Measuring Genetic Diversity of an Underutilized Tropical Fruit Tree Crop: *Artocarpus odoratissimus*

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Introduction

The world's food system is under pressure due to a rapidly growing world population and climate change (1). There are also concerns within the world's current crop system about lack of genetic diversity, which could make crops more susceptible to disease and climate stresses (2). The Irish potato famine is one catastrophic example of relying on a single crop with low genetic diversity. When the potato blight fungus hit, it wiped out the entire potato crop (3). To combat problems of reliance on a few major crops of low genetic diversity, researchers have been studying underutilized crops (4).

In Southeast Asia, some underutilized species of *Artocarpus* are being studied for cultivation on a larger scale. The genus *Artocarpus* contains about 70 species and belongs to the Moraceae family. Breadfruit (*A. atilis*) and jackfruit (*A. heterophyllus*) are the two most cultivated crops in the genus *Artocarpus*. This study focuses on another understudied species: *Artocarpus odoratissimus* (commonly called terap and marang). Terap is prized for its sweet taste and smell and is native to Borneo. It was likely introduced to the Philippines. The wild form, recognized informally as the *barbatus* form, is characterized by smaller fruits and longer trichomes (hairs) on buds, stems, and petioles (5). In this study, we used nuclear and chloroplast microsatellite markers to investigate the relationship between cultivated terap and the wild *barbatus* form in Sabah, Malaysia (north Borneo).

Hypotheses

1.) The *barbatus* form is genetically distinct from cultivated *A. odoratissimus.*
2.) The *barbatus* form is the wild progenitor of cultivated *A. odoratissimus* and will have greater levels of genetic diversity compared to the cultivated terap.

Figures 1: Terap tree in an orchard setting (right). The syncarp (fruit) of terap (left) is highly prized for its sweet aroma and taste (5).

Methods

I. Sampling: included a total of 107 individuals. For the cultivated *A. odoratissimus.* 94 samples were collected in Sabah, one from peninsular Malaysia, and one from Singapore. Eleven *barbatus* samples were collected from western Sabah.
II. DNA extractions: used a standard CTAB Extraction Protocol.
III. Nanodrop Spectrophotomer: used to confirm presence, quantity, and quality of DNA.
IV. PCR: amplified 9 nuclear and 9 chloroplast microsatellite regions using the protocol described in Withrup et. al (2013).
V. Presence of PCR product: samples were run on 1% agarose gel to verify that the PCR product was successfully amplified.
VI. Beckman Coulter CEQ 8000 Genetic Analysis System: obtained allele sizes from the PCR reactions.

Figure 2: Comparison of cultivated *A. odoratissimus* (left), which lacks long trichomes (hairs) with the *barbatus* form (right), which has long trichomes.

Data Analysis

The following programs were used to analyze the data: Structure Harvester (7), Strurture (8), Haplotype Analysis (9), Network (10), GenAlEx (11)

Results

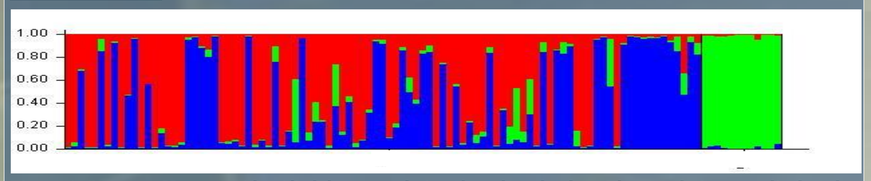

Figure 4: Structure Harvester identified three populations based on the data. The populations are displayed here, in red, green, and blue. Terap samples are on the left, and *barbatus* samples are on the right.

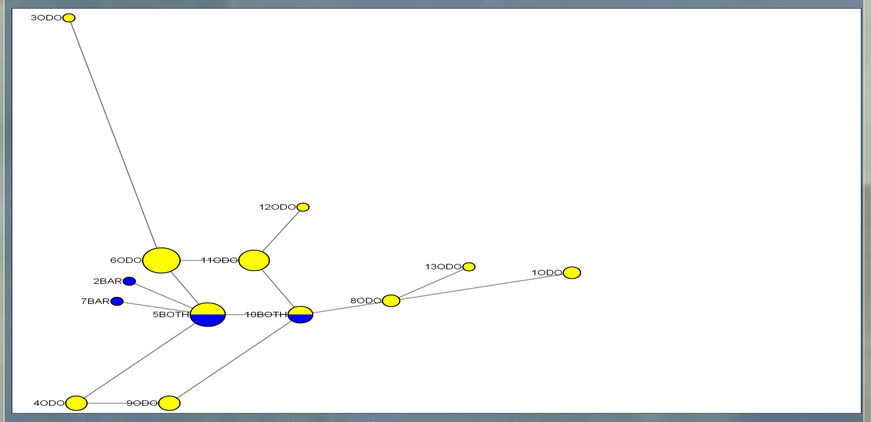

Figure 5: Haplotype Analysis identified 13 different haplotypes that were used to infer the median-joining network seen above. The size of the circles are proportional to the number individuals belonging to each haplotype. Yellow represents terap haplotypes and blue represents *barbatus* haplotypes.

Mean and SE over Loci for each Pop									
Pop		N	Na	Ne	I	Ho	He	uHe	F
odoratissimus	Mean	88.286	24.857	5.938	2.098	0.597	0.767	0.771	0.211
	SE	2.090	11.734	1.125	0.260	0.092	0.065	0.066	0.103
barbatus	Mean	8.571	6.429	3.958	1.422	0.522	0.636	0.670	0.133
	SE	1.510	1.674	0.882	0.292	0.134	0.117	0.124	0.172

Table 1: Genetic diversity statistics. N is the number of individuals, Na is number of alleles, Ne is number of effective alleles, I is the Shannon's Information Index, Ho is observed heterozygosity, He is expected heterozygosity, and F is Fixation Index (11).

Discussion and Conclusions

Hypothesis I: The *barbatus* form is genetically distinct from cultivated *A. odoratissimus*.

Based on the Structure analysis of the nuclear microsatellite data, all of the *barbatus* samples formed one distinct population (shown in green in figure 3) which suggests little gene flow between terap and *barbatus*. These findings support the first hypothesis that the *barbatus* form is genetically distinct from cultivated terap (Figure 3) .

Hypothesis II: The *barbatus* form is the wild progenitor of cultivated *A. odoratissimus* and will have greater levels of genetic diversity compared to the cultivated terap.

The network analysis shows that the *barbatus* haplotypes are in the center of the network, suggesting that cultivated terap may have been derived from it (Figure 4). However, some terap individuals share the same haplotypes as the *barbatus* forms in the center of the network. These could represent examples of terap that are early along the domestication gradient and have not yet differentiated greatly from the wild *barbatus*. The genetic diversity indices reveal little difference in genetic diversity between cultivated terap and wild *barbatus*. Given that the sample size of the *barbatus* form was substantially smaller than for terap, it is possible that furthter *barbatus* sampling will reveal greater diversity. With the current data, we are unable to support or reject hypothesis two and further study is warranted.

Figure 6: Terap and the *barbatus* form are native to Sabah, Malaysia in Southeast Asia.

Future Work

More *barbatus* samples from the rest of Borneo and the Phillipines should be collected to further test that the *barbatus* form is genetically distinct from terap, and to further test whether the *barbatus* form is the wild progenitor of cultivated terap.

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