Performance of Seven Tea Accessions in North-central Florida: Correlations between Potential Yield and Growth Parameters over 2 Years

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Summary. **Tea (*Camellia sinensis*) is a promising new specialty crop for production in Florida. However, few data exist on the establishment phase of tea plantings in this environment and on how early growth parameters may predict yield potential. We tested seven accessions of tea grown under field conditions in north-central Florida for leaf yield and growth parameters—namely, pruned biomass, trunk diameter, trunk height, trunk width, trunk height × width, and canopy area—in the second and third years after planting. Our analyses indicated that the accession Fairhope performed best overall. Pruned biomass and trunk diameter were the best predictors for leaf yield. The harvested leaves produced good-quality black tea, with caffeine levels comparable to commercially available tea. These data indicate that nondestructive measurements of growth can be useful to assess yield potential of tea, and that regionally adapted tea accessions can be identified during the establishment stage.**

Tea (*Camellia sinensis*) is grown in at least 66 countries around the world (Pettigrew, 2018) and is currently being explored as a new specialty crop in the southern United States (Orrock et al., 2017). Tea is the most consumed beverage in the world (Food and Agriculture Organization of the United Nations, 2018), and its consumption in the United States is increasing. From 2012 to 2018, U.S. wholesale tea sales increased from $9.79 billion to $12.66 billion (Statista, 2021), but domestically produced tea comprises only a negligible share of the market. Tea plants were introduced to the United States in 1799 (Pratt and Walcott, 2012), and tea has been grown historically in the southeastern part of the country. The first large-scale commercially successful tea venture was the Pinehurst Tea Planation/Charleston Tea Plantation, which was established in 1888 near Charleston, SC; however, interest in tea production waned by the 1900s, primarily as a result of increasing labor costs (Pettigrew, 2018; Shepard, 1899). With the introduction and subsequent improvements in mechanical harvesters for tea, and increasing interest in locally grown produce, opportunities for tea production are once again emerging in the United States. As of 2018, tea was cultivated in 17 states on 181 ha for an annual domestic production total of 5.6 t (Pettigrew, 2018; Pratt and Walcott, 2012). Florida is considered to have suitable areas for tea production, and tea has the opportunity to be a new specialty crop for the southeast region.

Yield maximization is one of the most important components of a successful tea plantation. Although yields can be improved by optimizing management practices, genetic limitations on yield can be problematic for a farmer, especially when costs for replanting with new varieties are high (Carr, 2018). Currently, there are no standard tea varieties available commercially in the United States that have been bred for high yield, and no information is available on yields of tea accessions grown under north-central Florida conditions. We use the term *accession* here to describe a collection of plants acquired from a single source under a particular name, without implying that the collection is homogeneous or fully characterized, as might be expected of a variety. A successful tea breeding program will need this information to develop and evaluate varieties adapted specifically to produce high yield under the growing conditions in Florida and other U.S. states adjacent to the Gulf of Mexico. However, calculating yield is labor intensive, and tea plants do not reach full yield potential until 5 to 6 years after planting. As the harvestable unit of the tea plant is the immature leaves, tea can be harvested at any stage of plant development. Identifying growth parameters that can predict yield from individual young plants would expedite the data collection needed to inform breeding efforts. Furthermore, if the relationships between plant growth and yield are understood for a particular location (e.g., Florida), we may be able to use specific measurements to optimize

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
29,574	fl oz	μL	3.3814×10^{-5}
0.3048	ft	m	3.2808
0.1242	gal/100 ft	$L \cdot m^{-1}$	8.0520
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1.1209	lb/acre	$kg \cdot ha^{-1}$	0.8922
1	micron(s)	μm	1
28.3495	oz	g	0.0353
1	ppm	$mg \cdot kg^{-1}$	1
0.9072	ton(s)	t	1.1023
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

cultural practices such as fertilizer application and irrigation.

In a commercial setting, a tea plant receives numerous prunings over the first 5 to 6 years in the field (Goswami, 2011). These initial prunings, called *formative prunings*, are critical for establishing a central trunk, with three evenly spaced branches that, as the tea plant matures, will grow into the traditional plucking table. In typical high-density commercial plantings, per-plant yield can be expected to increase with plant age until canopy closure, after which mature bushes may compete with neighboring plants, resulting in reduced yields per plant (Carr, 2018). Therefore, yield calculations in a mature planting are a product of both genetic potential and competition created by site-specific factors, and are thus difficult to generalize. To determine differences in genetic yield potential, it is preferable to evaluate tea plants just before canopy closure, reducing the influence of competition on the resulting data. Yields are reported to increase an average of 588% from year 1 to year 5 (Goswami, 2011), during which time competition may be expected to be minimal, and yield data should reflect genetic potential under prevailing environmental conditions.

In our previous work (Orrock et al., 2017), we evaluated the survival and establishment of eight tea accessions available commercially in the southeastern United States, at the Plant Science Research and Education Unit in Citra, FL. We determined that the accessions Big Leaf, Fairhope, Georgian, and Large Leaf had the most rapid early growth, measured as pruned biomass; however, the earlier data only examined survival and growth (Orrock et al., 2017). Building on our previous work, the research presented here examines yields of seven tea accessions in Florida over two growing seasons, describes correlations between plant growth measurements and yield, and confirms tea quality among the accessions using caffeine as an objective quality metric (Zhang et al., 2020a).

Materials and methods

Field site. In 2016, we obtained 1-year-old tea plants from Mississippi State University in Starkville, MS, and from Camellia Forest Nursery in Chapel Hill, NC. The accessions obtained from Mississippi State University were Small Leaf, Big Leaf, Fairhope, and Georgian. The accessions obtained from Camellia Forest Nursery were Large Leaf, Assamica, Quinquebracteata, and China Seed. Of these accessions, Quinquebracteata did not survive in the field trial and is not included in this study. We established the research plot at the Plant Science Research and Education Unit (lat. 29°24′27.3″N, long. 82°08′27.5″W). Soil at the plot is classified as Candler sand (major land resource area 154). Soil chemical analysis performed shortly after planting indicated total Kjeldahl nitrogen, 410.5 $mg\cdot kg^{-1}$; phosphorus, 18.69 $mg\cdot kg^{-1}$; and potassium, 34.87 $mg\cdot kg^{-1}$ (University of Florida/Institute of Food and Agricultural Sciences Analytical Services Laboratories, Gainesville, FL). The tea accessions were planted in Mar. 2016 in three blocks of eight accessions each, with 15 plants per accession in a randomized complete block design. Within-row spacing of 60 cm and between row-spacing of 1 m were used. Plants were drip-irrigated twice per day for 30 min at a rate of 15 gal/100 ft, and fertilizer was applied at a rate of 6 lb/acre nitrogen, 8.5 lb/acre sulfur, and 7 lb/acre potassium per week supplied in the form of ammonium sulfate and potassium sulfate. In July 2016, a plastic weed barrier fabric was added for weed suppression.

Yield. Tea leaves were hand-harvested monthly during the 2018 and 2019 growing seasons (Mar.–Oct. 2018, Feb.–Oct. 2019). Yield was calculated as fresh weight of shoots, which consisted of a single leaf and a bud, emulating the harvest technique for a premium tea (Tipton et al., 1990). Shoots were pooled within each experimental unit of 15 plants and weighed. Pooled shoot weights were then divided by the number of living plants out of the original 15 plants (Supplemental Table 1) to calculate a per-plant yield. Growth measurement data (as described next) were collected during the Sept. 2018 and Oct. 2019 harvests. Annual yield data were used for regression analyses, examining the relationship of yield with pruned biomass; trunk diameter, height, width, and height × width; and canopy area derived from computational analysis of images using an image analysis software (ImageJ 1.8.0; National Institutes of Health, Bethesda, MD). Data were analyzed to test differences between means using analysis of variance followed by Tukey's honestly significant difference method in statistical analysis software (JMP for Windows 7 Ultimate x64; SAS Institute, Cary, NC).

Growth measurements: Biomass. Following industry standard recommendations, in Nov. 2018, we decenter-pruned the tea plants to a standard height of 20 cm to encourage central trunk formation and apical branch development (Goswami, 2011). Branches originating below 10 cm were removed, and plants were pruned to a maximum height of 35 cm. We collected the pruned plant material, which consisted of leaves and stem, weighed the biomass for each treatment unit (15 plants), and normalized the data per number of living plants. Following the same standard (Goswami, 2011), we performed a second pruning in Nov. 2019, in which tea plants were pruned to a maximum height of 45 cm to strengthen the central trunk and encourage formation of three equally spaced branches. To select these branches, we identified the three largest and most evenly distributed offshoots from the central trunk that originated at least 30 cm distant from the plant crown. We collected and weighed the pruned biomass, which consisted of leaves, branches, flowers, and buds. Data were collected from each replicate of a treatment and normalized per number of living plants in that block.

Growth measurements: Trunk diameter. Trunk measurements were taken in Aug. 2018 and July 2019 using calipers to measure the diameter of the widest point at the base of the trunk, just above the soil level. Measurements were taken of every plant and were then averaged within each block.

Growth measurements: Height and width. Measurements of canopy height and width were taken of each tea plant in Aug. 2018 and July 2019. Height was measured at the tallest point of the plant, and width was measured at the widest point of the plant. Values were averaged within each block. Height and width averages were then multiplied to obtain the height × width values used in the regression analysis.

Growth measurements: Canopy area. Four representative plants were selected randomly from the central 13 plants in each block of 15, and in Aug. 2018 and July 2019 we photographed the canopy areas horizontally in profile using a portable white backdrop to improve contrast, and included a square size reference in each image. Image processing software (ImageJ) was used to calculate canopy area by selecting the canopy areas in each image based on color and contrast, and then quantifying the selected area. Areas were averaged for plants of each accession within each block.

Estimation of caffeine in prepared tea infusions. Small batches of harvested tea leaves were rolled by hand and dried in an electric dryer to produce black tea per the procedures described by Sato et al. (2007). Tea was prepared following the British brewing standard BS 6008:1980/ISO3103:1980 (International Organization for Standardization, 1980). Commercial black tea (Lipton Yellow Label Tea; Unilever, Englewood Cliffs, NJ) was used as a standard for comparison because this brand of commercial tea is a black tea blend widely available in the United States. The compounds in tea (10 μL) were separated using high-performance liquid chromatography (HPLC) followed by electrospray ionization mass spectrometry. For HPLC, a chromatography system (1100; Agilent Technologies, Santa Clara, CA) was used, consisting of an autosampler (GI313A, Agilent Technologies), degasser (G1322A, Agilent Technologies), binary pump (G7312A, Agilent Technologies), chromatography column (2.1 × 150-mm, 3-μm column; Hypersil Gold AQ, Thermo Fisher Scientific, Waltham, MA), and a security guard column (C-18; Phenomenex, Torrance, CA). For mass spectrometry, a quadrupole ion trap mass spectrometer (LCQ DECA, Thermo Fisher Scientific) equipped with electrospray ionization operating with data acquisition and interpretation software (XCALBUR 2.0.7.SP1, Thermo Fisher Scientific), and a (+)ESI spray voltage of 4.0 kV was used. The mobile phases in HPLC were water and methanol, and a gradient of 100:0 water:methanol to 0:100 water:methanol over 60 to 65 min was used for elutions. Caffeine (MilliporeSigma, St. Louis, MO) was used as an external standard to estimate the concentration of caffeine in tea infusions based on the peak area for m/z 195, the $[M+H]^+$ ion for caffeine.

Results

Tea accessions cultivated under field conditions were examined for mortality, and growth parameters and yield of live plants. Some plants in each accession died despite comparable management conditions. Weather data for the site were analyzed for 1339 d between 1 Mar. 2016 and 30 Oct. 2019 (Fig. 1). Daily maximum temperature 60 cm above the soil ranged from 6.6 to 39 °C; daily minimum temperature ranged from –4.5 to 24 °C. Within this period, the number of days when minimum temperatures were less than 0.6 °C ranged from 0 to 12 per year, with a total of 16 d below this threshold: 1 d in 2016, 3 d in 2017, 12 d in 2018, and 0 d in 2019. The number of days that recorded maximum temperatures above 37 °C also ranged from 0 to 12 per year, with a total of 20 d above this threshold: 12 d in 2016, 1 d in 2017, 0 d in 2018, and 7 d in 2019 (Florida Automated Weather Network, 2021). Rain totals during the study period were 74 cm in 2016, 141 cm in 2017, 157 cm in 2018, and 105 cm in 2019 (Fig. 1). When monthly mortality data were evaluated, most of the plant deaths were recorded between 7 Apr. and 17 May 2017—a period of low rainfall. Together these data suggested that both freezing and high-temperature stress are limitations, but high-temperature stress and drought are key impediments in establishing tea in north-central Florida.

In 2018, Fairhope had a significantly greater yield than Assamica and China Seed, and in 2019, Fairhope had a significantly greater yield than Assamica, China Seed, and Georgian [$P < 0.05$ (Fig. 2)]. All tea accessions showed an increase in yield from the 2018 to 2019 harvest: Small Leaf, 107%; Fairhope, 78%; Big Leaf, 71%; Large Leaf, 58%; Assamica, 52%; China Seed, 23%; and Georgian, 8%. Fairhope had a significantly greater number of shoots collected per plant than Large Leaf or Small Leaf (Fig. 3). The estimated time it took to perform each of the growth measurements on a single plant was trunk diameter, <1 min;

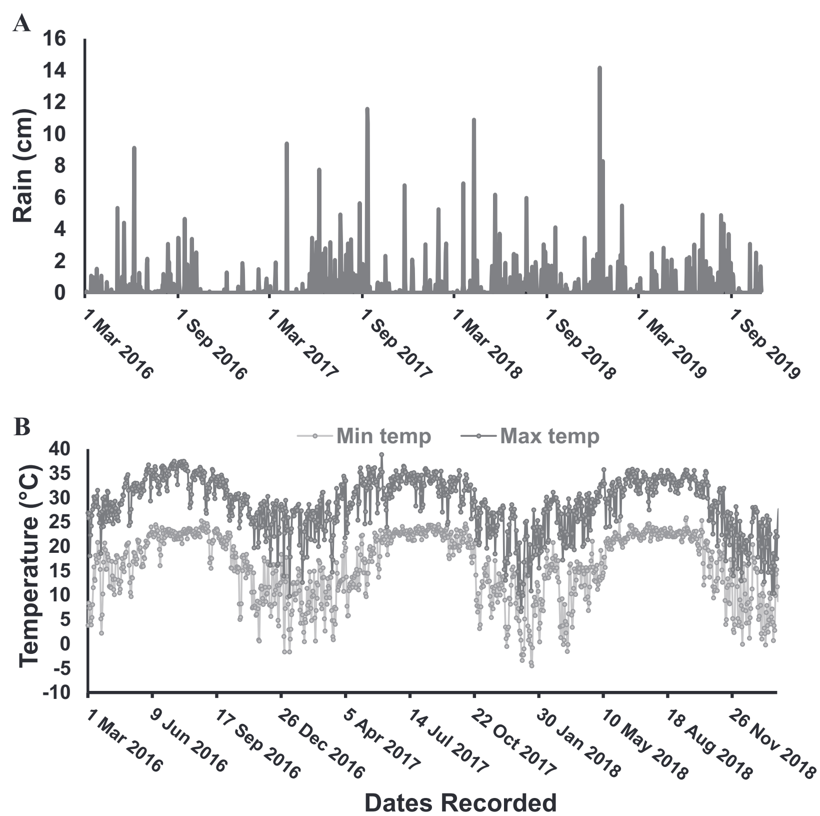

Fig. 1. (A) Rainfall and (B) daily minimum and maximum temperatures 60 cm aboveground recorded between 1 Mar. 2016 and 30 Oct. 2019 at Citra, FL, where tea plants were cultivated (Florida Automated Weather Network, 2021); 1 cm = 0.3937 inch, (1.8 × °C) + 32 = °F.

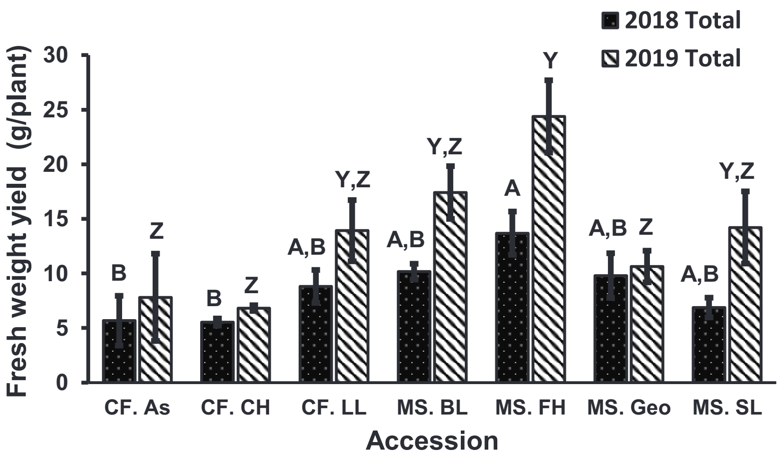

Fig. 2. Mean leaf yield (fresh weight) per living plant from harvests in 2018 and 2019 of seven tea accessions: Assamica (CF.As), China Seed (CF.Chn), Large Leaf (CF.LL), Big Leaf (MS.BL), Fairhope (MS.FH), Georgian (MS.Geo), and Small Leaf (MS.SL). Data were generated from within each block of surviving plants from the initial 15 planted in Mar. 2016. Values are reported as mean of three replicates with SE bars (N = 3). Statistical analyses were run separately for each year. Means not significantly different ($P < 0.05$) using Tukey's honestly significant difference test are marked by the same letters; 1 g = 0.0353 oz.

height, <1 min; width, <1 min; height × width, <2 min; pruned biomass, <5 min; and computer-measured canopy area, <6 min. All growth measurements were quicker than the average time spent harvesting a tea plant over a growing season (>10 min/plant), and the measurements were nondestructive. Our regression analyses show that pruned biomass and trunk diameter were the best predictors for yield in both 2018 and 2019 (Table 1).

Black tea prepared from different accessions of tea was of good quality based on visual inspection and taste (Orrock, 2020). Estimated caffeine concentrations in these teas varied from 0.91 mM in China to 1.9 mM in Assamica (Table 2). These were comparable to the concentration of caffeine of 1.69 mM found in tea made from commercially purchased black tea [Lipton® Yellow Label Tea (Table 2)].

Discussion

In this multiyear study, we focused on comparing mean yield among seven accessions of tea and identifying nondestructive, easy-to-perform measurements that could be used to predict yield during the early years of establishment, thereby shortening the time required for assessing yield potential in breeding lines. Yield data for currently available varieties are critical for growers who aim to establish new tea plantings in Florida, and yield-predicting parameters are critical for breeders who want to support growers with a rapid release of regionally adapted varieties.

A recent study in Mississippi examined growth characteristics and quality of field-grown tea (Zhang et al., 2020b). It focused on frost tolerance and quality characteristics of tea made from several accessions, but did not address yield differences. Our study included three of the same accessions studied by Zhang et al. (2020b)—Large Leaf, Small Leaf, and Assamica—but ours are different clones of these materials. In our study, the tea accession Fairhope had the greatest yields in both 2018 and 2019 (Fig. 2), and the accession China Seed had the lowest yield in both years. Assamica was low yielding under our growing conditions, whereas Large Leaf and Small Leaf were intermediate. Although Zhang et al. (2020b) identified frost tolerance as a key trait for adaptability in north-central Mississippi, we experienced most of our plant losses during the early summer (J.M. Orrock, B.S. Richter, and B. Rathinasabapathi, unpublished), and heat tolerance will likely be a more important trait in varieties bred for production in Florida and coastal regions, especially in light of predicted climate warming trends.

The data presented here are from the second and third years after planting. Tea plants are not considered mature until the fifth or sixth year (Goswami, 2011), so yields will likely increase as the tea plants reach maturity. It should also be noted that the yields collected for this study consisted of a bud and single leaf, as might be collected for a very high-end product, rather than the bud and two leaves typically harvested for a standard hand-harvested tea. Thus, comparisons of per-plant yield should be made only within the study, or with other studies using the single-leaf harvesting practice. Although these yield data are from immature plants, the consistent rankings among our accessions suggest that Fairhope, Big Leaf, and Large Leaf will

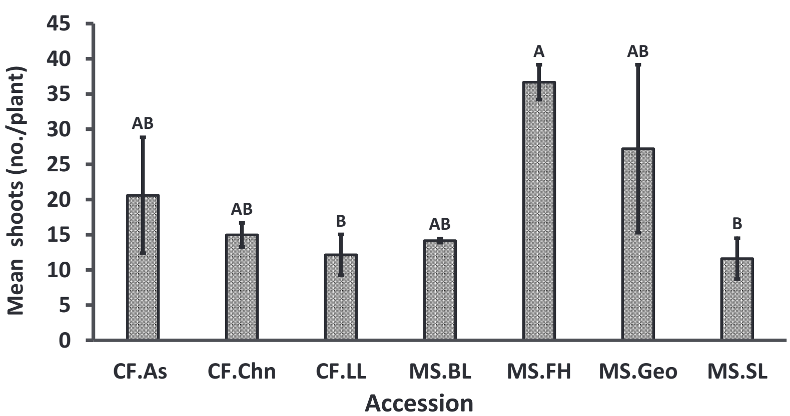

Fig. 3. Average total number of shoots harvested during Sept. and Oct. 2018 from seven tea accessions: Assamica (CF.As), China Seed (CF.Chn), Large Leaf (CF.LL), Big Leaf (MS.BL), Fairhope (MS.FH), Georgian (MS.Geo), and Small Leaf (MS.SL). Data were generated from within each block of surviving plants from the initial 15 planted in Mar. 2016. Values are reported as the mean of three replicate groups with SE bars (N = 3). Means not significantly different ($P < 0.05$) using Tukey's honestly significant difference test are marked by the same letters.

Table 1. Test statistics from a multiple linear regression analysis between leaf yield of tea and growth parameters: pruned biomass, trunk diameter, height × width values, canopy area, and canopy height and width. Data are from 2018 and 2019, averaged within an accession experimental block (N = 21), with regression coefficients (R^2), probability values, and t values (measures of precision for the regression coefficients) following a Student's *t* test on the multiple linear regression output. Probability values that indicate significant relationship ($P < 0.05$) are shown in bold.

	2018			2019		
Growth parameter	R^2	*P* value	*t* value	R^2	*P* value	*t* value
Pruned biomass	0.780	**0.002**	3.88	0.785	**0.001**	4.73
Trunk diameter	0.520	0.198	1.35	0.763	0.142	1.56
Height × width	0.471	0.908	–0.12	0.714	0.168	1.45
Height	0.393	0.800	–0.26	0.645	0.180	–0.41
Estimated canopy area[z]	0.353	0.875	–0.16	0.598	0.255	1.19
Width	0.430	0.680	0.42	0.440	0.609	–0.52

[z]Maximum of four selected plants in each experimental unit.

be beneficial to consider as parents in crosses when breeding for high-yielding varieties, whereas the Assamica and China Seed accessions may be less suitable for Florida production. Accessions Small Leaf and Georgian had intermediate yields, but also highly variable performance under our growing conditions; individuals among these accessions may also be worthy of selection for breeding purposes, especially those that show resilience against both high- and low-temperature conditions. Georgian, for example, was advertised for cold tolerance, but some individuals among this seed-grown population have also demonstrated excellent heat tolerance in the field.

Table 2. Estimated concentrations of caffeine found in black tea prepared from tea leaves harvested from different accessions of field-grown tea compared with commercial black tea (Lipton Yellow Label Tea; Unilever, Englewood Cliffs, NJ). Mean values are from three replicate tea preparations and estimated based on high-performance liquid chromatography-mass spectrometry by comparing to a caffeine standard.

Accession	Caffeine in tea infusion (mM)
Assamica	1.89 a[z]
Big Leaf	1.05 bc
China Seed	0.91 c
Fairhope	1.13 bc
Georgian	1.55 ab
Large Leaf	1.18 bc
Small Leaf	1.21 bc
Commercial black tea	1.69 ab

[z]Means that are significantly different from each other at $P = 0.05$ are indicated by different letters based on Tukey's test.

Our results indicate that pruned biomass is the best predictor for yield, using a linear regression analysis of yield and growth parameters (Table 1). Pruned biomass has been used previously as a predictor of yield. Visser (1969) reported R^2 correlation values of 0.92 and 0.89 for two different sets of 28 and 23 clones. Our R^2 values showed a similarly high level of correlation (Table 1). Growth prediction parameters did not differ among our accessions (data not shown), and correlation values improved in the second year. Tea plants need to be pruned annually to establish the plucking table; therefore, collecting biomass data may offer the best option for identifying high-yielding tea plants at an early growth stage. Trunk diameter has also been used in tea (Visser, 1969) and other crops for predicting canopy size (Smith, 2008), and may offer the simplest measurement for larger scale screening, whereas collecting and weighing biomass may be cumbersome. Although trunk diameter had a lower correlation coefficient value than pruned biomass (0.520 vs. 0.780), it is a faster measurement to collect and can be done at any time of year. Our lowest correlations were found for height × width and image-measured canopy areas. Canopy area has been used previously as a predictor of yield; however, as both poor- and high-yielding plants are found in either upright or spreading accessions, yield per unit area of the plant canopy is largely independent of the canopy area itself (Visser, 1969). Our sampling for canopy area analysis may also have had lower power as a result of small sample size and high phenotypic variability. Our tested accessions had varying degrees of genetic diversity, with the accessions Big Leaf, Fairhope, Large Leaf, and Small Leaf from more clonally derived plant material than Assamica, China Seed, and Georgian, which were selected from seed more recently (Orrock et al., 2017). However, this variability did not seem to impact our results (Table 2), as we observed correlation values similar to those reported previously (Visser, 1969).

Yield is a highly complex trait influenced by both genetic and environmental conditions. Data reviewed in Visser (1969) indicate that clones planted at different heights on a slope can result in yield differences of 3% to 44%. Although soil heterogeneity may be responsible for some of these differences (Visser, 1969), there could also be other contributing factors, such as pathogen damage or water availability, which may covary with slope. Given the degree of yield variability that can be observed even in clonal lineages within a single site, relative yields among accessions tested within a single study are more reliable than absolute reported yields compared from different times or locations. Our results identify the accession Fairhope as having the greatest yield among seven accessions in both 2018 and 2019 in a research plot in north-central Florida. These data, although from a single growing location, provide an important starting point for regional selections and for future studies on site characteristics and management practices. Given resource limitations in research, studies examining management practices are unlikely to include a large number of plant lines, repeated for each management treatment; data from this study will enable future workers to focus on those accessions most likely to yield well in north Florida and Gulf of Mexico coastal growing regions. Furthermore, we have confirmed that the black tea infusions prepared from the harvested material were of good quality and had caffeine levels comparable to commercially available tea (Table 2). Assamica and commercial black tea (Lipton Yellow Label Tea) had significantly greater caffeine levels than other accessions, with the lowest being in China Seed (Table 2). Caffeine content is one of several chemical analyses demonstrated to correlate well with tea quality (Liang et al., 2003). Many studies report caffeine in tea leaves in percentages by dry weight [e.g., 1.53% to 3.49% (Zhang et al., 2020b)], and about 50% to 85% of that caffeine is estimated to be transferred to the tea infusions (Tfouni et al., 2018). Although mass spectral signatures for the presence of theanine, theobromine, catechin, epigallocatechin, epigallocatechin gallate, and catechin gallate were observed in our analyses (data not shown), accurate quantification of these compounds were not possible because of different degrees of polymerization of the compounds in different replicate preparations of tea infusions. However, the mass spectral methods described here will be useful for refining methods to estimate tea quality based on phytochemical composition.

Our results indicate that pruned biomass or trunk diameter measurements taken during the first 3 years of establishment may be the best early predictors for yield in a tea breeding program. Our analyses show correlation values similar to those reported previously for other tea varieties (Visser, 1969), and we identified several potential high-yielding accessions for Florida. These results suggest there is potential to breed specific varieties of tea suited for Florida field production using available tea accessions. Typically, it takes 5 to 6 years for a tea plant to reach maturity. With our early selection techniques, we can identify potentially higher yielding varieties sooner, supporting an emerging local industry with better-adapted planting materials.

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Supplemental Table 1. Survival of tea accessions following field planting in Citra, FL. Mean number of living plants per block for years 2017 and 2018 are presented for each accession. Plants of each accession were planted in three different blocks following the randomized block design.

	Living plants (no./block)	
Accession	**2017**	**2018**
Assamica	13.3	3
China Seed	15	9.7
Large Leaf	14.6	13.3
Big Leaf	15	13.3
Fairhope	15	8.7
Georgian	13.7	7
Small Leaf	15	4.3