.07534	1.88310		
C. Total	182	361.32764			

Length of core

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	18.4927	6.16423	1.0157	0.3869
Error	186	1128.8027	6.06883		
C. Total	189	1147.2954			

Diameter of core

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	22.43949	7.47983	14.3230	<.0001*
Error	186	97.13393	0.52223		
C. Total	189	119.57342			

Scabbing between sections

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	4.109121	1.36971	3.8494	0.0105*
Error	187	66.539350	0.35583		
C. Total	190	70.648470			

Scabbing around center

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	1.763911	0.587970	2.3780	0.0713
Error	186	45.990216	0.247259		
C. Total	189	47.754127			

Flesh colour

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	7.207691	2.40256	7.1352	0.0002*
Error	166	55.895118	0.33672		
C. Total	169	63.102808			

Amount of latex

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	5.144217	1.71474	9.5157	<.0001*
Error	166	29.913463	0.18020		
C. Total	169	35.057680			

Length of peduncle

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	68.52227	22.8408	2.8610	0.0449*
Error	56	447.08141	7.9836		
C. Total	59	515.60369			

Seed count

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	394.99103	131.664	61.1701	<.0001*
Error	165	355.14898	2.152		
C. Total	168	750.14001			

Seed length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	1.1108684	0.370289	2.8878	0.0433*
Error	57	7.3088670	0.128226		
C. Total	60	8.4197354			

Seed diameter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	1.9115853	0.637195	5.1528	0.0032*
Error	57	7.0486593	0.123661		
C. Total	60	8.9602447			

Length of male inflorescence

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	452.0099	150.670	4.3122	0.0058*
Error	176	6149.4821	34.940		
C. Total	179	6601.4919			

Diameter of male inflorescence

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	6.256471	2.08549	5.4500	0.0013*
Error	176	67.348477	0.38266		
C. Total	179	73.604948			

Leaf length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	344.5249	114.842	2.6674	0.0494*
Error	167	7190.0887	43.054		
C. Total	170	7534.6136			

Leaf width

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	379.0986	126.366	4.3192	0.0058*
Error	167	4885.9505	29.257		
C. Total	170	5265.0491			

Width to length ratio

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	0.03903449	0.013011	4.2493	0.0062*
Error	201	0.61547276	0.003062		
C. Total	204	0.65450725			

Leaf margin

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	1.439102	0.479701	1.8814	0.1339
Error	201	51.248898	0.254970		
C. Total	204	52.688000			

Distance to widest point

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	199.5113	66.5038	3.1945	0.0250*
Error	167	3476.5979	20.8180		
C. Total	170	3676.1093			

Number of lobes

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	104.30435	34.7681	16.7335	<.0001*
Error	167	346.98524	2.0778		
C. Total	170	451.28959			

Lobe spacing

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	8.89655	2.96552	4.4617	0.0047*
Error	201	133.59789	0.66467		
C. Total	204	142.49444			

Distance to first lobe

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	10.1775	3.3925	0.2659	0.8500
Error	201	2564.9496	12.7609		
C. Total	204	2575.1271			

Lobe length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	208.3276	69.4425	4.2638	0.0061*
Error	199	3241.0418	16.2866		
C. Total	202	3449.3694			

Sinus depth

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	154.10234	51.3674	15.2704	<.0001*
Error	201	676.13737	3.3639		
C. Total	204	830.23971			

Distance to first sinus

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	58.2439	19.4146	2.8665	0.0377*
Error	201	1361.3686	6.7730		
C. Total	204	1419.6125			

Dissection ratio

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	4528.114	1509.37	9.2115	<.0001*
Error	201	32935.322	163.86		
C. Total	204	37463.436			

Petiole length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	3.00646	1.00215	1.4907	0.2193
Error	155	104.20478	0.67229		
C. Total	158	107.21124			

Petiole Diameter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	0.4755192	0.158506	5.0125	0.0023*
Error	201	6.3561064	0.031622		
C. Total	204	6.8316256			

Upper leaf hair amount

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	4.74886	1.58295	1.6434	0.1813
Error	167	160.86073	0.96324		
C. Total	170	165.60959			

Upper leaf hair length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	1.609130	0.536377	1.8342	0.1429
Error	167	48.836134	0.292432		
C. Total	170	50.445263			

Lower leaf hair amount

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	22.65912	7.55304	8.0956	<.0001*
Error	167	155.80766	0.93298		
C. Total	170	178.46678			

Lower leaf hair length

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region split 2	3	5.22480	1.74160	3.0212	0.0313*
Error	167	96.27040	0.57647		
C. Total	170	101.49520			

1-1-7 Statistical information for linear discriminant analysis of breadfruit cultivars grouped by geographic origin (domesticated cultivars only) using 29 quantitative desciptors

Eigenvalue	Percent	Cum Percent	Canonical Corr
1.37006537	77.6232	77.6232	0.76030961
0.23183608	13.1350	90.7583	0.43382448
0.16311799	9.2417	100.0000	0.37448898
6.054e-16	0.0000	100.0000	0
2.1036e-16	0.0000	100.0000	0
1.9063e-16	0.0000	100.0000	0
9.4726e-17	0.0000	100.0000	0
7.1273e-17	0.0000	100.0000	0
4.8861e-17	0.0000	100.0000	0
4.3318e-17	0.0000	100.0000	0
3.4033e-17	0.0000	100.0000	0
3.0362e-17	0.0000	100.0000	0
1.812e-17	0.0000	100.0000	0
1.0789e-17	0.0000	100.0000	0
1.8785e-18	0.0000	100.0000	0
-3.351e-18	0.0000	100.0000	0
-6.507e-18	0.0000	100.0000	0
-1.029e-17	0.0000	100.0000	0
-1.351e-17	0.0000	100.0000	0
-2.874e-17	0.0000	100.0000	0
-4.502e-17	0.0000	100.0000	0
-5.164e-17	0.0000	100.0000	0
-9.356e-17	0.0000	100.0000	0
-1.004e-16	0.0000	100.0000	0
-1.55e-16	0.0000	100.0000	0
-4.629e-16	0.0000	100.0000	0
-7.693e-16	0.0000	100.0000	0
-1.242e-15	0.0000	100.0000	0

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.2944849	27.7091	84	4610.8	0.0000*
Pillai's Trace	0.9065164	23.8624	84	4629	<.0001*
Hotelling-Lawley	1.7650194	32.3533	84	4130.2	0.0000*
Roy's Max Root	1.3700654	75.5004	28	1543	<.0001*

1-2 Statistics from chapter 5

1-2-1 ANOVA comparing the results of three colorometric protein assays (660nm, BCA, and Lowry) used to measure total protein in breadfruit

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
assay	2	30.893952	15.4470	320.2297	<.0001*
Error	69	3.328365	0.0482		
C. Total	71	34.222317			

1-2-2 ANOVA comparing the protein content of fresh breadfruit with breadfruit flour across all accessions

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
fresh or flour	1	171.4830	171.483	59.2380	<.0001*
Error	501	1450.3034	2.895		
C. Total	502	1621.7864			

1-2-3 ANOVA comparing the protein content of fresh fruit among all evaluated breadfruit accessions

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid	81	472.12588	5.82871	59.1664	<.0001*
Error	167	16.45183	0.09851		
C. Total	248	488.57771			

1-2-4 ANOVA comparing the protein content of flour among all evaluated breadfruit accessions

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid	93	847.03105	9.10786	12.7055	<.0001*
Error	160	114.69461	0.71684		
C. Total	253	961.72566			

1-2-5 ANOVA comparing protein content of raw, boiled and baked breadfruit

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
cooking	2	26.44992	13.2250	8.8039	0.0005*
Error	51	76.61078	1.5022		
C. Total	53	103.06071			

1-2-6 ANOVA comparing the protein content of breadfruit between two harvest seasons

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
season	1	0.008046	0.00805	0.0061	0.9383
Error	40	52.972820	1.32432		
C. Total	41	52.980866			

1-2-7 ANOVA comparing the protein content among Artocarpus altilis and A. altilis x A. mariannensis hybrids (fresh fruit)

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Species	1	1.285196	1.28520	3.0998	0.0819
Error	86	35.656169	0.41461		
C. Total	87	36.941365			

1-2-8 MANOVA with breadfruit accession as the independent variable and fruit composition (fruit weight, % edible portion, % peel, % core, and % seed) as the dependent variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0020053	7.4045	372	738.4	<.0001*
Pillai's Trace	2.9795831	5.8713	372	748	<.0001*
Hotelling-Lawley	19.839135	9.7349	372	680.53	<.0001*
Roy's Max Root	12.233851	24.5992	93	187	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1246.8432	57354.789	4	184	<.0001*

grid

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0020053	7.4045	372	738.4	<.0001*
Pillai's Trace	2.9795831	5.8713	372	748	<.0001*
Hotelling-Lawley	19.839135	9.7349	372	680.53	<.0001*
Roy's Max Root	12.233851	24.5992	93	187	<.0001*

1-2-9 ANOVA comparing the differences in fruit composition among breadfruit accessions

Fruit weight

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid	93	80065478	860919	9.8654	<.0001*
Error	187	16318849	87267		
C. Total	280	96384327			

Percent fruit flesh

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid	93	10570.222	113.658	17.1481	<.0001*
Error	187	1239.446	6.628		
C. Total	280	11809.668			

Percentage comprised of peel

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid	93	1014.7076	10.9108	6.8165	<.0001*
Error	187	299.3234	1.6007		
C. Total	280	1314.0311			

Percentage comprised of core

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid	93	2371.7910	25.5031	6.6182	<.0001*
Error	187	720.6032	3.8535		
C. Total	280	3092.3941			

Percentage comprised of peel

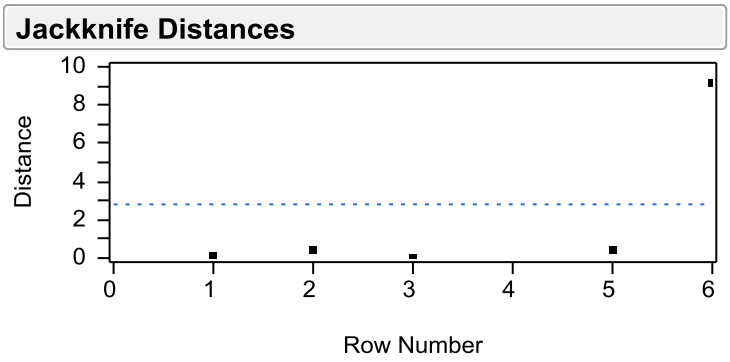
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid	93	1617.5711	17.3932	18.9318	<.0001*
Error	187	171.8028	0.9187		
C. Total	280	1789.3739			

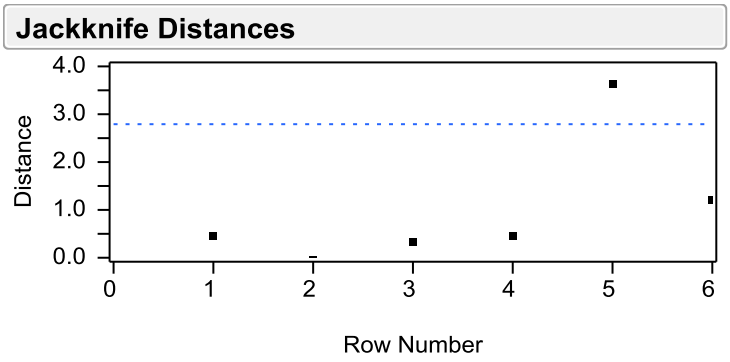
1-3 Statistics from Chapter 6

1-3-1 Comparison of soil parameters in 6 soil collection sites within Kahanu Garden using Jack-knifed distance outlier tests (numbers 1-6 correspond to soil sites A, B, C, D, E, and H, respectively)

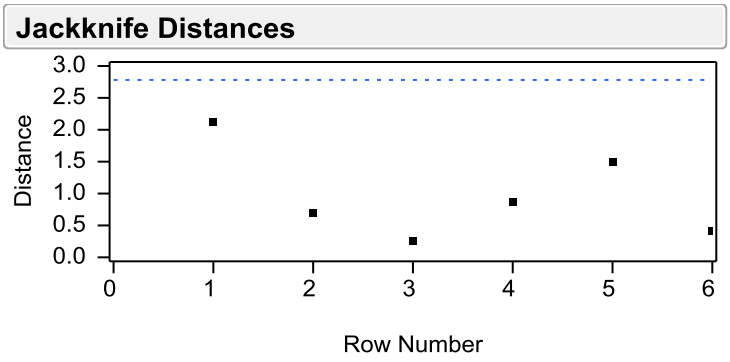
Aluminium



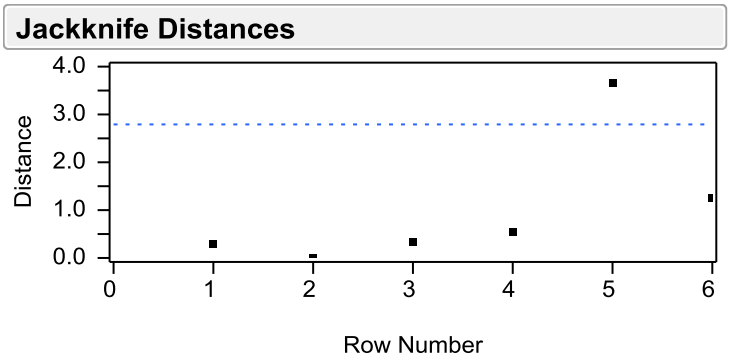
Arsenic



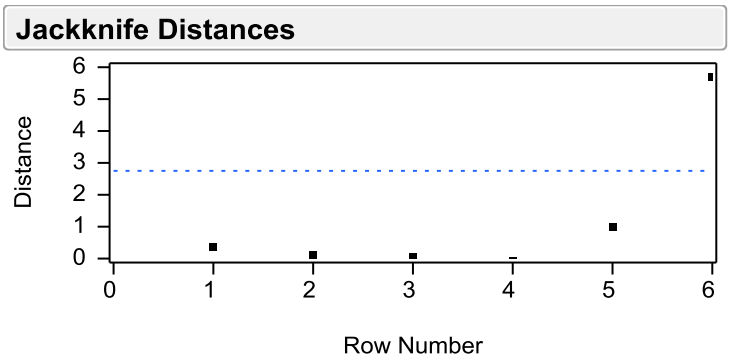
Boron



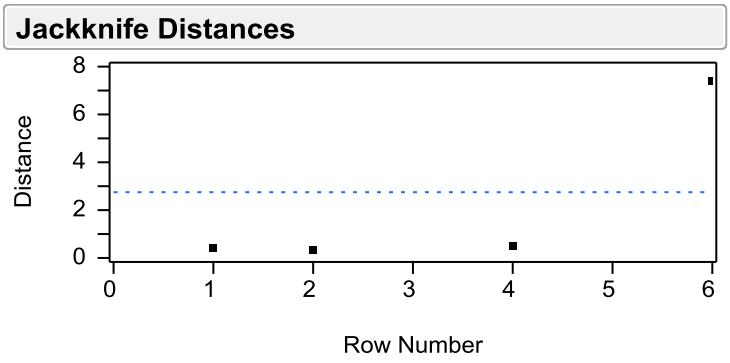
Cadmium



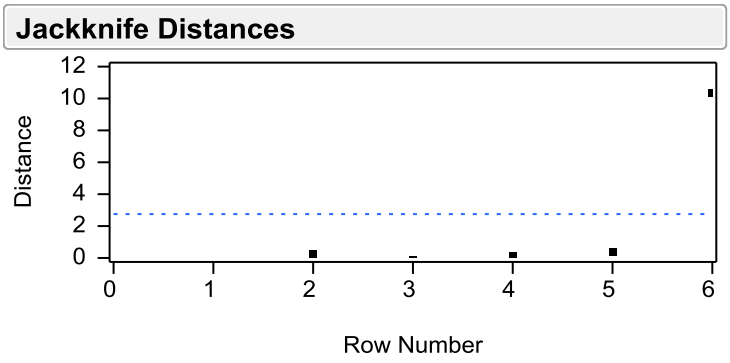
Calcium



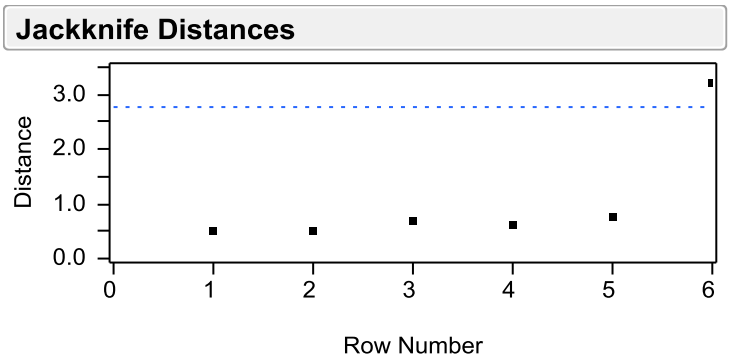
Chromium



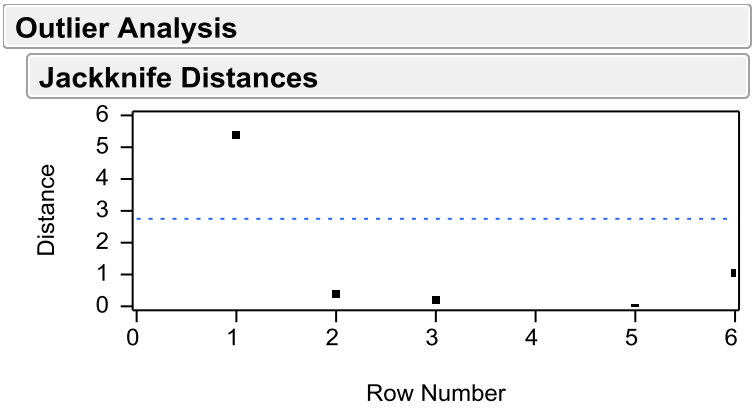
Cobalt



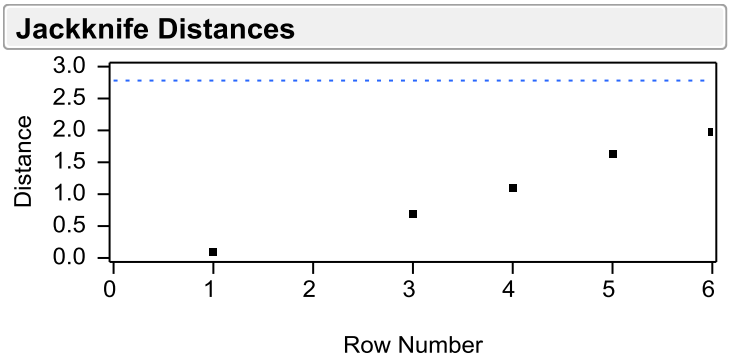
Copper



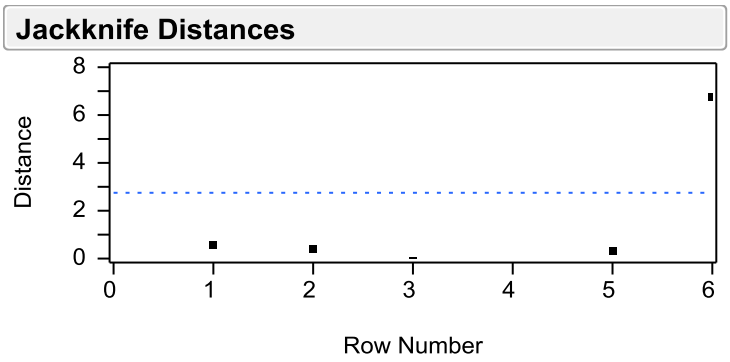
Iron



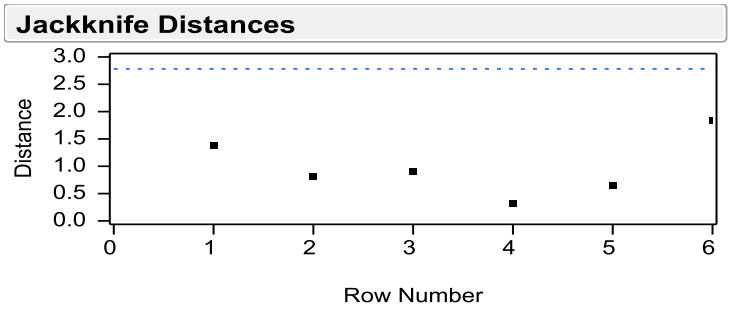
Lead



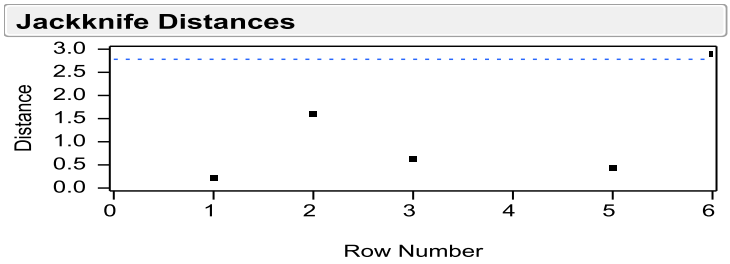
Magnesium



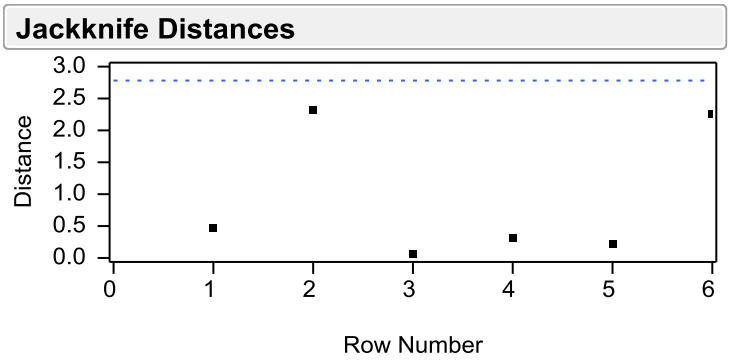
Manganese



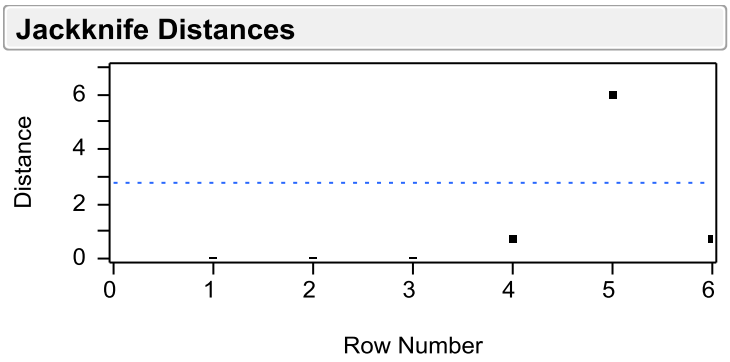
Nichol



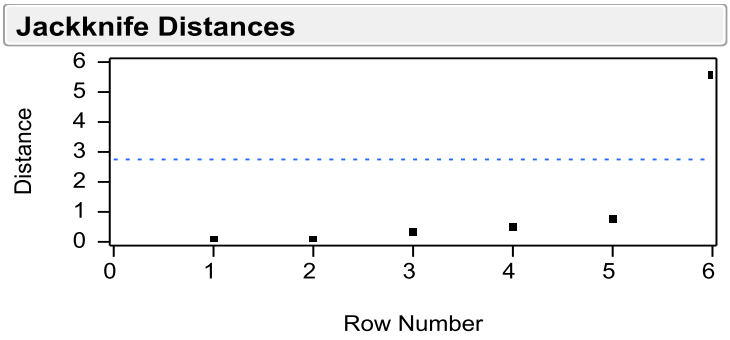
Organic matter



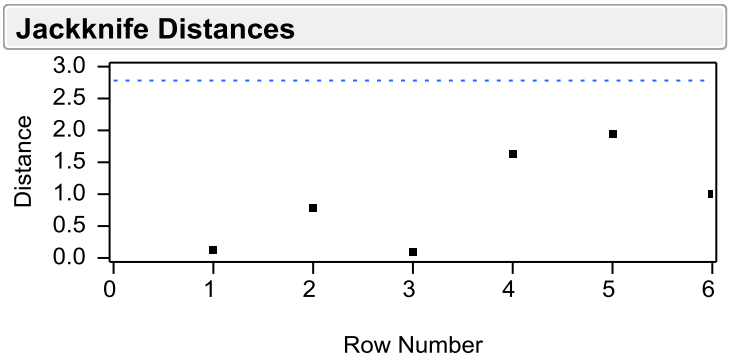
pH



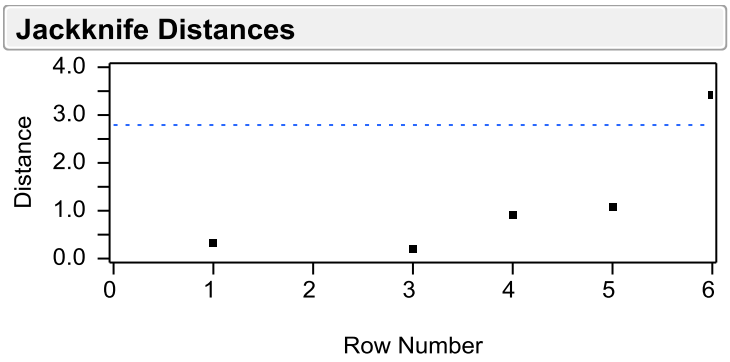
Phosphorous



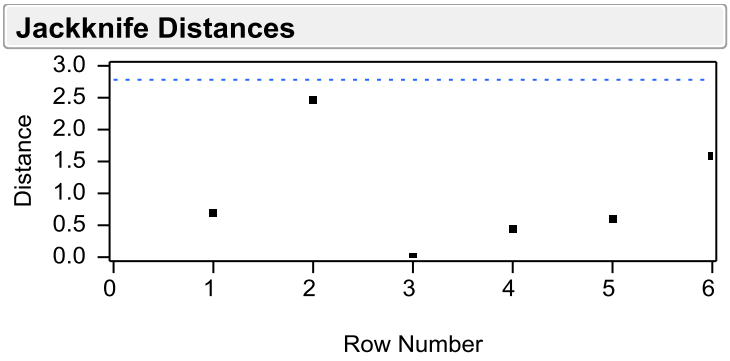
Potassium



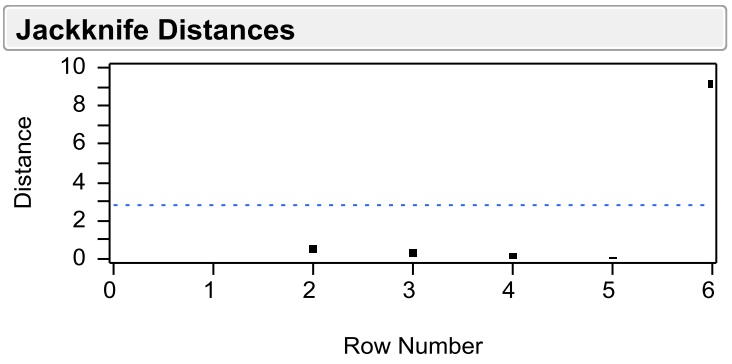
Selenium



Total nitrogen



Zinc



1-3-2 ANOVA comparing the individual mineral concentrations in fruit of breadfruit trees growing in the main grid (soil sites A-E) to trees growing in the smaller area (Soil site H)

Calcium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	52432.3	52432.3	1.3781	0.2437
Error	85	3233898.3	38045.9		
C. Total	86	3286330.7			

Cobalt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	0.00000642	6.417e-6	0.2280	0.6343
Error	85	0.00239268	0.000028		
C. Total	86	0.00239910			

Copper

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	3.826126	3.82613	7.8346	0.0063*
Error	85	41.510634	0.48836		
C. Total	86	45.336760			

Iron

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	0.00015382	0.000154	0.0000	0.9977
Error	85	1590.7732	18.7150		
C. Total	86	1590.7734			

Potassium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	1599917	1599917	0.6266	0.4308
Error	85	217036653	2553372		
C. Total	86	218636571			

Magnesium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	35608.0	35608.0	0.5474	0.4614
Error	85	5529667.2	65054.9		
C. Total	86	5565275.2			

Manganese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	0.000869	0.000869	0.0025	0.9606
Error	85	30.029256	0.353285		
C. Total	86	30.030125			

Sodium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	979684	979684	3.6296	0.0601
Error	85	22942849	269916		
C. Total	86	23922533			

Phosphorous

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	488126.2	488126	8.8865	0.0037*
Error	85	4668984.3	54929		
C. Total	86	5157110.5			

Zinc

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
soil site	1	0.31375	0.31375	0.1791	0.6732
Error	85	148.90307	1.75180		
C. Total	86	149.21682			

1-3-3 MANOVA with grid ID as the independent variable and mineral contents as the dependant variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	1.112e-13	61.4967	900	2061.5	0.0000*
Pillai's Trace	8.119534	21.6714	900	2115	0.0000*
Hotelling-Lawley	1534.0648	383.9485	900	1776.1	0.0000*
Roy's Max Root	1098.9323	2582.4909	100	235	0.0000*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	527.86486	13313.925	9	227	<.0001*

Grid ID

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	1.112e-13	61.4967	900	2061.5	0.0000*
Pillai's Trace	8.119534	21.6714	900	2115	0.0000*
Hotelling-Lawley	1534.0648	383.9485	900	1776.1	0.0000*
Roy's Max Root	1098.9323	2582.4909	100	235	0.0000*

1-3-4 ANOVAs of individual mineral concentrations among breadfruit accessions

Calcium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	12394584	133275	27.0216	<.0001*
Error	189	932181	4932		
C. Total	282	13326765			

Cobalt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	0.00715618	0.000077	6.9896	<.0001*
Error	189	0.00208068	0.000011		
C. Total	282	0.00923686			

Copper

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	156.47160	1.68249	85.7753	<.0001*
Error	189	3.70725	0.01962		
C. Total	282	160.17885			

Iron

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	5694.3405	61.2295	18.7719	<.0001*
Error	189	616.4740	3.2618		
C. Total	282	6310.8145			

Potassium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	664645485	7146726	6.6224	<.0001*
Error	189	203964120	1079175		
C. Total	282	868609605			

Magnesium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	20274659	218007	11.3800	<.0001*
Error	189	3620675	19157		
C. Total	282	23895334			

Manganese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	134.44627	1.44566	48.4831	<.0001*
Error	189	5.63556	0.02982		
C. Total	282	140.08183			

Sodium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	73848414	794069	668.8448	<.0001*
Error	189	224385	1187		
C. Total	282	74072799			

Phosphorous

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	16594951	178440	14.6699	<.0001*
Error	189	2298946	12164		
C. Total	282	18893898			

Zinc

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	93	487.56507	5.24264	47.9929	<.0001*
Error	189	20.64593	0.10924		
C. Total	282	508.21101			

1-3-5 MANOVA with species as the independant variable and mineral contents as the dependent variables

Whole Model

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.1469115	10.3222	9	81	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	42.894805	386.0532	9	81	<.0001*

species

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	1.1469115	10.3222	9	81	<.0001*

1-3-6 ANOVAs evaluating the difference in individual mineral contents among species

Calcium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	4216708.8	527089	13.6453	<.0001*
Error	108	4171804.4	38628		
C. Total	116	8388513.3			

Cobalt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	7	0.00074474	0.000106	4.3700	0.0003*
Error	104	0.00253197	0.000024		
C. Total	111	0.00327671			

Copper

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	106.85125	13.3564	27.8294	<.0001*
Error	108	51.83330	0.4799		
C. Total	116	158.68454			

Iron

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	4323.6866	540.461	43.7632	<.0001*
Error	108	1333.7627	12.350		
C. Total	116	5657.4493			

Potassium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	1433585254	179198157	85.0811	<.0001*
Error	108	227469927	2106203		
C. Total	116	1661055181			

Magnesium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	9809266	1226158	19.3847	<.0001*
Error	108	6831417	63254		
C. Total	116	16640683			

Manganese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	3483.3210	435.415	1319.814	<.0001*
Error	108	35.6299	0.330		
C. Total	116	3518.9509			

Sodium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	7	7926291	1132327	6.5139	<.0001*
Error	104	18078519	173832		
C. Total	111	26004810			

Phosphorous

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	50816371	6352046	122.1147	<.0001*
Error	108	5617839	52017		
C. Total	116	56434210			

Zinc

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	8	2724.2204	340.528	227.5032	<.0001*
Error	108	161.6548	1.497		
C. Total	116	2885.8752			

1-3-7 MANOVA with sample ID as the independent variable and mineral contents as the dependent variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	1.112e-13	61.4967	900	2061.5	0.0000*
Pillai's Trace	8.119534	21.6714	900	2115	0.0000*
Hotelling-Lawley	1534.0648	383.9485	900	1776.1	0.0000*
Roy's Max Root	1098.9323	2582.4909	100	235	0.0000*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	527.86486	13313.925	9	227	<.0001*

Grid ID

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	1.112e-13	61.4967	900	2061.5	0.0000*
Pillai's Trace	8.119534	21.6714	900	2115	0.0000*
Hotelling-Lawley	1534.0648	383.9485	900	1776.1	0.0000*
Roy's Max Root	1098.9323	2582.4909	100	235	0.0000*

1-3-8 ANOVAs of individual mineral concentrations among elite breadfruit cultivars and cereals

Calcium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	10383448	451454	35.0683	<.0001*
Error	51	656551	12874		
C. Total	74	11039999			

Cobalt

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	0.00569582	0.000248	2.8647	0.0009*
Error	51	0.00440874	0.000086		
C. Total	74	0.01010456			

Copper

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	147.93624	6.43201	29.7609	<.0001*
Error	51	11.02227	0.21612		
C. Total	74	158.95851			

Iron

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	5066.8225	220.297	40.4338	<.0001*
Error	51	277.8648	5.448		
C. Total	74	5344.6872			

Potassium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	1481247010	64402044	42.2491	<.0001*
Error	51	77741451.1	1524342.2		
C. Total	74	1558988461			

Magnesium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	15979694	694769	22.2298	<.0001*
Error	51	1593953	31254		
C. Total	74	17573647			

Manganese

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	3088.4104	134.279	512.8470	<.0001*
Error	51	13.3533	0.262		
C. Total	74	3101.7637			

Sodium

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	13472187	585747	1066.891	<.0001*
Error	51	28000	549		
C. Total	74	13500187			

Phosphorous

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	65792880	2860560	16.2664	<.0001*
Error	51	8968714	175857		
C. Total	74	74761594			

Zinc

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Grid ID	23	2329.2044	101.270	42.1175	<.0001*
Error	51	122.6272	2.404		
C. Total	74	2451.8316			

1-3-9 MANOVA with harvest season as the independent variable and mineral contents as the dependent variables

Whole Model

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2660003	1.6256	9	55	0.1308

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	13.969486	85.3691	9	55	<.0001*

harvest season

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.2660003	1.6256	9	55	0.1308

1-4 Statistics from Chapter 7

1-4-1 MANOVA with breadfruit accession as the independent variable and carotenoid concentrations (Total carotenoids, Lutein, beta-carotene, alpha carotene, retinol equivalents, and visual yellowness score) as the dependent variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0004715	1.8268	270	135.07	<.0001*
Pillai's Trace	3.2017314	0.9891	270	150	0.5357
Hotelling-Lawley	40.000071	3.6351	270	91.383	<.0001*
Roy's Max Root	26.630138	14.7945	54	30	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	15.710836	81.6963	5	26	<.0001*

grid ID

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0004715	1.8268	270	135.07	<.0001*
Pillai's Trace	3.2017314	0.9891	270	150	0.5357
Hotelling-Lawley	40.000071	3.6351	270	91.383	<.0001*
Roy's Max Root	26.630138	14.7945	54	30	<.0001*

1-4-2 ANOVAs of individual carotenoid contents among breadfruit accessions

Total carotenoid content by microplate reader

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	93	11194359	120369	21.2926	<.0001*
Error	188	1062787	5653		
C. Total	281	12257146			

Total carotenoid content by HPLC

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	99	2825614.3	28541.6	17.6133	<.0001*
Error	210	340294.8	1620.5		
C. Total	309	3165909.1			

Lutein content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	97	2129622.2	21954.9	15.8097	<.0001*
Error	196	272184.2	1388.7		
C. Total	293	2401806.4			

Beta-carotene content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	91	16938.183	186.134	8.1794	<.0001*
Error	163	3709.288	22.756		
C. Total	254	20647.471			

Alpha-carotene content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	79	4693.9739	59.4174	3.5029	<.0001*
Error	62	1051.6608	16.9623		
C. Total	141	5745.6347			

Retinol equivalents

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	92	702.69871	7.63803	11.5236	<.0001*
Error	162	107.37595	0.66281		
C. Total	254	810.07467			

Ratio of pVACs to total carotenoids

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	97	9.993810	0.103029	2.8550	<.0001*
Error	200	7.217554	0.036088		
C. Total	297	17.211364			

Visual yellow score

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
grid ID	92	84.66775	0.92030	-4.2e+15	0.0000*
Error	196	-4.263e-14	-2.2e-16		
C. Total	288	84.66775			

1-4-3 MANOVA with breadfruit species as the independent variable and carotenoid concentrations (Total carotenoids, Lutein, beta-carotene, alpha carotene, retinol equivalents, and visual yellowness score) as the dependent variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.2817151	13.7913	10	156	<.0001*
Pillai's Trace	0.7617918	9.7207	10	158	<.0001*
Hotelling-Lawley	2.3952497	18.5281	10	114.28	<.0001*
Roy's Max Root	2.328938	36.7972	5	79	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	3.4552606	53.9021	5	78	<.0001*

species

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.2817151	13.7913	10	156	<.0001*
Pillai's Trace	0.7617918	9.7207	10	158	<.0001*
Hotelling-Lawley	2.3952497	18.5281	10	114.28	<.0001*
Roy's Max Root	2.328938	36.7972	5	79	<.0001*

1-4-4 ANOVAs of individual carotenoid concentrations among breadfruit species

Total carotenoid content by microplate reader

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	3	4664829	1554943	56.9357	<.0001*
Error	278	7592317	27310		
C. Total	281	12257146			

Total carotenoid content by HPLC

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	3	635792.2	211931	25.6315	<.0001*
Error	306	2530116.8	8268		
C. Total	309	3165909.1			

Lutein content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	3	541840.4	180613	28.1607	<.0001*
Error	290	1859966.0	6414		
C. Total	293	2401806.4			

Beta-carotene content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	3	2334.102	778.034	10.6636	<.0001*
Error	251	18313.368	72.962		
C. Total	254	20647.471			

Alpha-carotene content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	3	45.3579	15.1193	0.3660	0.7776
Error	138	5700.2768	41.3064		
C. Total	141	5745.6347			

Retinol equivalents

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	3	66.62962	22.2099	7.4984	<.0001*
Error	251	743.44505	2.9619		
C. Total	254	810.07467			

Ratio of pVACs to total carotenoids

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	3	1.318142	0.439381	8.1279	<.0001*
Error	294	15.893223	0.054059		
C. Total	297	17.211364			

Visual yellow score

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
species	2	12.197288	6.09864	24.0679	<.0001*
Error	286	72.470464	0.25339		
C. Total	288	84.667752			

1-4-5 Manova with region of origin as the independent variable and carotenoid concentrations (Total carotenoids, Lutein, beta-carotene, alpha carotene, and retinol equivalents) as the dependent variables

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0896445	11.5769	20	216.53	<.0001*
Pillai's Trace	1.3347516	6.8109	20	272	<.0001*
Hotelling-Lawley	5.5382444	17.7064	20	135.73	<.0001*
Roy's Max Root	4.556099	61.9629	5	68	<.0001*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	4.4741153	58.1635	5	65	<.0001*

region

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.0896445	11.5769	20	216.53	<.0001*
Pillai's Trace	1.3347516	6.8109	20	272	<.0001*
Hotelling-Lawley	5.5382444	17.7064	20	135.73	<.0001*
Roy's Max Root	4.556099	61.9629	5	68	<.0001*

1-4-6 ANOVAs of carotenoid contents among breadfruit cultivars originating from different geographic regions

Total carotenoid content by microplate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	862116.4	287372	10.3731	<.0001*
Error	248	6870493.8	27704		
C. Total	251	7732610.2			

Total carotenoid content by HPLC

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	307112.4	102371	13.9342	<.0001*
Error	272	1998304.8	7347		
C. Total	275	2305417.2			

Lutein content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	242541.0	80847.0	14.1994	<.0001*
Error	257	1463276.0	5693.7		
C. Total	260	1705817.0			

Beta-carotene content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	2042.061	680.687	11.4009	<.0001*
Error	225	13433.500	59.704		
C. Total	228	15475.561			

Alpha carotene content

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	1077.3064	359.102	13.9070	<.0001*
Error	121	3124.4216	25.822		
C. Total	124	4201.7280			

Retinol equivalents

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	89.41025	29.8034	12.6196	<.0001*
Error	225	531.37927	2.3617		
C. Total	228	620.78952			

Ratio of pVACs to total carotenoids

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	0.111076	0.037025	0.6313	0.5954
Error	261	15.306560	0.058646		
C. Total	264	15.417636			

Visual yellow score

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
region	3	20.046151	6.68205	28.8013	<.0001*
Error	254	58.929311	0.23201		
C. Total	257	78.975462			

1-4-7 MANOVA with harvest season as the independent variable and carotenoid concentrations (Total carotenoids, Lutein, beta-carotene, retinol equivalents, and visual yellowness score) as the dependent variables

Mean

Whole Model

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1785065	0.3570	6	12	0.8923

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	18.844908	37.6898	6	12	<.0001*

harvest season

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	0.1785065	0.3570	6	12	0.8923

1-4-8 MANOVA with cooking method (raw, boiled or baked) as the independent variable and carotenoid concentrations (Total carotenoids, Lutein, beta-carotene, and retinol equivalents) as the dependent variables

Mean

Whole Model

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.2615534	3.3437	8	28	0.0082*
Pillai's Trace	0.826481	2.6410	8	30	0.0255*
Hotelling-Lawley	2.4867275	4.2014	8	17.83	0.0056*
Roy's Max Root	2.3430776	8.7865	4	15	0.0007*

Intercept

Test	Value	Exact F	NumDF	DenDF	Prob>F
F Test	25.904211	90.6647	4	14	<.0001*

COOKED

Test	Value	Approx. F	NumDF	DenDF	Prob>F
Wilks' Lambda	0.2615534	3.3437	8	28	0.0082*
Pillai's Trace	0.826481	2.6410	8	30	0.0255*
Hotelling-Lawley	2.4867275	4.2014	8	17.83	0.0056*
Roy's Max Root	2.3430776	8.7865	4	15	0.0007*

1-4-9 ANOVA of individual carotenoid concentrations among raw, boiled and baked breadfruit

Lutein

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
COOKED	2	87669.18	43834.6	6.1887	0.0090*
Error	18	127493.54	7083.0		
C. Total	20	215162.72			

Beta-carotene

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
COOKED	2	344.18295	172.091	6.8332	0.0066*
Error	17	428.13905	25.185		
C. Total	19	772.32200			

Retinol equivalents

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
COOKED	2	15.055238	7.52762	1.7736	0.1981
Error	18	76.397143	4.24429		
C. Total	20	91.452381			

Total carotenoid content by microplate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
COOKED	2	89150.00	44575.0	4.5288	0.0255*
Error	18	177166.09	9842.6		
C. Total	20	266316.09			

Total carotenoid content by HPLC

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
COOKED	2	112424.84	56212.4	5.5038	0.0136*
Error	18	183840.83	10213.4		
C. Total	20	296265.67			

Appendix 2: Provenance information including seasonality group, Tree ID, cultivar name, accession number, species, original location of collection, island of origin, and planting date for the breadfruit trees at the National Tropical Botanical Garden used in the study.

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
1	33	Aarue	780332001	*A.a.*	Society Islands	Huahine	9/12/1979
1	30	Ahani	780333001	*A.a.*	Society Islands	Moorea	9/12/1979
1	I7	Anahonaho	900249001	*A.a.*	Society Islands	Raiatea	3/27/1990
1	H8	Apuapua	890158002	*A.a.*	Society Islands	Raiatea	3/27/1990
1	Z6	Apuapua	890158001	*A.a.*	Society Islands	Raiatea	2/9/1989
1	D7	Araarahaari	900243001	*A.a.*	Society Islands	Tahaa	3/27/1990
1	23	Aumee	780335001	*A.a.*	Society Islands	Huahine	9/12/1979
1	T3	Enua	900256001	*A.a.*	Samoa	Cook Islands	3/27/1990
1	W4	Enua	890472001	*A.a.*	Samoa	Cook Islands	7/27/1989
1	40	Fafai	780330002	*A.a.*	Society Islands	Raiatea	9/12/1979
1	X4	Lemae	890162001	*A.a.*	Mariana Islands	Saipan	2/9/1989
1	10	Luthar	890184001	*A.a.* x *A.m.*	Yap, FSM	Yap	2/9/1989
1	R7	Maire	890459001	*A.a.*	Society Islands	Moorea	7/27/1989
1	M6	Manua	900262001	*A.a.*	Samoa	Savaii	3/27/1990
1	T5	Mei maoi	900239001	*A.a.*	Marquesas Islands	Nuku Hiva	3/27/1990
1	H6	Meikole	900254001	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	3/27/1990
1	R6	Meisaip	890167002	*A.a.*	Pohnpei, FSM	Pohnpei	7/27/1989
1	57	Momolega	770519001	*A.a.*	Samoa	Upolu	12/31/1978
1	P7	Ouo	890464001	*A.a.*	Society Islands	Tahaa	7/27/1989
1	9	Porohiti	790492001	*A.a.*	Society Islands	Moorea	9/12/1979
1	S7	Puurea	890152002	*A.a.*	Society Islands	Raiatea	7/27/1989
1	W1	Puurea	890152001	*A.a.*	Society Islands	Raiatea	2/9/1989
1	E9	Siviri2	910279001	*A.a.*	Vanuatu	Efate	7/10/1991
1	51	Tapehaa	780338001	*A.a.*	Society Islands	Tahaa	12/31/1978
1	V7	Teahimatoa	890465001	*A.a.*	Society Islands	Tahaa	7/27/1989

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
1	E7	Unidentified 02	900228001	*A.a.*	Samoa	Savaii	3/27/1990
1	R9	Unidentified 09	910287001	*A.a.*	Unknown	Unknown	7/10/1991
1	T8	Unidentified 11	910286001	*A.a.*	Unknown	Unknown	7/10/1991
1	V5	Unidentified 13	890455001	*A.a.*	Samoa	Upolu	7/27/1989
1	R4	Uto samoa	890477001	*A.a.*	Fiji	Viti Levu	7/27/1989
1	45	White	810290002	*A.a.*	Seychelles	Mahe	12/31/1981
1	48	White	810290003	*A.a.*	Seychelles	Mahe	12/31/1981
2	I8	Anahonaho	900249002	*A.a.*	Society Islands	Raiatea	3/27/1990
2	S4	Lemae	890162002	*A.a.*	Mariana Islands	Saipan	7/27/1989
2	O4	Meikalak	890478002	*A.a.*	Pohnpei, FSM	Pohnpei	7/27/1989
2	Q7	Meisei	890479001	*A.a.*	Pohnpei, FSM	Pohnpei	7/27/1989
2	W6	Niue	890454001	*A.a.*	Cook Islands	Aitutaki	7/27/1989
2	F8	Piipiia	910266002	*A.a.*	Society Islands	Tahaa	7/10/1991
2	S8	Pua	900244001	*A.a.*	Society Islands	Tahaa	7/10/1991
2	J6	Pulupulu	900233001	*A.a.*	Samoa	Rotuma	3/27/1990
2	K8	Puou	910275001	*A.a.*	Vanuatu/Wallis	Efate	7/10/1991
2	O8	Puou	880691001	*A.a.*	Tonga	-	3/27/1990
2	S9	Sagosago	890475002	*A.a.*	Samoa	Savaii	7/10/1991
2	P9	Samoan 2	900261002	*A.a.*	Fiji	Viti Levu	7/10/1991
2	N6	Samoan 1	900234001	*A.a.*	Fiji	Viti Levu	3/27/1990
2	F7	Samoan 2	900261001	*A.a.*	Fiji	Viti Levu	3/27/1990
2	K9	Siviri3	910276001	*A.a.*	Vanuatu	Efate	7/10/1991
2	P4	Tehelewa	900281001	*A.a.*	Samoa	Solomon Islands	7/27/1989
2	R10	Timbul	910283001	*A.c.*	Indonesia	Bogor	7/10/1991
2	H7	Tuutou auena	900246001	*A.a.*	Society Islands	Raiatea	3/27/1990
2	17	Ulu sina	890155001	*A.a.*	Samoa	Savaii	2/9/1989
2	E4	Unidentified 01	900268001	*A.a.*	Unknown	Unknown	3/27/1990
2	N8	Unidentified 05	900267001	*A.a.*	Unknown	Unknown	3/27/1990

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
2	A8	Uto ni viti	900264001	*A.a.*	Fiji	Viti Levu	3/27/1990
2	S6	Uto vula	890476002	*A.a.*	Fiji	Viti Levu	7/27/1989
3	D6	Abareba	900236001	*A.a.*	Solomon Islands	Malaita	3/27/1990
3	42	Apu	890157001	*A.a.*	Society Islands	Raiatea	2/9/1989
3	L7	Aue	890147002	*A.a.*	Society Islands	Raiatea	3/27/1990
3	N9	Dugdug	900252003	*A.m.*	Mariana Islands	Rota	7/10/1991
3	X7	Fafai	890151001	*A.a.*	Society Islands	Tahaa	2/9/1989
3	G8	Huero	900245001	*A.a.*	Society Islands	Raiatea	3/27/1990
3	C6	Malphang	900259001	*A.a.*	Samoa	Vanuatu	3/27/1990
3	46	Mamaha	890149001	*A.a.*	Society Islands	Raiatea	2/9/1989
3	F6	Mei aueka	900241001	*A.a.*	Marquesas Islands	Nuku Hiva	3/27/1990
3	F5	Mei kiiahi	900238001	*A.a.*	Marquesas Islands	Nuku Hiva	3/27/1990
3	U2	Mei puau	890462001	*A.a.*	Marquesas Islands	Nuku Hiva	7/27/1989
3	G9	Meitehid	910273002	*A.a.*	Pohnpei, FSM	Pohnpei	7/10/1991
3	M7	Pakok	910285001	*A.c.*	Hawaii	Oahu	3/27/1990
3	V3	Patara	890463001	*A.a.*	Society Islands	Tahaa	7/27/1989
3	D4	Tehelewa	900281002	*A.a.*	Samoa	Solomon Islands	3/27/1990
3	I6	Tuutou ooa	900247001	*A.a.*	Society Islands	Raiatea	3/27/1990
3	ZZ5	Ulu afa	890172002	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
3	7	Ulu afa 2	890171002	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
3	6	Ulu afa hamoa	900230001	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
3	ZZ3	Ulu hamoa	890170002	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
3	K6	Ulu sina	890155002	*A.a.*	Samoa	Savaii	3/27/1990
3	L5	Unidentified 03	900235001	*A.a.*	Solomon Islands	Malaita	3/27/1990
3	O9	Unidentified 06	910289001	*A.a.*	Unknown	Unknown	7/10/1991
4	32	Afara	780325001	*A.a.*	Society Islands	Tahiti	9/12/1979
4	V8	Afara	910267001	*A.a.*	Society Islands	Tahaa	7/10/1991
4	A7	Atu	900232001	*A.a.*	Cook Islands	Rarotonga	3/27/1990

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
4	U4	Enua	890472002	*A.a.*	Samoa	Cook Islands	7/27/1989
4	56	Fafai	780330001	*A.a.*	Society Islands	Raiatea	12/31/1978
4	Y1	Hamoa (Maopo)	890154001	*A.a.*	Society Islands	Tahaa	2/9/1989
4	47	Havana pataitai	780291001	*A.a.*	Society Islands	Huahine	9/12/1979
4	27	Huehue	790487001	*A.a.*	Society Islands	Tahaa	9/12/1979
4	P8	Kea	880690001	*A.a.*	Tonga	-	3/27/1990
4	U9	Lemae	890163002	*A.a.* x *A.m.*	Mariana Islands	Rota	7/10/1991
4	36	Mahani	800269001	*A.a.*	Society Islands	-	9/12/1979
4	F9	Meinpohnsakar	910272001	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	7/10/1991
4	B5	Meinpwahr	900255001	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	3/27/1990
4	Y3	Midolab	890183001	*A.a.* x *A.m.*	Palau	Babeldaob	2/9/1989
4	39	Otea	780327001	*A.a.*	Society Islands	Moorea	9/12/1979
4	22	Sewan	890164001	*A.a.* x *A.m.*	Chuuk, FSM	Moen	2/9/1989
4	T9	Sewan	890164002	*A.a.* x *A.m.*	Chuuk, FSM	Moen	7/10/1991
4	Y4	Tahitian	890156001	*A.a.*	Cook Islands	Aitu	2/9/1989
4	B7	Tuutou taatoe	890186002	*A.a.*	Society Islands	Raiatea	3/27/1990
4	Z10	Ulu	040444001	*A.a.*	Hawaii	Maui	pre-1970s
4	28	Ulu elise	890185001	*A.a.* x *A.m.*	Tokelau	Fakaofo	2/9/1989
4	43	White	810290001	*A.a.*	Seychelles	Mahe	12/31/1981
4	38	Yellow	810289002	*A.a.*	Seychelles	Mahe	12/31/1981
5	21	Aue	890147001	*A.a.*	Society Islands	Raiatea	2/9/1989
5	50	Unknown	770444001	*A.c.*	Society Islands	Tahiti	9/12/1979
5	Y8	Ioio	890150001	*A.a.*	Society Islands	Raiatea	2/9/1989
5	M10	Kamansi	910281001	*A.c.*	Philippines	Luzon	7/10/1991
5	R8	Lipet 2	890480003	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	7/10/1991
5	Z3	Mei chon	890165001	*A.a.*	Chuuk, FSM	Moen	2/9/1989
5	B6	Mei puou	900237001	*A.a.*	Marquesas Islands	Nuku Hiva	3/27/1990
5	B9	Meikole	910280001	*A.c.*	Pohnpei, FSM	Pohnpei	7/10/1991

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
5	49	Meinuwe	790497002	*A.a.*	Pohnpei, FSM	Pohnpei	12/31/1981
5	41	Meisaip	890167001	*A.a.*	Pohnpei, FSM	Pohnpei	2/9/1989
5	O7	Meisei	890479002	*A.a.*	Pohnpei, FSM	Pohnpei	7/27/1989
5	4	Meitehid	790493001	*A.a.*	Pohnpei, FSM	Pohnpei	12/31/1981
5	O5	Meitehid	890481001	*A.a.*	Pohnpei, FSM	Pohnpei	7/27/1989
5	V4	Meriaur	890159002	*A.a.*	Palau	Koror	7/27/1989
5	X5	Meriaur	890159001	*A.a.*	Palau	Koror	2/9/1989
5	2	Paea	890153002	*A.a.*	Cook Islands	Aitutaki	3/27/1990
5	8	Pulupulu	900233002	*A.a.*	Samoa	Rotuma	3/27/1990
5	29	Rare	780329001	*A.a.*	Society Islands	Tahiti	9/12/1979
5	20	Roihaa	790486001	*A.a.*	Society Islands	Huahine	9/12/1979
5	V9	Rotuma	910265001	*A.a.* x *A.m.*	Society Islands	Tahaa	7/10/1991
5	13	Tuutou	790491001	*A.a.*	Society Islands	Tahiti	9/12/1979
5	L10	Unidentified 04	910290001	*A.a.*	Unknown	Unknown	7/10/1991
5	P5	Unidentified 07	900226001	*A.a.*	Unknown	Unknown	7/27/1989
5	S5	Unidentified 10	900225001	*A.a.*	Unknown	Unknown	7/27/1989
5	Y6	Unidentified 14	890148001	*A.a.*	Society Islands	Raiatea	2/9/1989
5	W3	Uto dina	890471001	*A.a.*	Samoa	Fiji	7/27/1989
6	H5	Dugdug	900252001	*A.m.*	Mariana Islands	Rota	3/27/1990
6	X8	Ebechab	890160001	*A.a.* x *A.m.*	Palau	Koror	2/9/1989
6	M9	Forari	910278001	*A.a.*	Vanuatu	Efate	7/10/1991
6	V6	Furau	890470001	*A.a.*	Samoa	Rotuma	7/27/1989
6	G5	Huero ninamu	900248001	*A.a.*	Society Islands	Raiatea	3/27/1990
6	C5	Karawa	900265001	*A.a.*	Fiji	Viti Levu	3/27/1990
6	J7	Maire	890459002	*A.a.*	Society Islands	Moorea	3/27/1990
6	C7	Mei kakano	890461002	*A.a.*	Marquesas Islands	Nukuhiva	3/27/1990
6	W2	Mei kakano	890461001	*A.a.*	Marquesas Islands	Nukuhiva	7/27/1989
6	Q9	Mei koeng	890466002	*A.a. x A.m.*	Chuuk, FSM	Losap	7/10/1991

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
6	B8	Mei kopumoko	900242001	*A.a.*	Marquesas Islands	Nuku Hiva	3/27/1990
6	R5	Meikalak	890478001	*A.a.*	Pohnpei, FSM	Pohnpei	7/27/1989
6	D9	Meiuhpw	910271001	*A.a.*	Pohnpei, FSM	Pohnpei	7/10/1991
6	L8	Midolab	890183002	*A.a. x A.m.*	Palau	Babeldaob	7/10/1991
6	L6	Niue	900231001	*A.a.*	Cook Islands	Rarotonga	3/27/1990
6	25	Paea	890153001	*A.a.*	Cook Islands	Aitutaki	2/9/1989
6	T6	Puaa	890460001	*A.a.*	Society Islands	Moorea	7/27/1989
6	53	Puou	770520001	*A.a.*	Samoa	Upolu	12/31/1978
6	A6	Rauulu	900257001	*A.a.*	Samoa	Rotuma	3/27/1990
6	W8	Sagosago	890475001	*A.a.*	Samoa	Savaii	7/27/1989
6	K5	Samoan (Ulu fiti)	900260002	*A.a.*	Fiji	Viti Levu	3/27/1990
6	K7	Samoan (Ulu fiti)	900260001	*A.a.*	Fiji	Viti Levu	3/27/1990
6	E8	Tedailir	910277001	*A.a.*	Vanuatu	Efate	7/10/1991
6	D5	Toro	890456002	*A.a.*	Samoa	Solomon Islands	3/27/1990
6	26	Ulu afa	890178001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
6	Z4	Ulu afa 4	890181001	*A.a.* x *A.m.*	Tokelau	Fakaofo	3/27/1990
6	ZZ6	Ulu elise 2	890182002	*A.a.* x *A.m.*	Tokelau	Fakaofo	3/27/1990
6	24	Ulu hamoa	890180001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
6	Q8	Unidentified 08	910288001	*A.a.*	Unknown	Unknown	7/10/1991
6	U7	Unidentified 12	900224001	*A.a.*	Unknown	Unknown	7/27/1989
7	A4	Dugdug	900252002	*A.m.*	Mariana Islands	Rota	3/27/1990
7	U8	Errud	910652001	*A.a.* x *A.m.*	Palau	Babeldaob	7/10/1991
7	A9	Faine	910269001	*A.a.* x *A.m.*	Chuuk, FSM	Uman	7/10/1991
7	N5	Furau	890470002	*A.a.*	Samoa	Rotuma	3/27/1990
7	W5	Karawa	890457001	*A.a.*	Samoa	Rotuma	7/27/1989
7	S3	Kukumu tasi	890469002	*A.a.*	Samoa	Solomon Islands	7/27/1989
7	X2	Lemae	890163001	*A.a.* x *A.m.*	Mariana Islands	Rota	2/9/1989
7	55	Ma'afala	770517001	*A.a.*	Samoa	Upolu	12/31/1978

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
7	J8	Masee	900263001	*A.a.*	Samoa	Savaii	3/27/1990
7	J5	Mei chocho	900253002	*A.a.* x *A.m.*	Chuuk, FSM	Nama	3/27/1990
7	Q10	Nahnmwal	910274001	*A.a.*	Pohnpei, FSM	Pohnpei	7/10/1991
7	H9	Piipiia	910266001	*A.a.*	Society Islands	Tahaa	7/10/1991
7	U6	Puou	890474001	*A.a.*	Samoa	Upolu	7/27/1989
7	C8	Siviri2	910279002	*A.a.*	Vanuatu	Efate	7/10/1991
7	L9	Te bukiraro	890468002	*A.a.* x *A.m.*	Kiribati	Tarawa	7/10/1991
7	U3	Te mai	890452001	*A.a.* x *A.m.*	Kiribati	Tarawa	7/27/1989
7	T7	Toro	890456001	*A.a.*	Samoa	Solomon Islands	7/27/1989
7	B4	Ulu afa 3	890181002	*A.a.* x *A.m.*	Tokelau	Fakaofo	3/27/1990
7	34	Ulu afa hamoa	890175001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
7	35	Ulu fiti	890258001	*A.a.*	Samoa	Rotuma	2/9/1989
7	C4	Ulu fiti	900368001	*A.a.*	Samoa	Rotuma	3/27/1990
7	Q4	Ulu fiti	890458002	*A.a.*	Samoa	Rotuma	7/27/1989
7	T4	Ulu fiti	890458001	*A.a.*	Samoa	Rotuma	7/27/1989
7	54	Ulu tala	770524001	*A.a.*	Samoa	Upolu	12/31/1978
8	E5	Meiarephe	900266002	*A.a.*	Pohnpei, FSM	Pohnpei	3/27/1990
8	M5	Meiarephe	900266001	*A.a.*	Pohnpei, FSM	Pohnpei	3/27/1990
8	Z9	Meinpadahk	790494001	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	12/31/1981
8	W9	Meinpwuht	890467001	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	7/27/1989
8	J9	Meion	910268001	*A.a.* x *A.m.*	Chuuk, FSM	Moen	7/10/1991
8	1	Piipiia	790489001	*A.a.*	Society Islands	Raiatea	9/12/1979
8	14	Puaa	780328001	*A.a.*	Society Islands	Moorea	9/12/1979
8	16	Puupuu	790485001	*A.a.*	Society Islands	Huahine	9/12/1979
8	15	Rotuma	790490001	*A.a.* x *A.m.*	Society Islands	Tahaa	9/12/1979
8	12	Toneno	790488001	*A.a.*	Society Islands	Tahaa	9/12/1979
8	Z2	Tuutou taatoe	890186001	*A.a.*	Society Islands	Raiatea	2/9/1989
8	52	Ulu ea	770521001	*A.a.*	Samoa	Upolu	12/31/1978

Seasonality Group	Tree ID	Cultivar Name	NTBG Accession Number	Species[1]	Location of Collection[2]	Island of Origin	Planting Date
8	A5	Yap	900250001	*A.a.* x *A.m.*	Palau	Koror	3/27/1990
9	D8	Lipet	910270001	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	7/10/1991
9	N7	Lipet	890480002	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	3/27/1990
9	O6	Lipet	890480001	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	7/27/1989
9	Q6	Mei chocho	900253001	*A.a.* x *A.m.*	Chuuk, FSM	Nama	7/27/1989
9	Y7	Mei koeng	890166001	*A.a.* x *A.m.*	Chuuk, FSM	Nama	2/9/1989
9	E6	Meinpohnsakar	910272002	*A.a.* x *A.m.*	Pohnpei, FSM	Pohnpei	7/10/1991
9	W7	Tahitian	890156002	*A.a.*	Cook Islands	Aitu	7/27/1989
9	V2	Te bukiraro	890468001	*A.a.* x *A.m.*	Kiribati	Tarawa	7/27/1989
9	18	Ulu afa	890174001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
9	Q5	Ulu afa	890453001	*A.a.* x *A.m.*	Tokelau	Nukunonu	7/27/1989
9	19	Yuley	890161001	*A.a.* x *A.m.*	Yap, FSM	Yap	2/9/1989
10	V1	Kukumu tasi	890469001	*A.a.*	Samoa	Solomon Islands	7/27/1989
10	U5	Manang	890473001	*A.a.*	Samoa	Vanuatu	7/27/1989
10	M8	Mei kauhiva	900240001	*A.a.*	Marquesas Islands	Nuku Hiva	3/27/1990
10	G6	Patara	890463002	*A.a.*	Society Islands	Tahaa	3/27/1990
10	3	Ulu afa	890177003	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
10	31	Ulu afa	890177001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
10	37	Ulu afa	890172001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
10	44	Ulu afa	890179001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
10	Z8	Ulu afa	890176001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
10	ZZ8	Ulu afa	890176002	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
10	ZZ9	Ulu afa	890168002	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
10	5	Ulu afa 1	890177002	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990
10	11	Ulu afa elise	890173001	*A.a.* x *A.m.*	Tokelau	Nukunonu	2/9/1989
10	ZZ7	Ulu afa elise	890173002	*A.a.* x *A.m.*	Tokelau	Nukunonu	3/27/1990

Appendix 3: Mineral content and provenance information of a diverse group of breadfruit (*Artocarpus*, Moraceae) cultivars growing in the National Tropical Botanical Garden's breadfruit germplasm repository. Means represent the average mineral content in three replicate flour samples quantified by ICP-OES.

Cultivar name	Accession number	Grid ID	Species	Geographic origin		Mineral content (µg/g dry weight)									
						Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
Piipiia	790489001	1	*Artocarpus altilis*	Society Islands	Mean	496	0.020	4.4	14.1	11518	990	2.3	844	1684	3.3
					SEM	±11.1	±0.0008	±0.09	±0.09	±11.1	±11.1	±0.09	±11.1	±11.1	±0.09
Pulupulu	900233002	8	*Artocarpus altilis*	Rotuma	Mean	589	0.006	1.6	8.5	9029	1045	1.6	352	1169	2.5
					SEM	±18.7	±0.0031	±0.06	±0.28	±295.7	±39.8	±0.04	±8.6	±58.3	±0.11
Porohiti	790492001	9	*Artocarpus altilis*	Society Islands	Mean	566	0.004	3.3	9.2	11575	1124	1.9	130	1532	3.0
					SEM	±0.8	±0.0039	±0.06	±0.02	±399.7	±52.8	±0.01	±15.6	±67.4	±0.13
Luthar	890184001	10	*A. altilis × A. mariannensis*	Yap, FSM	Mean	987	0.000	4.4	20.7	13476	1228	2.3	1864	2117	4.0
					SEM	±17.2	±0.0000	±0.06	±0.78	±534.8	±32.5	±0.03	±20.7	±49.2	±0.11
Tuutou	790491001	13	*Artocarpus altilis*	Society Islands	Mean	446	0.012	3.3	11.8	16212	1143	2.8	132	1844	2.3
					SEM	±8.0	±0.0017	±0.10	±0.19	±572.9	±14.2	±0.03	±16.5	±31.1	±0.08
Puupuu	790485001	16	*Artocarpus altilis*	Society Islands	Mean	331	0.013	3.1	9.4	11078	924	2.6	147	1406	1.8
					SEM	±3.7	±0.0026	±0.13	±0.06	±280.2	±36.1	±0.03	±3.7	±39.9	±0.02
Yuley	890161001	19	*A. altilis × A. mariannensis*	Yap, FSM	Mean	844	0.007	2.7	20.1	10518	1172	2.2	3866	1386	2.9
					SEM	±41.6	±0.0047	±0.09	±0.67	±401.3	±39.3	±0.07	±125.2	±30.9	±0.11
Roihaa	790486001	20	*Artocarpus altilis*	Society Islands	Mean	600	0.004	1.9	7.4	10152	843	0.9	228	1250	1.8
					SEM	±7.9	±0.0014	±0.01	±0.07	±153.9	±14.9	±0.01	±7.7	±18.6	±0.12
Huehue	790487001	27	*A. altilis × A. mariannensis*	Society Islands	Mean	294	0.006	3.3	15.8	13281	1064	1.1	1968	1527	3.4
					SEM	±12.9	±0.0004	±0.07	±1.06	±609.6	±49.9	±0.05	±74.4	±62.4	±0.17
Rare	780329001	29	*Artocarpus altilis*	Society Islands	Mean	330	0.006	2.4	7.6	9606	729	0.9	180	1202	1.5
					SEM	±5.2	±0.0007	±0.06	±0.11	±234.0	±4.6	±0.01	±3.3	±51.6	±0.02
Ahani	780333001	30	*Artocarpus altilis*	Society Islands	Mean	443	0.010	2.3	8.4	10069	821	1.3	150	1122	2.1
					SEM	±8.9	±0.0009	±0.10	±0.11	±362.7	±37.3	±0.01	±15.0	±26.0	±0.08
Ulu fiti	890258001	35	*Artocarpus altilis*	Rotuma	Mean	1491	0.019	5.6	18.3	14375	2281	3.8	180	2379	10.7
					SEM	±57.0	±0.0010	±0.06	±0.50	±681.6	±79.0	±0.09	±3.2	±82.2	±0.45
Mahani	800269001	36	*Artocarpus altilis*	Society Islands	Mean	648	0.021	2.9	9.1	9681	925	1.6	135	1198	2.2
					SEM	±6.0	±0.0016	±0.01	±0.05	±355.9	±51.9	±0.01	±1.5	±27.0	±0.03
Yellow	810289002	38	*Artocarpus altilis*	Seychelles	Mean	687	0.031	4.9	11.5	14962	1136	1.7	106	1881	2.9
					SEM	±24.7	±0.0011	±0.01	±0.18	±999.7	±69.1	±0.04	±7.8	±83.8	±0.13
Otea	780327001	39	*Artocarpus altilis*	Society Islands	Mean	541	0.020	2.7	10.6	9140	757	1.3	74	1238	2.1
					SEM	±4.9	±0.0004	±0.02	±0.08	±376.8	±44.5	±0.01	±6.8	±51.2	±0.05

Cultivar name	Accession number	Grid ID	Species	Geographic origin		Mineral content (µg/g dry weight)									
						Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
Fafai	780330002	40	*Artocarpus altilis*	Society Islands	Mean	646	0.007	2.5	9.0	11292	942	1.0	98	1252	1.7
					SEM	±10.1	±0.0005	±0.06	±0.03	±533.6	±37.4	±0.02	±5.1	±19.7	±0.07
Apu	890157001	42	*Artocarpus altilis*	Society Islands	Mean	565	0.007	2.3	9.5	11422	904	1.4	70	1402	2.0
					SEM	±14.3	±0.0013	±0.02	±0.20	±254.9	±7.1	±0.02	±3.1	±4.7	±0.18
White	810290001	43	*Artocarpus altilis*	Seychelles	Mean	604	0.007	3.5	9.9	11577	875	1.3	160	1429	1.6
					SEM	±25.1	±0.0008	±0.05	±0.29	±784.4	±58.7	±0.04	±10.5	±78.2	±0.03
Mamaha	890149001	46	*Artocarpus altilis*	Society Islands	Mean	608	0.004	2.4	9.6	9434	818	1.3	148	1207	1.9
					SEM	±8.2	±0.0009	±0.00	±0.09	±465.6	±40.8	±0.01	±13.1	±72.7	±0.07
Havana pataitai	780291001	47	*Artocarpus altilis*	Society Islands	Mean	619	0.014	3.1	13.5	12356	950	1.5	81	1384	2.2
					SEM	±22.8	±0.0024	±0.06	±1.23	±1047.8	±87.3	±0.04	±5.0	±96.1	±0.14
Mein uwe	790497002	49	*Artocarpus altilis*	Pohnpei, FSM	Mean	505	0.005	2.6	8.1	12068	870	1.1	105	1525	1.7
					SEM	±8.9	±0.0007	±0.02	±0.01	±312.7	±40.4	±0.01	±3.1	±51.1	±0.00
breadnut		50	*Artocarpus acamansi*		Mean	848	0.009	7.3	15.3	10457	1224	3.2	93	3170	8.1
					SEM	±82.9	±0.0022	±0.45	±1.53	±726.8	±44.3	±0.22	±4.3	±113.6	±0.98
Tapehaa	780338001	51	*Artocarpus altilis*	Society Islands	Mean	422	0.008	2.9	8.4	11033	681	1.0	156	1268	1.5
					SEM	±23.9	±0.0012	±0.14	±0.44	±83.7	±3.5	±0.05	±36.4	±19.9	±0.10
Puou	770520001	53	*Artocarpus altilis*	Samoa	Mean	552	0.013	2.5	15.5	9495	917	1.3	222	1180	2.8
					SEM	±7.4	±0.0013	±0.00	±0.67	±444.8	±93.4	±0.02	±4.0	±73.5	±0.16
Maafala	770517001	55	*Artocarpus altilis*	Samoa	Mean	482	0.018	2.8	21.2	12423	1106	1.8	169	1305	2.5
					SEM	±18.3	±0.0067	±0.01	±0.78	±1169.5	±115.3	±0.05	±4.4	±87.3	±0.06
Momolego	770519001	57	*Artocarpus altilis*	Samoa	Mean	1131	0.009	3.4	29.4	10992	1509	2.4	852	1804	5.4
					SEM	±32.8	±0.0002	±0.04	±3.87	±540.8	±49.4	±0.05	±3.5	±51.3	±0.14
Dugdug	900252002	A4	*Artocarpus mariannensis*	Mariana Islands	Mean	1188	0.014	5.0	17.5	10702	1783	5.3	869	1583	3.8
					SEM	±279.9	±0.0017	±0.19	±0.83	±719.8	±401.5	±0.80	±47.9	±73.4	±0.43
Yap	900250001	A5	*A. altilis × A. mariannensis*	Palau	Mean	543	0.014	3.6	19.4	12012	974	2.3	213	1317	2.7
					SEM	±7.4	±0.0013	±0.02	±6.59	±212.1	±31.1	±0.10	±13.6	±21.1	±0.05
Uto ni viti	900264001	A8	*Artocarpus altilis*	Fiji	Mean	905	0.016	2.4	11.0	13094	1583	3.0	182	1251	4.0
					SEM	±19.6	±0.0023	±0.33	±1.13	±288.4	±26.2	±0.22	±5.9	±28.0	±0.47
Faine	910269001	A9	*A. altilis × A. mariannensis*	Truk, FSM	Mean	454	0.005	2.6	10.1	10473	898	1.6	126	1309	3.0
					SEM	±0.9	±0.0005	±0.01	±0.07	±171.4	±20.1	±0.00	±2.8	±15.3	±0.05
Mein pwahr	900255001	B5	*A. altilis × A. mariannensis*	Pohnpei, FSM	Mean	625	0.009	2.7	12.6	11716	980	2.2	224	1277	3.1
					SEM	±28.2	±0.0005	±0.05	±0.50	±727.4	±77.3	±0.06	±6.4	±73.5	±0.25
Mei puou	900237001	B6	*Artocarpus altilis*	Marquesas Islands	Mean	580	0.012	3.1	11.6	14265	1045	1.4	108	1501	2.4
					SEM	±10.3	±0.0030	±0.05	±0.21	±152.4	±16.4	±0.02	±5.8	±14.8	±0.16
Tuutou, taatoe	890186002	B7	*Artocarpus altilis*	Society Islands	Mean	504	0.011	2.5	9.6	9693	726	1.1	136	1143	2.2
					SEM	±7.8	±0.0005	±0.01	±0.15	±476.3	±51.3	±0.01	±4.6	±31.8	±0.04
Mei kopumoko	900242001	B8	*Artocarpus altilis*	Marquesas Islands	Mean	526	0.009	2.4	9.1	10844	852	1.2	97	1275	1.8
					SEM	±9.1	±0.0010	±0.01	±0.10	±176.7	±8.8	±0.01	±6.0	±18.1	±0.08

Cultivar name	Accession number	Grid ID	Species	Geographic origin		Mineral content (µg/g dry weight)									
						Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
Meikole	910280001	B9	*Artocarpus acamansi*	Pohnpei, FSM	Mean	467	0.005	5.4	12.9	11296	1451	2.5	117	2863	5.7
					SEM	±106.0	±0.0005	±0.22	±0.24	±129.8	±48.9	±0.14	±14.6	±62.1	±0.32
breadnut	-	BNUT	*Artocarpus acamansi*	-	Mean	434	0.056	7.5	24.2	13906	1527	4.7	123	5043	13.8
					SEM	±37.1	±0.0006	±0.47	±0.44	±1535.9	±147.7	±0.19	±18.9	±399.3	±1.19
Karawa	900265001	C5	*Artocarpus altilis*	Fiji	Mean	474	0.009	2.1	10.6	10683	990	1.7	156	1215	2.0
					SEM	±3.6	±0.0009	±0.04	±0.13	±192.6	±29.1	±0.02	±4.6	±20.5	±0.05
Siviri2	910279002	C8	*Artocarpus altilis*	Vanuatu	Mean	651	0.019	3.9	20.5	13463	1127	2.0	258	1634	5.1
					SEM	±19.8	±0.0007	±0.02	±0.68	±154.2	±14.1	±0.05	±18.3	±23.5	±0.32
Lipet	910270001	D8	*A. altilis × A. mariannensis*	Pohnpei, FSM	Mean	463	0.011	3.2	12.8	11089	876	2.1	1188	1313	2.3
					SEM	±13.8	±0.0004	±0.04	±0.59	±688.5	±47.5	±0.07	±15.4	±61.4	±0.06
Mei uhpw	910271001	D9	*Artocarpus altilis*	Pohnpei, FSM	Mean	592	0.004	2.6	9.8	10688	920	1.2	109	1274	2.1
					SEM	±15.9	±0.0056	±0.05	±0.24	±442.0	±23.1	±0.02	±4.6	±47.7	±0.12
Unknown	900268001	E4	*Artocarpus altilis*	Unknown	Mean	451	0.009	2.3	11.4	10269	822	1.0	126	1161	2.0
					SEM	±8.0	±0.0002	±0.04	±0.17	±572.2	±64.0	±0.01	±18.1	±70.4	±0.11
Mei arephe	900266002	E5	*Artocarpus altilis*	Pohnpei, FSM	Mean	851	0.016	3.2	18.2	13152	1139	2.2	265	1594	4.5
					SEM	±7.6	±0.0009	±0.05	±0.18	±157.0	±9.9	±0.01	±6.3	±16.0	±0.20
Unknown	900228001	E7	*Artocarpus altilis*	Samoa	Mean	1462	0.016	3.1	12.5	13275	1559	2.7	144	1469	3.0
					SEM	±37.9	±0.0011	±0.07	±0.21	±528.8	±77.8	±0.06	±8.0	±45.4	±0.23
Mei aueka	900241001	F6	*Artocarpus altilis*	Marquesas Islands	Mean	423	0.011	2.3	11.7	9370	630	1.1	80	1189	1.9
					SEM	±10.7	±0.0010	±0.10	±0.36	±98.5	±20.5	±0.01	±15.5	±3.8	±0.09
Samoan	900261001	F7	*Artocarpus altilis*	Fiji	Mean	539	0.009	1.1	9.6	8053	855	1.5	157	846	2.2
					SEM	±17.4	±0.0005	±0.12	±0.21	±520.6	±39.9	±0.03	±22.3	±43.3	±0.06
Mein pohnsakar	910272001	F9	*A. altilis × A. mariannensis*	Pohnpei, FSM	Mean	740	0.015	3.3	18.9	15331	1067	2.6	366	1643	5.1
					SEM	±5.2	±0.0020	±0.01	±1.08	±391.3	±22.8	±0.04	±6.1	±36.0	±0.30
Huero	900245001	G8	*Artocarpus altilis*	Society Islands	Mean	462	0.011	2.9	9.4	9871	814	1.3	112	1191	1.8
					SEM	±4.2	±0.0028	±0.06	±0.18	±533.9	±55.6	±0.01	±5.5	±56.8	±0.09
Mei tehid	910273002	G9	*Artocarpus altilis*	Pohnpei, FSM	Mean	480	0.008	2.8	13.4	11575	884	1.5	90	1264	2.5
					SEM	±5.4	±0.0005	±0.01	±0.16	±250.5	±27.4	±0.01	±5.3	±25.9	±0.24
Mei kole	900254001	H6	*A. altilis × A. mariannensis*	Pohnpei, FSM	Mean	405	0.010	2.5	10.0	10289	841	1.6	204	1141	1.9
					SEM	±3.3	±0.0034	±0.03	±0.16	±870.6	±61.5	±0.01	±6.7	±73.2	±0.01
Tuutou, auena	900246001	H7	*Artocarpus altilis*	Society Islands	Mean	474	0.008	2.2	9.4	10201	888	1.2	105	1108	2.0
					SEM	±3.2	±0.0007	±0.02	±0.18	±679.0	±67.1	±0.01	±4.1	±56.3	±0.04
Apuapua	890158002	H8	*Artocarpus altilis*	Society Islands	Mean	474	0.009	2.4	11.7	11540	873	1.0	97	1197	2.0
					SEM	±7.7	±0.0005	±0.04	±0.35	±672.2	±31.6	±0.00	±7.4	±36.6	±0.15
Hawaiian	-	HAW001	*Artocarpus altilis*	Hawaii	Mean	613	0.009	2.2	10.0	10942	1103	1.3	262	1190	2.1
					SEM	±27.2	±0.0008	±0.21	±0.34	±534.6	±33.1	±0.05	±12.7	±54.2	±0.25
Hawaiian	-	HAW002	*Artocarpus altilis*	Hawaii	Mean	474	0.008	2.6	9.4	12630	1287	1.4	495	1303	2.7
					SEM	±5.5	±0.0004	±0.03	±0.11	±575.8	±42.9	±0.02	±3.8	±31.7	±0.16

Cultivar name	Accession number	Grid ID	Species	Geographic origin		Mineral content (µg/g dry weight)									
						Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
Tuutou, ooa	900247001	I6	*Artocarpus altilis*	Society Islands	Mean	410	0.010	2.4	9.6	12030	987	1.5	92	1354	2.2
					SEM	±11.8	±0.0014	±0.06	±0.22	±217.0	±30.1	±0.03	±5.8	±31.4	±0.09
Anahonaho	900249002	I8	*Artocarpus altilis*	Society Islands	Mean	425	0.006	2.0	10.9	11253	812	1.0	118	1101	1.7
					SEM	±13.8	±0.0003	±0.03	±0.11	±1051.5	±84.5	±0.02	±13.2	±88.2	±0.14
Meion	910268001	J9	*A. altilis × A. mariannensis*	Truk, FSM	Mean	392	0.023	3.2	34.0	13010	927	2.4	1193	1413	5.5
					SEM	±12.0	±0.0022	±0.01	±1.95	±294.7	±27.0	±0.06	±7.8	±34.5	±0.74
Samoan	900260001	K7-2	*Artocarpus altilis*	Fiji	Mean	935	0.028	4.0	12.9	12507	2131	3.0	135	1737	7.0
					SEM	±43.5	±0.0010	±0.07	±0.70	±365.1	±56.7	±0.08	±5.8	±18.7	±0.19
Siviri3	910276001	K9	*Artocarpus altilis*	Vanuatu	Mean	595	0.008	2.1	10.7	10931	1146	1.9	167	1080	2.2
					SEM	±12.5	±0.0004	±0.07	±0.02	±458.1	±73.5	±0.01	±12.2	±48.3	±0.04
		KM1	*Artocarpus altilis*		Mean	383	0.008	2.8	9.3	10746	949	1.5	178	1288	1.8
					SEM	±5.0	±0.0018	±0.06	±0.18	±306.3	±63.9	±0.03	±23.8	±38.7	±0.03
		KM3	*Artocarpus altilis*		Mean	803	0.013	2.0	11.0	10282	1253	2.4	143	1341	3.4
					SEM	±25.9	±0.0008	±0.07	±0.29	±404.4	±17.0	±0.07	±13.9	±18.8	±0.21
Unknown	910290001	L10	*Artocarpus altilis*	Unknown	Mean	601	0.013	3.1	11.8	11098	908	2.0	93	1278	2.9
					SEM	±6.3	±0.0002	±0.03	±0.13	±543.7	±53.1	±0.01	±4.8	±47.6	±0.26
Aue	890147002	L7	*Artocarpus altilis*	Society Islands	Mean	452	0.011	2.2	11.6	11216	908	1.2	135	1108	2.0
					SEM	±16.9	±0.0005	±0.06	±0.14	±699.2	±60.5	±0.02	±10.4	±59.5	±0.26
Manua	900262001	M6	*Artocarpus altilis*	Samoa	Mean	449	0.012	2.3	11.9	12056	996	1.1	129	1221	1.8
					SEM	±6.5	±0.0041	±0.07	±0.11	±305.1	±11.7	±0.02	±22.1	±19.2	±0.20
Samoan	900234001	N6	*Artocarpus altilis*	Fiji	Mean	533	0.011	1.8	10.8	7578	808	2.1	137	984	2.2
					SEM	±6.5	±0.0003	±0.05	±0.15	±767.8	±86.7	±0.04	±8.0	±83.2	±0.04
Mei sei	890479002	O7	*Artocarpus altilis*	Pohnpei, FSM	Mean	409	0.009	2.1	9.7	10747	877	1.3	108	1167	2.0
					SEM	±15.1	±0.0004	±0.00	±0.55	±382.2	±30.4	±0.04	±6.0	±31.2	±0.15
Unknown	900226001	P5	*Artocarpus altilis*	Unknown	Mean	370	0.009	1.4	9.2	10510	1028	1.8	146	920	2.2
					SEM	±16.3	±0.0003	±0.09	±0.50	±1368.7	±146.0	±0.08	±5.0	±103.5	±0.09
Ouo	890464001	P7	*Artocarpus altilis*	Society Islands	Mean	361	0.009	2.0	10.6	9684	703	1.1	176	1193	1.8
					SEM	±3.7	±0.0010	±0.10	±0.19	±289.6	±33.5	±0.00	±34.1	±39.5	±0.08
Kea	880690001	P8	*Artocarpus altilis*	Tonga	Mean	283	0.007	2.6	12.8	13550	874	1.8	114	1396	1.6
					SEM	±3.0	±0.0006	±0.04	±0.39	±831.5	±63.6	±0.02	±1.8	±76.3	±0.01
Uto samoa	890477001	R4	*Artocarpus altilis*	Fiji	Mean	512	0.013	2.2	9.6	8981	1090	1.4	220	1194	1.9
					SEM	±5.6	±0.0048	±0.03	±0.16	±585.3	±108.4	±0.01	±4.9	±83.9	±0.04
Mei saip	890167002	R6	*Artocarpus altilis*	Pohnpei, FSM	Mean	528	0.004	2.0	7.9	9773	864	0.9	173	1057	1.6
					SEM	±12.1	±0.0004	±0.04	±0.29	±618.3	±48.4	±0.01	±7.8	±64.9	±0.16
Lipet	890480003	R8	*A. altilis × A. mariannensis*	Pohnpei, FSM	Mean	437	0.006	2.2	10.4	10372	754	1.5	126	1236	1.8
					SEM	±15.5	±0.0011	±0.05	±0.18	±459.3	±39.8	±0.05	±4.0	±37.4	±0.17
Unknown	900225001	S5	*Artocarpus altilis*	Unknown	Mean	596	0.009	2.6	9.1	11786	1021	1.3	136	1287	2.1
					SEM	±8.4	±0.0008	±0.03	±0.05	±745.7	±80.6	±0.01	±11.3	±74.7	±0.14
Puurea	890152002	S7	*Artocarpus*	Society	Mean	406	0.010	2.8	9.8	11146	842	1.7	142	1342	1.9

Cultivar name	Accession number	Grid ID	Species	Geographic origin		Mineral content (µg/g dry weight)									
						Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
			altilis	Islands	SEM	±22.6	±0.0031	±0.06	±0.45	±621.8	±53.4	±0.06	±13.5	±74.9	±0.09
Sagosago	890475002	S9	*Artocarpus altilis*	Samoa	Mean	589	0.016	2.9	16.1	13197	1473	2.3	204	1354	3.4
					SEM	±11.3	±0.0005	±0.01	±0.45	±547.1	±13.7	±0.02	±3.4	±8.2	±0.18
Enua	900256001	T3	*Artocarpus altilis*	Cook Islands	Mean	541	0.009	2.3	10.6	10547	762	1.0	129	1197	1.6
					SEM	±16.5	±0.0003	±0.04	±0.20	±483.6	±43.3	±0.03	±13.5	±38.9	±0.12
Ulu fiti	890458001	T4	*Artocarpus altilis*	Rotuma	Mean	475	0.014	2.6	10.0	11789	1193	2.2	166	1336	3.3
					SEM	±8.6	±0.0001	±0.02	±0.12	±215.7	±24.5	±0.03	±4.9	±30.8	±0.10
Mei maoi	900239001	T5	*Artocarpus altilis*	Marquesas Islands	Mean	484	0.011	2.6	9.3	11732	901	1.1	112	1236	1.9
					SEM	±15.3	±0.0017	±0.02	±0.33	±796.5	±76.7	±0.03	±6.0	±75.4	±0.09
Puaa	890460001	T6	*Artocarpus altilis*	Society Islands	Mean	446	0.010	2.7	12.9	9904	642	1.3	92	1117	1.6
					SEM	±6.0	±0.0019	±0.14	±0.49	±351.7	±19.9	±0.03	±7.8	±37.6	±0.02
Unknown	910286001	T8	*Artocarpus altilis*	Unknown	Mean	482	0.008	2.6	10.0	11172	828	1.5	135	1369	1.9
					SEM	±2.5	±0.0015	±0.04	±0.08	±369.5	±45.7	±0.00	±5.5	±75.6	±0.07
Sewan	890164002	T9	*A. altilis × A. mariannensis*	Truk, FSM	Mean	532	0.008	3.3	11.8	11583	806	2.5	380	1466	3.3
					SEM	±24.1	±0.0004	±0.02	±0.38	±821.6	±68.6	±0.09	±4.0	±93.2	±0.11
Mei puau	890462001	U2	*Artocarpus altilis*	Marquesas Islands	Mean	551	0.006	2.9	10.0	10380	1019	1.4	82	1294	2.4
					SEM	±5.1	±0.0063	±0.02	±0.20	±601.9	±83.1	±0.03	±3.5	±82.2	±0.16
Enua	890472002	U4	*Artocarpus altilis*	Cook Islands	Mean	562	0.011	2.2	8.8	11242	950	1.2	134	1159	1.6
					SEM	±14.1	±0.0014	±0.03	±0.32	±1086.0	±92.4	±0.03	±3.9	±84.5	±0.10
Unknown	900224001	U7	*Artocarpus altilis*	Unknown	Mean	558	0.008	2.2	11.1	10166	956	2.2	149	1172	2.1
					SEM	±13.9	±0.0017	±0.03	±0.11	±471.2	±67.2	±0.02	±14.3	±54.3	±0.05
Lemae	890163002	U9	*A. altilis × A. mariannensis*	Mariana Islands	Mean	565	0.008	2.6	11.3	9804	840	1.3	1268	1230	2.1
					SEM	±7.5	±0.0014	±0.02	±0.99	±427.3	±43.6	±0.02	±5.4	±61.9	±0.03
Patara	890463001	V3	*Artocarpus altilis*	Society Islands	Mean	369	0.012	2.7	11.1	12104	924	2.2	106	1712	2.2
					SEM	±4.0	±0.0005	±0.07	±0.15	±756.8	±84.9	±0.02	±9.5	±112.7	±0.12
Meriaur	890159002	V4	*Artocarpus altilis*	Palau	Mean	498	0.007	1.9	6.2	9671	998	1.2	151	1084	1.6
					SEM	±6.0	±0.0010	±0.02	±0.14	±468.1	±64.8	±0.01	±6.1	±50.7	±0.03
Furau	890470001	V6	*Artocarpus altilis*	Rotuma	Mean	705	0.014	2.8	9.9	10137	1223	2.4	155	1597	3.9
					SEM	±19.2	±0.0007	±0.04	±0.08	±504.7	±83.7	±0.04	±8.8	±85.8	±0.11
Teahimatoa	890465001	V7	*Artocarpus altilis*	Society Islands	Mean	922	0.008	3.0	7.3	11861	1181	1.8	137	1379	2.6
					SEM	±41.1	±0.0010	±0.08	±0.86	±595.4	±59.5	±0.08	±1.9	±97.0	±0.16
Afara	910267001	V8	*Artocarpus altilis*	Society Islands	Mean	529	0.006	2.2	8.2	11114	791	1.0	109	1273	1.7
					SEM	±23.7	±0.0006	±0.05	±0.37	±498.3	±7.6	±0.04	±15.2	±19.1	±0.08
Uto dina	890471001	W3	*Artocarpus altilis*	Fiji	Mean	513	0.012	1.6	8.5	9925	1010	2.0	202	974	2.9
					SEM	±5.7	±0.0015	±0.04	±0.11	±585.2	±79.9	±0.01	±10.5	±44.4	±0.05
Lemae	890163001	X2	*A. altilis × A. mariannensis*	Mariana Islands	Mean	538	0.008	2.6	11.9	9083	831	1.6	644	1293	2.6
					SEM	±4.3	±0.0015	±0.03	±0.08	±510.5	±34.9	±0.01	±4.8	±59.7	±0.15
Lemae	890162001	X4	*Artocarpus altilis*	Mariana Islands	Mean	471	0.005	2.2	8.9	10673	840	1.4	138	1258	1.8
					SEM	±11.9	±0.0002	±0.04	±0.67	±987.2	±85.1	±0.03	±8.0	±99.5	±0.10

Cultivar name	Accession number	Grid ID	Species	Geographic origin		Mineral content (µg/g dry weight)									
						Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	Zn
Ulu elise	890182001	X9	*A. altilis × A. mariannensis*	Tokelau	Mean	660	0.015	3.4	25.1	12189	1058	3.0	315	1587	4.4
					SEM	±4.2	±0.0005	±0.07	±0.17	±689.5	±94.6	±0.03	±12.0	±98.4	±0.10
Maopo (Hamoa)	890154001	Y1	*Artocarpus altilis*	Society Islands	Mean	595	0.014	2.7	11.5	9188	845	1.6	127	1236	2.2
					SEM	±4.8	±0.0011	±0.03	±0.14	±365.4	±46.0	±0.01	±4.3	±65.9	±0.01
Midolab	890183001	Y3	*A. altilis × A. mariannensis*	Palau	Mean	547	0.011	3.8	15.6	13211	881	1.7	224	1840	3.5
					SEM	±19.7	±0.0004	±0.08	±0.19	±1290.6	±95.3	±0.05	±9.5	±141.9	±0.11
Mein padahk	790494001	Z9	*A. altilis × A. mariannensis*	Pohnpei, FSM	Mean	532	0.011	3.7	12.1	10575	862	1.6	1322	1450	2.5
					SEM	±18.5	±0.0006	±0.03	±0.26	±350.3	±57.7	±0.04	±6.5	±71.1	±0.06