POTENTIAL USE OF MORPHOLOGICAL CHARACTERISTICS IN EVALUATING NATURAL VARIATION OF "BARAKO"

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ABSTRACT

The Philippines is one of only three countries in the world capable of producing *Coffea liberica*, and grow two other species of coffee, *Coffea canephora, Coffea arabica. C. liberica*, commonly "Barako", is the least cultivated coffee species. Its unique taste has high potential to grow in the market if production was increased. However, the Taal Volcano eruption in 2020 resulted in thousands of damaged Barako trees in 2020 and 2021. As new seedlings are produced, the question of variety among planting material comes up. Farmers need a method to evaluate natural differences of current Barako trees, to answer whether seedlings from farms cultivating Barako are naturally different. The purpose of this research is to determine if the morphological characteristics of cultivated seedlings from different farms can be classified through image and statistical analysis. Basic knowledge on how varieties—which produce different flavors, aroma, and market value of coffee—differ among farms is needed for a strategy to increase the number of seedlings. The initial set of data from this study indicate statistically significant differences on the average seedling height per node and the leaf area per length of 31, 10-month old coffee seedlings; from two different farms, grown in a common environment. The process may be developed further for use in evaluating natural variation among C. liberica as seedlings.

INTRODUCTION

Coffea liberica, or "Barako" coffee, is part of the Rubiaceae family. The Philippines, with a national production of 1% and a global production of 3-4%, has a competitive advantage in the market for *C. liberica*; being one of three countries—besides Ethiopia and Malaysia, that is able to produce this (Wallengren, 2018; Philippine Statistics Authority, 2019). Barako is on the verge of extinction due to the 2020 Taal eruption in Batangas; struggling to keep up in the coffee market due to the demand for instant coffee, making farmers replace them with Robusta and short, hybrid coffee trees. (Kapeng Barako, 2018).

Market prices vary due to the differences in the nature of seedlings (i.e. species, variety or stock), affecting other qualities such as tastes, aroma, etc. Identifying phenotypic characters determines varieties easily, therefore, providing long-term benefits for farmers and plant breeders due to its practicality (Kordrostami & Rahimi, 2015). With the demand for Barako in the market, information on natural differences and distinct qualities of Barako stocks can help increase seedling production. Genetic markers determine varieties and species best, but this would not be feasible for farmers. Thus, the use of seedling morphology and leaf characters in this process.

Morphological clusters from the data gathered was used in image analysis and statistical programs. ImageJ—an image processing program—calculates, measures, and processes the parameters of the plant images (Bankhead, 2014). The study differentiated the morphological characteristics of 41 *C. liberica* (Barako) seedlings from two farms, grown in a controlled environment, by using image analysis. However, DNA markers were not used to verify whether the Barako seedlings are of different varieties. The study can help in assisting farmers, sellers, buyers of coffee in easily determining *Coffea liberica* varieties. This can strategize the increase in production of Barako and the income as well of those involved in the industry and encourage them to produce and preserve Barako.

METHODOLOGY

Sample Collection

41 ten-month-old C. liberica seedlings from 2 farms in Malaysia were used as data for the morphological characteristics—31 for leaf area per leaf length and 41 for seedling height per seedling node—grown together in a controlled environment.

Data Collection

Seedling images with ruler alongside were used to gather height and number of nodes data—collecting four to five leaves each, pictured in a parallel manner. All the seedlings were named and segregated by category. Data collected from the coffee seedlings and their leaves are put into Microsoft Excel for data analysis.

Data Analysis

Mean and standard deviation were applied to the raw data of the four main seedling characteristics to see the overall view and its closeness in groups Farm 1, Farm 2, and All Samples. A histogram —for the formation of a bimodal graph, and a scatter plot—for morphological characteristics correlation, was made to determine groups in the sample seedlings. Application of Paired T-test determines possible significant difference between the variability of the two farms based on the standardized values of Seedling Height per Seedling Node and Leaf Area per Leaf Length. If the T-test value is greater than the distribution table value, then null hypothesis "paired population is equal" is rejected; otherwise, accepted.

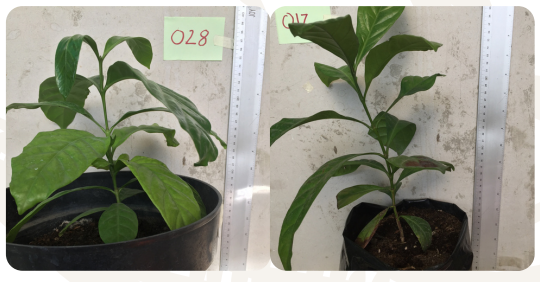

Figure 1
Sample Seedlings with Ruler

Figure 2
Sample Leaf with Ruler

Figure 3
ImageJ Logo

RESULTS

Correlation between "Leaf Length vs Leaf Area" is high, and so the values cannot be separated and instead can be standardized to combine the two parameters. On the other hand, the correlation between "Seedling Height vs Seedling Nodes" is low, and it is difficult to compare the sets of data unless they are standardized together. Therefore both areas have been standardized per farm and will be combined for comparison (Figure 3).

Figure Title	R^2 value
Leaf Length vs Leaf Area	0.8931
Seedling Height vs Seedling Nodes	0.0396

Table 1
Correlation Values of "Leaf Length vs Leaf Area" and Seedling Height vs Seedling Nodes

Seedling Data for Height per Nodes

When the seedling height by nodes of 14 seedlings from Farm 1 (XA = 3.1, SA = 1.4) and the data of the other 13 seedlings from Farm 2 (XB = 4.6, SB = 1.05) were compared, the resulting t-test value score was $t(12) = 2.306$, $p < .05$. This shows differences between the means, as the p-value or threshold acquired from the t-test table (2.306) is lower than that of the t-test score result (3.0802). Therefore, the null hypothesis stating that there is no significant difference between the two farms, is rejected.

Leaf Data for Area per Length

Comparing the leaf area by length of 55 leaves from 11 seedlings from Farm 1 (XA = 3.4, SA = 0.9) with the data of the other 63 leaves coming from 13 seedlings of Farm 2 (XB = 4.1, SB = 1.09); the calculated t-test value score was $t(54) = 3.7892$, $p < .05$, indicating significant differences between the means. As the p-value or threshold acquired from the t-test table (2.0154) is lower than that of the t-test score result (3.7892), the null hypothesis that there is no significant difference between the two farms, is rejected.

	Seedling Height per Nodes		Leaf Area per Length	
	Farm 1	**Farm 2**	**Farm 1**	**Farm 2**
Mean ($\overline{X}$)	3.1	4.6	3.4	4.1
Sample size (N)	14	13	55	63
Standard deviation (S)	1.4	1.05	0.9	1.09
Paired T-Test Value for Seedling Height per Nodes	±3.0802		±3.7892	

Table 2
T-test data for Seedling Height per Nodes and Leaf Area per Length

Once the overall variables were standardized and combined, they appear to form two overlapping groups (Figure 3), as some variables from Farm 1 and Farm 2 have merged in the middle of the group (between the range of 3 to 5). The two groups that formed are Farm 1 (blue points) located on the leftmost and Farm 2 (orange points) on the rightmost. They are not enough to completely separate them into varieties. It is possible to enable further investigation on the properties that would make the groups distinct, by introducing more seedlings, characters, and farms.

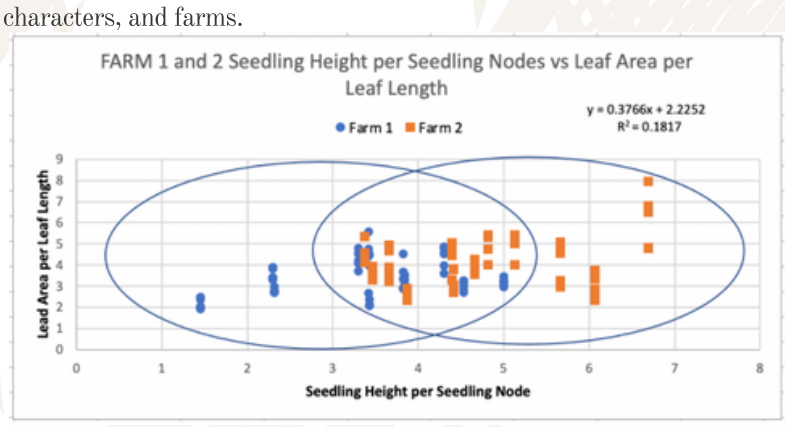

Figure 3
Comparison of data points from results of Farm 1 and Farm 2

CONCLUSION

The four parameters: seedling height, seedling nodes, leaf length, and leaf area are best used as standardized values since they partially separate the groups—qualifying as characteristics needed to carry out a paired t-test calculation. The seedling samples classified into two groups showed a significant difference where two overlapping groups are formed from the scatter plot. This indicates a potential of differentiation by using standardized morphological characteristics of seedlings grown in a controlled environment.

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