Agriculture, Forestry and Fisheries
2023; 12(6): 180-185
http://www.sciencepublishinggroup.com/j/aff
doi: 10.11648/j.aff.20231206.12
ISSN: 2328-563X (Print); ISSN: 2328-5648 (Online)

Characters Association Study of Tea (*Camellia sinensis*) Clones Using Morphological Markers in South West Ethiopia

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To cite this article:
Mohammedsani Zakir, Lemi Beksisa, Melaku Addisu. Characters Association Study of Tea (Camellia sinensis) Clones Using Morphological Markers in South West Ethiopia. *Agriculture, Forestry and Fisheries*. Vol. 12, No. 6, 2023, pp. 180-185.doi: 10.11648/j.aff.20231206.12

Received: August 2, 2023; **Accepted**: October 26, 2023; **Published**: November 17, 2023

Abstract: In order to choose high yielding cultivars, it is crucial to understand the complex quantitative character of tea yield and how it is influenced by traits that are related to yield. On experimental plots installed in RCBD with three replications, the study was carried out during the 2017–2018 cropping season. Number of days between medium pruning and the first harvest, height to the first branch, stem diameter, leaf serration density, leaf width, leaf size, petiole length, leaf ratio, internode length, shoot length, number of shoots, canopy diameter, hundred shoot weights, and fresh leaf yield per tree are just a few of the morphological traits that were recorded. Leaf length, leaf width, the number of days between medium pruning and the first harvest, leaf size, and hundred-shoot weight all showed positive and significant correlations with tea yield. The results of a path coefficient analysis showed that leaf size (p=0.783) had a positive direct effect on the yield of fresh tea leaves, whereas leaf length, leaf width, height to the first branch, the number of days between medium pruning and the first harvest, and the weight of a hundred shoots had a negative direct effect. Leaf size had positive direct effects on fresh tea leaf yield per plant. The phenotypic correlation between fresh tea leaf yield per plant and leaf size was both positive and significant, supporting the claim that correlation explains true relationships and indicating the importance of these traits for direct selection to increase tea yield.

Keywords: Association, Morphological Path, Correlation Coefficient, Tea

1. Introduction

Around the current world daily than three billion cups of Tea (*Camellia sinensis* (L.) O. Kuntze) are consumed, making it one of the most popular and least expensive types of beverages [1]. Three different types of tea are categorized primarily based on the characteristics of the leaf [2]. Kitamura and later Sealy identified two intra-specific forms of tea based on leaf pose and growth habit as *Camellia sinensis var. sinensis (L)* and *Camellia sinensis var. assam.* Tea yield is a complex quantitative characteristic that is influenced by a number of yield-related characteristics. Because of this, it's crucial for tea breeding programs to understand the relationships between traits that affect yield. Strongly positive character-to-yield correlations in crop plants should be the main focus of selection for high-yielding genotypes [3, 4].

Phenotypic, genotypic, and environmental correlations are the three types of correlations that are typically discussed in quantitative genetics [5]. Phenotypic correlations (rp) are associations between two characters that are capable of direct observation. The degree to which the two observed characters are linearly related is quantified by phenotypic correlations. According to [5], it is established through measurements of the two characters in a variety of population members. Breeding values are associated through genetic correlation (rg) thus, additive genetic variance between the two individuals. Pleiotropy, a gene property that allows it to affect two or more characters, is the main genetic cause of correlation. Pleiotropy results in simultaneous variation in the characters that a gene affects when it segregates. Additionally, linkage is another factor that contributes to transient correlation, especially in populations that are the result of crosses between divergent strains. Genetic correlation measures how much co-variation (simultaneous variations) is caused by the same genes or genes that are closely linked to each other in two different characters [5].

For breeding program selection and combining several desirable characteristics, association of characters between yield, its components, and other economic traits is important. The benefit of a selection strategy that considers multiple characters at once is implied [6]. When breeding for high yield, it makes sense to look at a variety of factors and pay closer attention to those that have the biggest impact on yield because correlation coefficient is a measure of the degree of linear association between two variables.

A path coefficient, which measures the direct impact of one variable on another [7], is just a standardized partial regression coefficient. Estimates of correlation coefficients are useful in identifying the constituent parts of a complex trait like yield, but they do not accurately depict the relative weight or direct and indirect effects of each of the component characters on yield [8]. The pattern of interaction of other known factors can be more precisely clarified through path analysis. It allows for the identification of both direct and indirect causes of association and quantifies each character's relative importance [9]. Direct selection must be used when correlation coefficients are positive and comparable to the direct effects, according to [10], whereas indirect selection may be used when correlation coefficients are positive but the direct effects are insignificant or negative. It is clear that yield is the result of characters that correlate with yield and a few other ambiguous factors. So, in order to use this method, there must be a relationship between the variables' causes and effects [7]. The goal of the exercise was to comprehend how the morphological traits of tea plants that affect yield are associated.

2. Material and Methods

2.1. An Explanation of the Study Sites

The experiment was conducted in 2017–2018 at the Jimma Agricultural Research Centers (JARC) at Melko and Gera.

Melko, which has an elevation of 1750 meters above sea level, is situated at 7°46′ N and 36°E in latitude and longitude, respectively. with altitude of 1750 m a. s. l., average of last five year temperature was minimum11.7°C and maximum 25.9°C, rain fall of 1511.7 mm, 68.4% relative humidity, wind speed at 1m 2.448 km/hrs, monthly mean soil temperature at 5cm 24.9°C and average annual sunshine duration of 73 to 95 hours. Melko is distinguished by Eutric Nitosol (reddish brown) with a pH of approximately 5.2 [11].

Gera, which has an elevation of 1940 m a, can be found at latitudes of 7 7′ N and 36 E, respectively, with altitude 1940 m a. s. l., average of last five year (Table 1) temperature was minimum 11.1°C and maximum 23.9°C, rain fall of 1558.9 mm, 71.7% relative humidity, wind speed at 1m 1.92 km/hrs, monthly mean soil temperature at 5 cm 22.46°C and 61.76 hrs average annual sun shine. Gera, station also characterized by red soil, which was loam type and quite fertile [12].

Table 1. *Melko and Gera 2012-2016 metrological data.*

Location	annual RF (mm)	monthly temp mean (°C)		monthly RH (%)	monthly wind speed at 1m	monthly wind speed at 2m	monthly soil tem (°C) at 5cm	monthly sunshinehrs	soil type
		min	max						
Jimma	1512	12	26	68.4	2.5	8.1	25	6.16	Eutric Nitosol (reddish brown)
Gera	1559	11	24	71.7	1.9	1.6	23	5.15	Loam type (red soil)

2.2. Materials for Experiments

The experiment was conducted on thirteen newly introduced Assam-type tea clones that were gathered from various tea farms (Wushwush, Gumero, and Chewaka) and JARC established at Melko and Gera research stations (Table 2).

Table 2. *Description of the tea clones that were used in the study.*

Serial no.	country of introduction	tea clones	sources of tea clones
1	Kenya	Mlk-2	JARC
2	India	L6	Gummaro
3	Kenya	Mlk-1	JARC
4	India	B9	Gummaro
5	Kenya	11/56	Wushwush
6	India	Chai	Gummaro
7	Kenya	S-15/10	Chewaka
8	Kenya	FNF	Wushwush
9	India	BB-35	Gummaro
10	India	SR-18	Gummaro
11	Kenya	11/4	Wushwush
12	Kenya	6/8	Wushwush
13	Kenya	31/11	Chewaka

Source: Jimma Agricultural Research Center (JARC, 2005)

2.3. Experimental Design and Management

At the Gera and Melko research station, the experiment was conducted using RCBD with three replications over the effectively settled tea plantation in 2005. According to [13], 12 years old tea bushes were medium pruned in December 2017 at a 50 cm height from the ground. Following these treatments, tea plants returned to their typical cycle of shoot replacement or plucking stage; from mid-March to May, during the pre-monsoon season, all quantitative data from five sampled plants per plot were recorded.

2.4. Collected Data

Following the established protocols for tea descriptors [14], fifteen quantitative data points (Table 3) were gathered from each tea clone. For the following characters, the data was gathered from five representative plants that were hand-picked for each plot.

Table 3. *Lists of quantitative characters studied and their descriptions.*

no.	characters	unit	description
1	Internode length	cm	Measured distance between the 5th and 6th leaves from top of a flush growth, average of 10 shoots exposed to full sunlight
2	Length of mature leaf	cm	Recorded on the 5th leaf below the apical bud, average of five leaves
3	Width of mature leaf	cm	Measured on the 5th leaf from the apical bud of flushing shoot at the maximum breadth, average of five leaves
4	Length of mature leaf petiole	mm	Recorded on the 3rd leaf from the apical bud of flushing shoot, average of five leaves
5	Height up to 1st branching position	cm	Measured length of stem starting from the ground up to the first branch
6	Shoot length	cm	Recorded by measuring the length of harvested shoot of two leaves and a bud at 2/3 height of node between the 2nd and 3rd leaf
7	Canopy diameter	cm	Measuredfrom North-South and East-West using tape meter and taking the average as (NS +EW)/2
8	Fresh tea leaf yield	g	Recorded from five representative plants and divided by number of sampled plants to obtain yield per tree by undertaking fine plucking
9	Leaf serration density	no./cm	Counted number of serration per one cm at 5th leaves, average of single leave from five sampled plants
10	Flushing duration from medium pruning	no.	Counted number of days from medium pruning to reach at two leaves and a bud stage on plot based
11	length leaf to width ratio	cm	Measured by dividing the leaf length value by the leaf width, average of five leaves
12	Leaf size	cm^2	Computed by multiplying leaf length by leaf width values
13	Hundred shoot weight	g	Measured by taking weight of hundred harvestable shoot per plot
14	Number of shoot	no.	Recorded by counting the harvestable shoots from sampled five plants and averaged to get per tree
15	Stem diameter	mm	Measured by using caliper at 10cm height of stem above ground

2.5. Phenotypic and Genotypic Correlation Coefficient Analysis

The phenotypic and genotypic correlation coefficients estimated using the formula suggested by [10].

$$rp = Pcovxy/V\ (Vpx.\ Vpy)$$

$$rg = Gcovxy/V\ (Vgx.\ Vgy)$$

Where,
rp = Phenotypic correlation coefficient
rg = Genotypic correlation coefficient
Pcovxy = Phenotypic covariance between traits x and y
Gcovxy = Genotypic covariance between traits x and y
Vpx = Phenotypic variance of trait x
Vgx = Genotypic variance of trait x
Vpy = Phenotypic variance of trait y
Vgy = Genotypic variance of trait y

2.6. Path Coefficient Analysis

Path coefficient analysis was made for morphological and biochemical traits following the method described by [7].

$$rij = Pij + \Sigma rikPkj$$

Where:
rij=Mutual association between the independent character (i) and dependent Character (j) as measured by genotypic correlation coefficient, Pij=Component of direct effects of the independent character (i) on dependent character (j) as measured by genotypic path coefficient and Σrikpkj= Sum of components of indirect effect of a given independent character (i) on a given dependent character (j) via all other independent character k, rij = Mutual association between the independent character (i) and dependent character (j) as measured by genotypic correlation coefficient.

The residual effect: estimated as described by [7].

$1 - \sqrt{R^2}$ Where: - $R^2 = \Sigma pijrij$

pij=Component of direct effects of the independent character (i) and dependent character (j) as measured by the path coefficient. rij=Mutual association between the independent character (i) and dependent character (j) as measured by the correlation coefficient.

3. Discussion of the Results

3.1. Phenotypic Correlation of Yield with Other Quantitative Traits

For most traits, the magnitude of the genotypic correlation significance was greater than the phenotypic correlation significance. This demonstrated that the environment has an impact on how phenotypes are expressed. However, traits like stem diameter, leaf serration density, leaf length, leaf width, leaf size, shoot length, number of shoots, and canopy diameter showed lower rp to rg ratios, indicating that the genetics played a significant role in how these traits were expressed. Phenotypic correlation showed that the number of days from medium pruning to the first fresh leaf harvest, the leaf length, width, and size, and the hundred shoot weights all had a positive and significant relationship with fresh tea leaf yield. This implies that improving those characteristics would increase the yield of fresh tea leaves produced by each plant. Fresh leaf yield per plant also showed negative and significant correlation height to first branch, thus, selection for a long height to the first branch will have a significant negative impact on the improvement of fresh tea leaf yield per plant.

3.2. Correlation Among Morphological Traits

Phenotypically the number of days from medium pruning to the first fresh leaf harvest showed a positive significant correlation with the stem diameter, leaf length, leaf width, leaf size, shoot length, number of shoots, and hundred shoot weight. The length, width, and size of the leaves, the petiole, the number of shoots, and the weight of a hundred shoots all significantly correlated positively with the stem diameter. With regard to leaf width, leaf size, petiole length, shoot length, and hundred shoot weights, leaf length showed a positive significant correlation. Leaf width and size showed a significant positive correlation with shoot length, petiole length, and hundred shoot weights. Both shoot length and shoot number showed positive significant with hundred shoot weight, indicating the simultaneous breeding program can be followed for the above listed traits.

At the phenotypic level, there was a negative and significant correlation between the number of days from medium pruning to the first fresh leaf harvest and the diameter of the canopy and the height to the first branch. The stem diameter, leaf length, leaf width, leaf size, shoot length, number of shoots, and hundred-shoot weight all showed negative and significant correlation with height to first branch. Stem diameter and leaf length manifested negative significant with canopy diameter. Leaf width and leaf size exhibited negative and significant association with leaf ratio and canopy diameter. These variables also indicated negative and significant correlation with leaf serration density. Conversely, there was a negative and significant correlation between canopy diameter and shoot length and hundred shoot weights. Therefore, a separate breeding program for the enhancement of the aforementioned characters is implied.

Genotypically positive and significant correlations were found between the number of days from medium pruning to the first fresh leaf harvest and the leaf ratio; the height to the first branch and the density of leaf serrations; the diameter of the stem with the leaf width and petiole length; the length of the leaf with the leaf width and leaf size; and finally the number of shoots with the canopy diameter. This showed that improving one attribute would inevitably improve the other. This was partially agreed with [15] findings that leaf length and width were positively and significantly correlated with one another. Negative and significant phenotypic correlation was manifested between number of days from medium pruning to first fresh leaf harvest and canopy diameter; leaf serration density and leaf width; number of shoots with leaf length, leaf width and leaf size; indicating a separate breeding program for the traits.

3.3. Genotypic Correlations for Morphological Traits and Yield

The relationship of yield with other traits in genotypic form presented in Table 4. Genotypically, stem diameter was negatively and significantly correlated with green tea leaf yield, this indicated separate improvement of the traits and large stem diameter was low yielder thus simultaneous improvement of stem diameter and fresh tea leaf yield was not possible. However, height to first branch, leaf serration density, leaf ratio, number of shoot, canopy diameter showed positive and non-significant correlation. This shows that the yield of fresh tea leaves and the above-mentioned characteristics were simultaneously improved. While number of days from medium pruning to first harvest, leaf length, leaf width, leaf size, petiole length, internode length and shoot length showed negative and insignificant correlations with green tea leaf production per plant.

Table 4. *Phenotypic (lower diagonal) and genotypic (upper diagonal) correlation coefficients of 15 morphological traits.*

Traits	ND	HFB	SD	LSD	LL	LW	LS	PL	LR	IL	SL	NS	CD	HSW	YLD
ND		0.29	-0.04	0.05	-0.06	-0.16	-0.15	0.18	0.66**	-0.52	-0.31	-0.41	-0.59*	0.05	-0.18
HFB	-0.73**		-0.17	0.75**	-0.03	-0.53	-0.27	0.36	0.62*	0.29	-0.16	0.23	-0.06	-0.24	0.26
SD	0.77**	-0.71**		-0.47	0.16	0.55*	0.34	0.60 *	0.06	0.18	0.03	-0.05	-0.26	-0.49	-0.59*
LSD	-0.19	0.31**	-0.16		-0.08	-0.58*	-0.31	0.04	0.54*	0.32	-0.17	0.19	0.07	-0.16	0.45
LL	0.44**	-0.45**	0.39**	-0.17		0.57*	0.92**	0.47	-0.15	0.19	0.25	-0.55*	-0.34	-0.16	-0.29
LW	0.65**	-0.63**	0.56**	-0.3**	0.55**		0.84**	0.13	-0.47	-0.08	0.25	-0.55*	-0.33	-0.46	-0.51
LS	0.59**	-0.59**	0.52**	-0.26*	0.90**	0.84**		0.36	-0.35	0.08	0.32	-0.59*	-0.34	-0.29	-0.40
PL	0.22	-0.06	0.35**	0.01	0.31**	0.28**	0.34**		0.46	0.34	0.24	0.09	-0.24	-0.13	-0.38
LR	-0.04	0.11	0.04	0.44**	-0.16	-0.34**	-0.27**	0.14		0.09	-0.42	0.03	-0.45	-0.09	0.09
IL	-0.18	0.04	-0.11	0.08	-0.06	-0.07	-0.09	0.12	-0.11		0.20	0.42	0.32	-0.25	-0.05
SL	0.53**	-0.35**	0.33**	-0.13	0.34**	0.52**	0.48**	0.29**	-0.24 *	0.15		0.25	0.47	0.16	-0.29
NS	0.32**	-0.37**	0.53**	-0.08	-0.09	0.06	-0.01	0.05	0.09	0.07	0.15		0.72**	0.29	0.35
CD	-0.86**	0.65**	-0.62**	0.12	-0.41**	-0.59**	-0.54**	-0.23*	0.01	0.20	-0.49**	-0.18		0.29	0.25
HSW	0.75**	-0.77**	0.74**	-0.26*	0.31**	0.52**	0.45**	0.18	-0.03	-0.11	0.50**	0.50**	-0.60**		0.16
YLD	0.24*	-0.49**	0.12	-0.03	0.28**	0.38**	0.37**	-0.10	-0.08	-0.12	0.11	0.18	-0.18	0.31**	

**=highly significant (P<0.01),*= significant (P<0.05), ND= number of days from medium pruning to first harvest, HFB=height to first branch, SD= stem diameter, LSD= leaf serration density, LL= leaf length, LW=leaf width, LS=leaf size, SL=shoot length, NS=number of shoot, CD=canopy diameter, HSW=hundred shoot weight, PL= petiole length of mature leaf, LR=leaf ratio, IL= internode length and YLD= fresh tea leaf yield

3.4. Path Coefficient Analysis

Path coefficient analysis was performed at the phenotypic level considering fresh tea leaf yield per plant as the dependent trait and yield attributes as independent traits. Each component has a two-way impact, specifically a direct impact on tea leaf yield and an indirect impact through the component that correlation studies have not revealed. The direct and indirect phenotypic effects of different traits on fresh tea leaf yield are presented in Table 5.

Path coefficient analysis showed that only leaf size had a positive direct impact on fresh tea leaf yield per tree (p=0.783). However, height to first branch (p=-0.611), leaf length (p=-0.445), number of days from average height to first fresh leaf harvest (p=-0.355), width leaves (p=-0.177) and hundreds of shoot weights (p=-0.020) showed a direct negative impact on fresh tea leaf yield per plant. This contrast with [16] on a hundred shoot weight reported a direct positive effect on fresh tea leaf yield. Leaf size (p=0.783) had a positive direct effect on fresh tea leaf yield per plant and showed a positive and significant phenotypic correlation with fresh tea leaf yield per plant. This demonstrates that correlation explains the true relationships and shows that these traits are important for direct breeding to improve tea yield. Leaf length, number of days from average size to first fresh leaf harvest, leaf width and hundred shoot weight showed positive and significant phenotypic correlation coefficients and directly negatively affected yield of fresh tea leaves. This indicates that these traits are important for indirect breeding to improve the yield of fresh tea leaves. Therefore, the positive correlation coefficient is largely due to their respective positive indirect effects on the other traits. Height to first branch had a negative direct effect and a negative correlation coefficient with fresh tea leaf yield, which demonstrated a correlation that explains the true relationship between traits.

Table 5. *Estimates of morphological traits direct (bold) and indirect (off) effects on fresh tea leaves at phenotypic level.*

Traits	ND	HFB	LL	LW	LS	HSW	rp to YLD
ND	-0.355	0.448	-0.195	-0.114	0.466	-0.015	0.235
HFB	0.260	-0.611	0.200	0.110	-0.461	0.015	-0.486
LL	-0.155	0.275	-0.445	-0.098	0.705	-0.006	0.276
LW	-0.230	0.382	-0.245	-0.177	0.660	-0.010	0.380
LS	-0.211	0.359	-0.400	-0.149	0.783	-0.009	0.373
HSW	-0.267	0.471	-0.136	-0.093	0.353	-0.020	0.309
Residual0.043							

ND= number of days from medium pruning to first harvest, HFB=height to first branch, LL= leaf length, LW=leaf width, LS=leaf size, HSW=hundred shoot weights and YLD= fresh tea leaf yield

4. Synopsis and Conclusion

For most traits, the magnitude of the genotypic correlation significance was greater than the phenotypic correlation significance. This suggested that environmental factors affect how phenotypes manifest themselves. However, the ratio of rp to rg was lower for traits like stem diameter, leaf serration density, leaf length, leaf width, leaf size, shoot length, number of shoots, and canopy diameter, indicating that the genetic component played a significant role in how these traits were expressed. Phenotypic correlation showed that the number of days from medium pruning to the first fresh leaf harvest, leaf length, leaf width, leaf size, and the hundred shoot weights all had a positive and significant relationship with fresh tea leaf yield. This suggests that improving those traits would lead to a higher yield of fresh tea leaves per plant. Genotypically, stem diameter showed a negative and significant correlation with fresh tea leaf yield. Thus, a large stem diameter was a low yielder, so simultaneous improvement of stem diameter and fresh tea leaf yield was not possible. However, height to the first branch, number of shoots, leaf ratio, density of the leaf serrations, and diameter of the canopy showed positive but non-significant correlations. This suggests that both the traits and the yield of fresh tea leaves can be improved concurrently. Leaf size had positive direct effects on fresh tea leaf yield per plant, exhibited positive and significant phenotypic correlation with fresh tea leaf yield per plant. This supports the claim that correlation explains real relationships and implies the significance of these traits for direct selection to increase tea yield.

Leaf length, the number of days from medium pruning to the first fresh leaf harvest, leaf width, and hundred shoot weights all showed positive and significant phenotypic correlation coefficients as well as negative direct effects on fresh tea leaf yield. As a result, it can be concluded that these traits are crucial for indirect selection to increase fresh tea leaf yield. Therefore, their respective positive indirect effects of other traits played a large role in the positive correlation coefficient.

Acknowledgments

Our great gratitude goes to Almighty Allah for giving us health and time to conduct this study and then to the Coffee and Tea breeding staff of Jimma Agricultural Research Center for their technical assistance throughout the study as well as Ethiopian Institute of Agricultural Research for funding the study.

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