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**RESEARCH ARTICLE**

# EVALUATION OF MORPHOLOGICAL TRAITS AND BIOCHEMICAL PARAMETERS OF TEA (*Camellia sinensis*) GENOTYPES FOR THE QUALITY AND YIELDS

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## ABSTRACT

**An extensive knowledge on morphological traits and biochemical properties is a prerequisite in utilizing the existing tea germplasm for its improvement incurring grower acceptance and market profitability. The aim of the present study was to evaluate the five clones of tea genotypes (SH/D/11/333, H/B/6/4, TV-23, SH/D/11/13 and H/B/2/3) based on their morphological and biochemical responses in the environment of Sylhet, Bangladesh to identify superior clone. The experiment was laid out in a Latin Square Design (LSD) and Completely Randomized Design (CRD) with five replications and collected data were analyzed by using SPSS. The findings demonstrated that the greater amounts of theaflavins (TF), thearubigins (TR), a ratio of TF and TR, high polymerized substances, caffeine content and the highest amount of liquor colour index were observed in the superior quality of tea genotypesTV-23 and H/B/2/3. Meanwhile, another test clone SH/D/333 showed better performance in morphological traits along with yield attributes. In overall, the test clone H/B/2/3 and standard clone TV-23 can be considered for quality tea production while, SH/D/11/333 can be used for commercial plantations and future utilization to shape up the Bangladesh tea industry sustainably.**

Keywords: morphology, biochemical properties, tea genotypes, total liquor color (TLC), caffeine content (CC), test clones.

## INTRODUCTION

Tea (*Camellia sinensis* (L) O. Kuntze) is the most popular consumed beverage in the world. Globally, tea consumption has increased from \$1.84 billion in 1990 to \$12.66 billion in 2018 and in the next 10 years it will be projected to highly increase the demand for its industrial value (Goggi, 2018). Tea was first used in China as a traditional medicinal drink and later become a popular beverage in the world. As an important 'health drink' in view of its purported medicinal value, it has been gaining further popularity. Nearly 2/3$^{rd}$ of the world's population takes tea daily as a morning drink (Nasir and Shamsuddhoa, 2011). Tea has numerous health promoting beneficial compounds like catechin, caffeine, theanine and polyphenols as well as antimicrobial and antioxidant properties (Reygaert, 2014; Yan *et al*. 2020). Polyphenols also helps to prevent certain types of cancer, slow down ageing and reduce the risk of cardiovascular diseases (Yang *et al*. 2009).

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Tea is a predominantly agro-based export-oriented evergreen crop in Bangladesh. There are three moderately divergent ecological zones of tea cultivation in Bangladesh such as Surma Valley in greater Sylhet, Halda Valley in Chittagong and Karatoa Valley in Panchagarh districts (Mamun, 2011). According to the Bangladesh Tea Board (BTB), 67.38 million kg of tea was produced in 2015 in Bangladesh but it has increased to 96.5 million kg in 2021, the highest in 168 years of commercial production in the tea industry (Anonymous, 2022). A decade back Bangladesh exported 9.01 million kg of tea but in the financial year 2015-2016, it was exported only 0.39 million kg because of increased domestic consumption from 162 gardens. The quality of black tea depends mainly on the components and colour of the tea infusions.

Choosing tea cultivars based on the high yield with adequate cup quality for various end products, and high functional components are the pre-requisites for tea improvement. And most tea breeding institutions have developed their varieties through conventional breeding. Identification of taxa in variation in floral structures is uninformative, where leaf morphology could play an important role (Meade and Parnell, 2003). The morphological characterization of a species is very useful in the separation of populations into different morphotypes and proper utilization of genetic resources in plant breeding programmes (Piyasundara *et al*. 2009). Several outstanding accessions from natural populations were released by Bangladesh Tea Research Institute (BTRI) through breeding methods (Alam and Haque, 2001). The characterization of eleven released clones of BTRI was statistically described by Sarwar and Dutta (2002) based on the leaf morphology. Therefore, characterization and preservation of tea for future crop improvement programs are the most important factors to support the industry for better growth.

Many researchers have tested the tea germplasms using morphological characterization, biochemical compositions and molecular evaluations (Feng *et al*. 2014; Li *et al*. 2016b). Assessment of morphological characteristics viz. leaf size, leaf area and fresh weight are frequently used traits in breeding programs for yield improvement. Because the quality of tea is highly co-related with leaf biochemical composition like polyphenols, carbohydrates, amino acids (AA), soluble solids, theanine and caffeine (Li *et al*. 2016a; Tang *et al*. 2019). An extensive variation in physical characteristics and chemical composition has also been reported in leaves of different tea germplasms (Gai *et al*. 2019). Still no systematic study was carried out based on morphological and biochemical aspects of tea varieties in Bangladesh. Hence, analysis of morphological characteristics and biochemical parameters of tea can helps systematically define and narrate for conservation of tea diversity and future utilization which will shape the sustainability of the Bangladesh tea industry. Four tea clones like SH/D/11/13, SH/D/11/333, H/B/6/4, H/B/2/3 and TV-23 are the leading future tea genotypes in Bangladesh (Aziz *et al*. 2011). Therefore, the present experiment was conducted to study the variations in morphological traits and biochemical properties of leaves and the yield of five tea clones for varietal improvement of tea in Bangladesh.

## MATERIALS AND METHODS

### Sample collection

The clone of five different tea genotypes namely SH/D/11/333, H/B/6/4, TV-23, SH/D/11/13 and H/B/2/3 were collected from the experimental Farm of the Bangladesh Tea Research Institute (BTRI). The tea samples were collected with a regular plucking interval of 15 days during June and October 2016.

### Determination of morphological traits

The randomly selected 30 plants from each tea genotypes were used to examine morphological parameters in Latin Square Design (LSD) with five replications. The whole experimental area was divided into 25 equal blocks and size of a plot was 22.95 × 12.6 m$^2$. Leaf length and breadth, length of leaf pedicel, and length of leaf pedicel to first serration of 4$^{th}$ or 5$^{th}$ number leaf at the tip of the main shoot was measured in centimeters. The internodal length was measured from the well-developed young shoots which were ready to be plucked. Shoot components were schematic from maintenance foliage to scale leaf (MF×SL) as first-order, scale leaf to fish leaf (SL×FL) as second-order, fish leaf to mother leaf (FL×ML) as third-order, mother leaf to third leaf (ML×3L) as fourth-order, third leaf to second leaf (3L×2L) as fifth-order, second leaf to first leaf (2L×1L) as sixth-order and first leaf to bud (1L×Bud) as seventh-order. The number of leaf veins and serratulation on the leaf margin was measured by counting in pair numbers. The leaf angle formed between the branches and the lamina was measured in degree by a graduated degree scale. The ten tea shoots from each genotype were taken randomly for measuring the fresh and dry weight. After measuring fresh weight (g), the samples were oven dried for 48 hours at 65˚C and dry weights wet and dry ratio were recorded.

**Sample preparation for biochemical trait analysis**

About two grams of black tea sample was added to 100 ml of boiled water and allowed

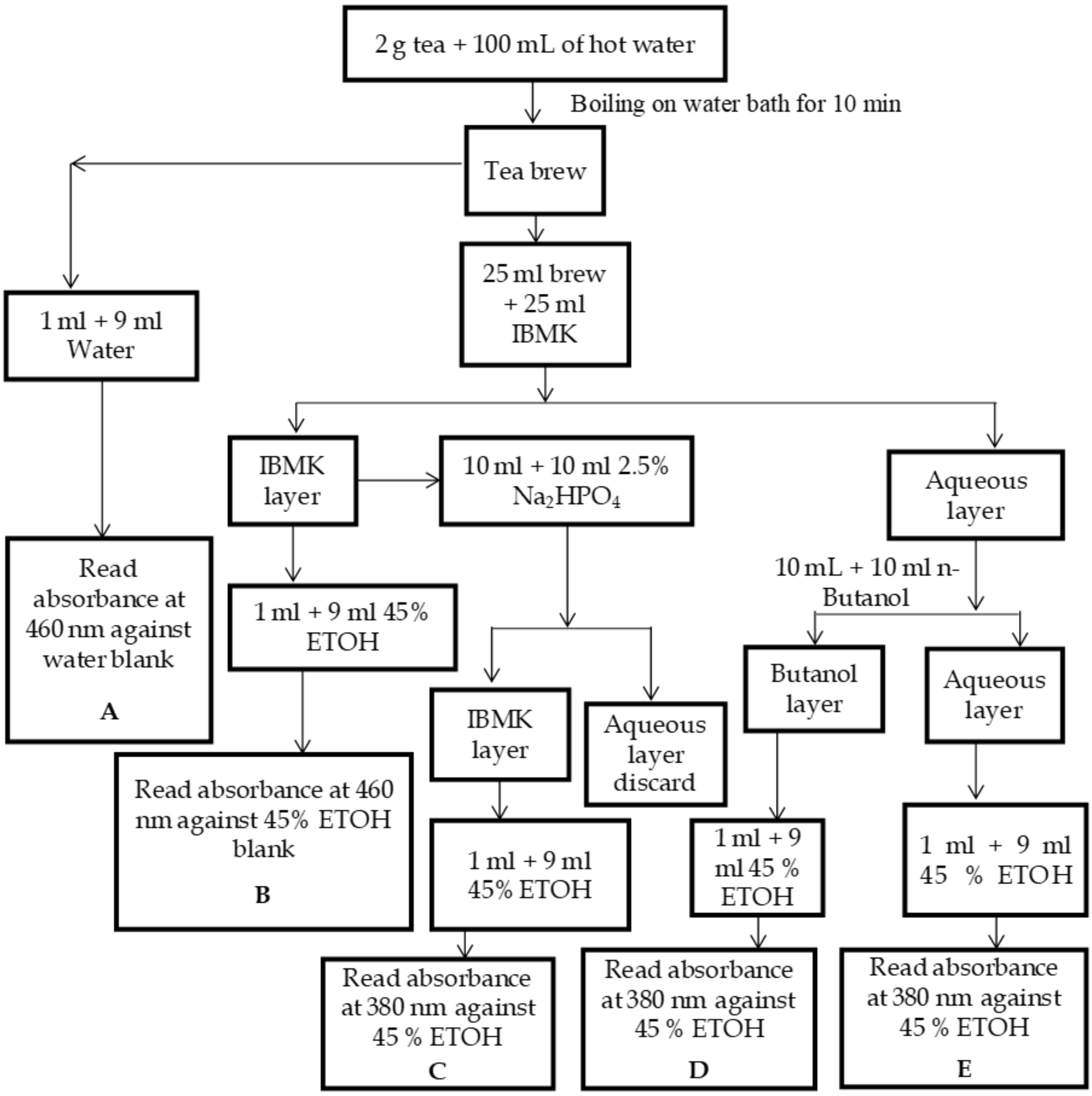


**Figure 1: The method followed to determine Theaflavin (TF), Thearubigin (TR), Total liquor colour (TLC) and High polymerised substances (HPS)**

to brew for 10 min with intermittent shaking. The mixture was filtered and the liquor was then cooled to room temperature and weighed. An aliquot of liquor was removed and evaporated to constant weight in an oven at 65˚C for 48 h. From the weight of residue, the amount of soluble solids in the extract was obtained according to the method of Liang and Xu (2001). The theaflavin (TF), thearubigins (TR), total liquor color (TLC) and high polymerized substances (HPS) content of the each black tea sample were analyzed by the method of Al Mamun *et al*. 2016 (Figure 1). The contents were infused over the boiling water bath for 10 min. After filtering, the analysis was carried out as per the scheme furnished. Solvent extraction of tea was carried out with adequate shaking at every stage. Contents of TF, TR, HPS and TLC were calculated from the absorbance values as followed in Figure 1.

Where,
The theaflavin (TF), thearubigin (TR), total liquor colour (TLC), and high polymerised substances (HPS) level were calculated by using the following formulas and expressed in percentage (%).

$$TF\ (\%) = \frac{4.313 \times C \times 2 \times 100}{\text{Sample weight} \times \text{DMC}}$$

$$TR\ (\%) = \frac{13.643 \times (B + D - C) \times 2 \times 100}{\text{Sample weight} \times \text{DMC}}$$

$$TLC\ (\%) = \frac{10 \times A \times 2 \times 100}{\text{Sample weight} \times \text{DMC}}$$

$$PS\ (\%) = \frac{13.643 \times E \times 2}{\text{Sample weight} \times \text{DMC}}$$

Multiplication factors of TF and TR were derived from molar extinction coefficients of pure compounds and dilution factors (Roberts and Smith, 1963). In the case of TLC, value 10 is the dilution factor (Thanaraj and Seshadari, 1990).

The colour index level was calculated according to the method of Ramaswamy (1986):

$$CI = \frac{TF \times 100}{TR + HPS}$$

**Determination of caffeine**

For determining caffeine at first heat 50 ml distilled water at 40˚C and then a 100 mg tea sample was added to the hot water and stirred for 30 minutes with a magnetic stirrer. Then filtered it and cooled it to room temperature. Poured 50 ml of chloroform into the tea infusion and stirred for 10 minutes with magnetic stirrer. Separate the water phase from the organic phase (Chloroform) with a separating funnel. Pour the organic solution into quartz of UV cell and take absorbance in 260 nm. The Quantum of caffeine present in tea leaves was computed using the standard calibration curve derived from known concentrations (0 to 20 ppm) of caffeine and the results were expressed as percent caffeine equivalents (Maidon *et al*. 2012). The pure caffeine standard curve was needed to determine caffeine content.

**Data analysis**

Collected morphological data were analyzed for having an overall mean, minimum, maximum value and standard deviation for calculation of CV%, means of each treatment (variety) and compare means value. And the mean values of TF, TR, HPS, TLC, CI and CC were also analyzed and mean separation was done using Duncan's New Multiple Range Test and one way ANOVA was used to measure the level of significance. Correlate value was assigned to have the Pearson's correlation coefficients and SPSS were applied to get the correlation data/values.

## RESULTS AND DISCUSSION

**Analysis of morphological traits**

Five genotypes (SH/D/11/333, H/B/6/4, TV-23, SH/D/11/13 and H/B/2/3) of tea were differed significantly in the leaf length, leaf breadth and leaf length and breadth ratio. The result was demonstrated on a general basis where the leaf length and leaf breadth was very essential for overall productivity in tea and leaf length and leaf breadth ratio were potentially limited for effective photosynthesis.

The highest leaf length and leaf breadth was reported in the genotype SH/D/11/333, while the shortest was found in the genotype H/B/6/4 (Table 1). The longest leaf length and breadth of the tea genotypes can potentially affect the yield. Since leaves serve as the sites for photosynthetic activities in the tea plant, the shortest leaf length and breadth may have severe implications for the production of assimilates in the plant. Consequently, a greater length and breadth of them in any particular tea genotypes would be assumed to produce a better yield due to the higher photosynthetic capacity that is brought to bear by an increased leaf area index and a resultant higher fraction of intercepted radiation and its utilization efficiency. Longer leaf length and breadth of teas produced and displayed a more elaborated, varied and complex array of aromas, colours and mouth-feel. However, in leaf length, the germplasm closer to the Assam type exhibited a length of young shoots compared to the germplasm resembling the China type (Smith and Barua, 2011). Similarly, in respect of leaf breadth all clones were significantly differed and for commercial cultivation in Bangladesh considering different morphological and yield characters of MZ/39 which was found the most suitable followed by B2 × T1 and SDL/1 (Aziz *et al*. 2011). The better performance was showed by SH/D/11/333 in the current study because of yielded the longer leaf length and breadth which may have enabled this genotype to produce greater assimilates during their photosynthetic activities, resulting the higher yield.

A leaf having a lower ratio of leaf length and leaf breadth indicated that they were somewhat broader in leaf shape and obviate in shape including H/B/6/4 and H/B/2/3. The genotypes H/B/6/4 and H/B/2/3 possess the medium leaf size and SH/D/11/333, TV-23, SH/D/11/13 possess the smallest leaves (Table 1). On the basis of the ratio of leaf length and leaf breadth of leaves, the genotypes would be grouped into three distinct groups elliptic, lanceolate and obviate. However, it was reported that the leaf length was found to be less important in distinguishing some Sri Lankan tea clones (Ariyarathna *et al*. 2011; Kottawa-Arachchi *et al*. 2013). The highest ratio of leaf length and leaf breadth indicated that the leaf is long and narrow with an obovate shape and the lowest ratio indicates the leaf is wide relative to its length with lanceolate shape. On the basis of parametric and non-parametric characteristics of the eleven BTRI released clones, it was observed that the tea clones are not usually possible to recognize on the basis of a single character but rather a combination of characters is required (Al Mamun *et al*. 2016; Sarwar *et al*. 2002).

Variation was also observed in the leaf angle of the studied tea genotypes. The highest leaf angle was found in the genotype TV-23 and can be categorized as oligophile. The lowest leaf angle was found in the genotype H/B/2/3 and can be categorized as ereectophile. On the basis of leaf angle, tea can be classed as erectophile (leaf angle <50°), planophile (leaf angle >70°) or oligophile leaf angle (50-70°).

**Table 1: Performance of morphological trait analysis of different genotypes of tea**

| **Genotypes** | **Leaf length (cm)** | **Leaf breadth (cm)** | **Leaf length and breadth ratio** | **Length of leaf pedicel to first serration (cm)** | **Internodal length (cm)** | **Leaf angle (degree)** |
|---|---|---|---|---|---|---|
| **SH/D/11/333** | $16.14^{a}$ | $7.90^{a}$ | $2.06^{de}$ | $1.87^{e}$ | $7.06^{a}$ | $49.16^{c}$ |
| **H/B/6/4** | $13.28^{e}$ | $4.97^{e}$ | $2.69^{b}$ | $2.41^{a}$ | $5.14^{de}$ | $45.00^{d}$ |
| **TV-23** | $14.31^{bc}$ | $6.49^{bc}$ | $2.20^{c}$ | $2.41^{a}$ | $6.25^{b}$ | $57.40^{a}$ |
| **SH/D/11/13** | $13.69^{d}$ | $6.64^{b}$ | $2.08^{d}$ | $2.33^{bc}$ | $5.66^{d}$ | $57.00^{ab}$ |
| **H/B/2/3** | $14.78^{b}$ | $5.27^{d}$ | $2.80^{a}$ | $2.04^{d}$ | $6.18^{bc}$ | $32.00^{e}$ |
| **LS** | *** | *** | *** | *** | *** | *** |

LS-level of significance, $^{abcde}$ Means with the different letters showed significant difference within the row (P<0.05) and ***-p<0.001.

Similarly, leaf angle varied significantly among tea varieties like, MZ/39, B2×T1, D/13 and SDL/1 in Bangladesh (Aziz *et al*. 2011). The highest leaf angle has a significant impact on the reflectance, transmittance and absorption of solar light in the vegetation layer, and thus also on its growth and development.

Among the tea genotypes studied significant variation in internodal length (INL) was observed in fourth to fifth leaf. The greater INLwas found in SH/D/11/333 (Table 2) and lowest was found in H/B/6/4. Similarly, internodal length of fish leaf to mother leaf, shoot density and yield varied significantly among their studied tea clones (Al Mamun *et al*. 2016; Aziz *et al*. 2011). The greater length of leaf and internodes can influence the yield potentially of tea.

The greater amount of shoot fresh weight was found in SH/D/11/333 and the lowest amount of shoot fresh weight was found in H/B/2/3 (Table 2). In the determination of tea yield and yield variations, fresh shoot weight has been reported to be less influential than shoot population density. The germplasm is closer to the Assam type and exhibited a higher fresh weight of young shoots compared to the germplasm resembling the china type (Smith and Barua, 2011). Nevertheless, it has been observed that clones with a moderate shoot population density can still out-yield those with high shoot population densities when the former is producing bigger shoots (Samarina *et al*. 2020). Hence, it is also possible to secure a higher yield by harvesting bigger shoots.

Dried leaves of tea are the marketable product and therefore the dry matter content of tea shoots has greater importance. The significantly highest amount of shoot dry weight (SDW) was found in SH/D/11/333 while the lowest amount was found in H/ B/2/3 (Table 2). In our present study, the result indicated that the genotypes which possess the highest leaf length and internodal length, enhanced the production of higher amount of shoot dry weight. But, the germplasm is closer to the Assam type exhibited higher dry weight of young shoots compared to the germplasm resembling the China type (Smith and Barua, 2011).

The highest ratio between shoot fresh and dry weight was found in TV-23 (Table 2). From the results of the present study, it can be concluded that in addition to the superior character of yield components, a high yielding tea genotype possesses a relatively higher ratio with superior growth parameters. The leaf fresh weights were reported from 0.80 to 0.99 g per leaf, and dry weights from 0.37 to 0.44 g per leaf (data not shown). Similarly, the leaf fresh weights were reported from 0.80 to 0.99 g per leaf, and dry weights from 0.37 to 0.44 g per leaf for tea varieties in Mississippi, United States (Zhang *et al*. 2020) which is corroborated with the present study.

**Table 2: Performance of morphological traits of different genotypes of tea**

| Genotypes | Shoot fresh weight (g) | Shoot dry weight (g) | Ratio of shoot fresh and dry weight | Number of leaf veins in pair | Number of leaf margin serrulated form in pair | Length of leaf pedicel (cm) |
|---|---|---|---|---|---|---|
| **SH/D/11/333** | $14.55^{a}$ | $3.32^{a}$ | $4.38^{c}$ | $7.53^{bc}$ | $64.83^{a}$ | $0.46^{e}$ |
| **H/B/6/4** | $12.05^{bc}$ | $2.58^{cd}$ | $4.73^{ab}$ | $7.30^{cd}$ | $47.83^{d}$ | $0.51^{cd}$ |
| **TV-23** | $12.35^{b}$ | $2.65^{bc}$ | $4.80^{a}$ | $7.80^{b}$ | $54.60^{b}$ | $0.54^{bc}$ |
| **SH/D/11/13** | $11.16^{d}$ | $2.74^{b}$ | $4.18^{cd}$ | $6.83^{e}$ | $40.93^{e}$ | $0.74^{a}$ |
| **H/B/2/3** | $9.60^{e}$ | $2.46^{de}$ | $4.07^{e}$ | $11.26^{a}$ | $48.23^{bc}$ | $0.57^{b}$ |
| **LS** | *** | *** | ** | *** | *** | *** |

LS-level of significance, $^{abcde}$ Means with the different letters showed significant difference within the row (P<0.05), **-p<0.01 and ***-p<0. 001

Genetic variation was also observed in leaf vein in pair (LVP), the number of serratulation on leaf margin serrulated form in pair (LMSP), length of leaf pedicel and length of leaf pedicel to first serration (LLPFS). The highest leaf vein pair was found in H/B/2/3 and the lowest was found in SH/D/11/13.The highest leaf margin serrulated form in pair was found in SH/D/11/333 and the lowest was found in SH/D/11/13 (Table 2). The highest length of leaf pedicel was found in SH/D/11/13 and the lowest was found in SH/D/11/333. The highest length of leaf pedicel to first serration was found in H/B/6/4 and the lowest was found in SH/D/11/333 (Table 2). These morphological characteristics of tea are often considered the most important character for selective breeding throughout the world (Feng *et al*. 2014; Li *et al*. 2016b; Mohammedsani *et al*. 2021). However, the genetic variation of these characters was continuous to distinguish or identify the tea genotypes from the studied clones. Assessing all the morphological traits, the clone SH/D/333 produced the largest leaves in terms of individual leaf area, fresh, dry weights, and new shoot weight.

**Analysis of biochemical parameters**

The biochemical components are responsible for the taste, aroma, strength, colour, briskness and pungency of the black tea infusion. The major quality attributes of tea are flavor, aroma, color, and strength (Bhattacharyya *et al*. 2008). In high-quality black tea, having a high quality green leaf is the first prerequisite during manufacturing. The greater amount of TF was recorded in H/B/2/3 (0.68%) which was followed by SH/D/11/13, TV-23, H/B/6/4 and SH/D/11/333 (0.45%) (Table 3). The greater amount of TF is responsible for the astringency, brightness, orange colour and briskness of the black tea. The low amount of theaflavin in black tea may be due to their genetic variation. Likewise, variation in TF composition in tea varieties was reported by many researchers (Li *et al*. 2016a; Tang *et al*. 2019).The rapid estimation of TF thus may resolve the problem of certain uncertainty or ambiguity that may arise during the quality assessment of tea by the tea tasters.

The highest TR was recorded in TV-23 (6.99%) which was followed by H/B/2/3, H/B/6/4, SH/D/11/333 and SH/D/11/13 (3.47%) (Table 3). TR reduces the brightness of tea liquor. The greater amount of thearubigin contributes mostly to the ashy taste of the liquor with minor improvement in astringency. TR contribute to the mouth feel (thickness) and brownish colour of the tea (Zhang *et al*. 2020). The significant variation in rate of fermentation, crude fibre content, total polyphenols, total catechins, chlorophyll-a, chlorophyll-b and total carotenoids indicated the high genetic diversity of tea germplasm in Sri Lanka (Ariyarathna *et al*. 2011; Kottawa-Arachchi *et al*. 2013). The rapid estimation TR thus may resolve the problem of certain uncertainty or ambiguity that may arise during quality assessment of tea by the tea tasters.

The TF: TR ratio of genotypes SH/D/11/333, H/B/6/4, TV-23, SH/D/11/13 and H/B/2/3 are 8.33, 8.67, 10.58, 5.24 and 9.33 (Table 3). The best TF: TR ratio is considered to be 1:10 by Hossain *et al*. (2022). It is established that the ratio between TR and TF must be 1:10 for better quality tea production. The result of this study, TF: TR ratio of all the genotypes belongs to the standard ratio of 1:10 and that indicated, all of the genotypes have a possible chance to produce better quality tea. Young tea shoots were extremely rich in polyphenolic compounds and they were the major phenolic constituents, which were responsible for the formation of theaflavins and thearubigins during the fermentation process (Kottawa-Arachchi *et al*. 2013).

The greater amount of high polymerized substances (HPS) was recorded in TV-23 (12.03%) which was followed by H/B/2/3, SH/D/11/333, H/B/6/4 and SH/D/11/13 (6.11%) (Table 3). A high polymerized substance is very effective in evaluating and classifying the quality of tea. Together with HPS, TR in tea liquor which contributes to the colour and mouth feel. The greater amount of HPS was found in TV-23 which influenced the better quality of tea. Maximum similarity value i.e. 1.000 could be found between AS I-AS 18, AS 6- AS 16, AS 3 China and AS 20-

**Table 3. Biochemical properties of different genotypes of tea**

| Genotypes | Theaflavin (TF, %) | Thearubigin (TR, %) | Ratio of TF and TR | High polymerized substances (%) | Total liquor color (%) |
|---|---|---|---|---|---|
| **SH/D/11/333** | $0.45^{e}$ | $3.74^{de}$ | $8.33^{cd}$ | $7.55^{c}$ | $2.24^{de}$ |
| **H/B/6/4** | $0.62^{d}$ | $5.41^{bc}$ | $8.67^{c}$ | $7.53^{cd}$ | $3.02^{c}$ |
| **TV-23** | $0.66b^{c}$ | $6.99^{a}$ | $10.58^{a}$ | $12.03^{a}$ | $4.22^{a}$ |
| **SH/D/11/13** | $0.66^{bc}$ | $3.47^{e}$ | $5.24^{e}$ | $6.11^{e}$ | $2.22^{e}$ |
| **H/B/2/3** | $0.68^{a}$ | $6.38^{ab}$ | $9.33^{b}$ | $10.55^{b}$ | $3.67^{b}$ |
| **LS** | *** | *** | *** | *** | *** |

LS-level of significance, $^{abcde}$Means with the different letters showed significant difference within the row ($P<0.05$) and ***-$p<0.001$.

China, whereas the lowest similarity value (0.606) was observed between AS 2 and AS 19 in the similarity coefficient matrix, utilizing young shoot characters (Smith and Barua, 2011). A lower amount of high polymerized substances (HPS) was found in SH/D/11/13 (Table 3) and the discussion might be indicative that SH/D/11/13 was a low quality tea producing genotype than others.

To determine the total liquor color of black tea, both TF and TR levels indicate the importance of biochemical components. The highest TLC was recorded in TV-23 (4.22%) which was followed by H/B/2/3, H/B/6/4, SH/D/11/333 and SH/D/11/13 (2.22%) (Table 3).TF and TR levels indicated the importance of both biochemical components in determining the total liquor colour of black tea (Li *et al*. 2016a; Tang *et al*. 2019).

The color index plays a vital role in the assessment of quality tea. Tea genotypes SH/D/11/13 was found to have the highest color index of 6.85, followed by H/B/6/4 (4.83), H/B/2/3 (4.05), SH/D/11/333 (3.92) and TV-23 (3.47) (Figure 2). The attractive colour of tea infusion is due to TF and it emerges as an important quality index of black tea and contributes significantly to the production of quality tea. Hazra *et al*. (2021) reported that the tea colour is one of the important scales which can attract and increase value by the consumers.

The highest caffeine content (CC) was recorded in TV-23 (150.03 mg/100 g of tea) which was followed by SH/D/11/333, H/B/2/3, H/B/6/4 and SH/D/11/13 (45.13 mg/100 g of tea) (Figure 3). Caffeine decreases with the maturity of the tea shoots. The amount of caffeine also varies depending

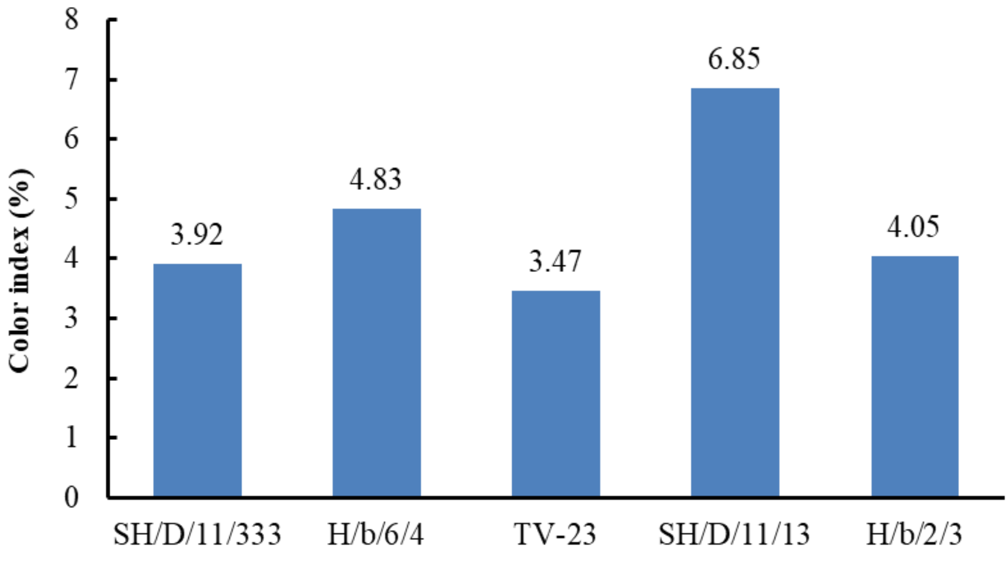


**Figure 2: Color index of tea genotypes**

on the variety of tea and brand of tea and is also directly attributed to the processing and leaf maturity (Hyun *et al*. 2020; Rahimi *et al*. 2021).

Green tea contained less caffeine content when compared to black tea and hence was is good for health (Kumar and Kumar, 2014). But, the research demonstrated that the normal ranged from 2-5% (dry weight) together with a small quantity of theobromine and the content of caffeine differs from variety to variety (Tang *et al*. 2019).Earlier studies proved that caffeine content is associated with origin, genetic and environmental variability, harvest time and processing manner of plant material (Ahmed *et al*. 2019), and can range from 24% to 40%. The displayed results confirmed that caffeine content depends on the age of tea leaves.

**Correlation of morphological and biochemical parameters**

The correlation analysis was measured to explore the relationship between morphological traits with biochemical constituents of five tea genotypes. Strong and medium levels of negative correlation were reported for TF with LB (0.62), LMSP (0.64), SFW (0.56) and SDW (0.48) while TR reported a positive correlation with LLR and LVP (Table 4). However, a strong, lower and negative correlation was reported for TF with LA, SFW and SDW. The significant variation in the rate of fermentation, crude fibre content, total polyphenols, total catechins, chlorophyll-a, chlorophyll-b and total carotenoids indicated the high genetic diversity of tea germplasm in Sri Lanka (Kottawa-Arachchi *et al*. 2013). Also a strong and lower level of positive correlation was reported for RTFTR with LLR (0.28), LVP (0.36) and LMSP (0.39) and a negative value between RTFTR and LLP (Table 4). During the fermentation process, theaflavins and thearubigins forms from polyphenolic compounds, found in extremely high amount in young tea shoots (Kottawa-Arachchi *et al*. 2013).

Significant and positive correlation were reported for HPS with LVP (medium level; 0.47) and LMSP (lower level; 0.18) but the same were lower level and negative values with LB (0.18), LLP (0.16) and SDW (0.21). It can be observed that similarity value ranged from 0.606 to 1.000 in the similarity coefficient matrix, utilizing young shoot characters, indicating a medium degree of variation amongst the germplasms (Smith and Barua, 2011). Significant and positive correlations were reported for TLC with LLR (0.34), LVP (0.42) and LLPFS (0.18) (Table 4). Significant, lower levels and positive correlations were reported for CI with LLP (0.42), LLPFS (0.16) and LA (0.21) but the same was negative and medium (with LMSP; 0.59) and lower (with LL, LLR, LVP, INL

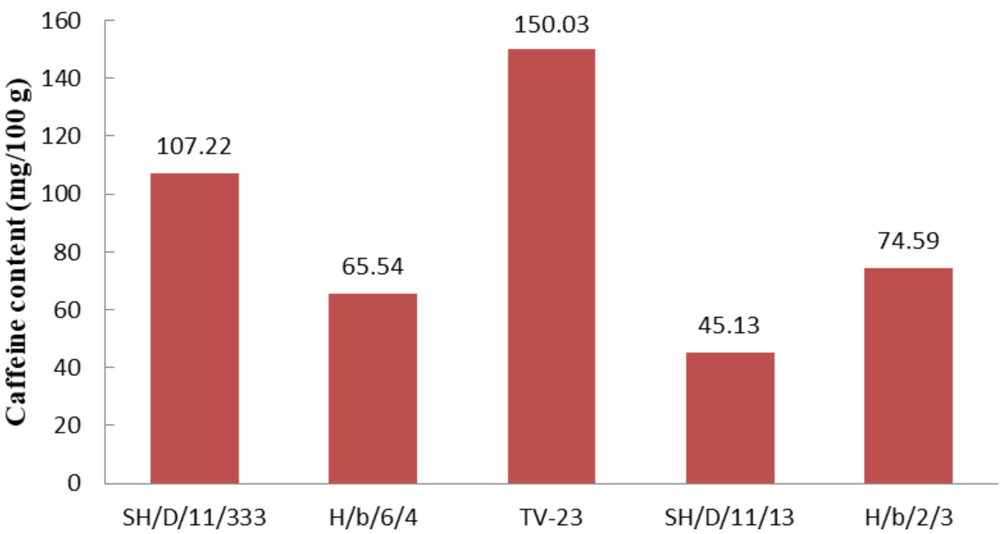


**Figure 3: The caffeine content of tea genotypes**

and SFW) level. On the other hand, biochemical parameters such as plant pigments in green leaves were importantin explaining the biochemical variation (Kottawa -Arachchi *et al.* 2013). Moreover, significant, positive and medium and lower level correlations were reported for CCwith LMSP (0.55), LL, LB, INL, LA and RFD (Table 4) but the same were negative and lower level LLR and LLP.

Strong (p<0.01) and negative correlation of theaflavin, thearubigin, and the ratio of theaflavin and thearubigin with morphological traits indicates that the increase of one trait will decrease the other but significant (p<0.01, 0.05) and positive correlation of high polymerized substances, total liquor colour, colour index and caffeine content with morphological traits indicates that increase of one trait will increase the other.

## CONCLUSIONS

The five cultivars tested in this study varied in plant morphological traits (including leaf length, width, area, fresh and dry weights) and leaf biochemical compounds (including TP, TR and caffeine). Clone SH/D/333 had the largest leaves in terms of individual leaf area, fresh, dry weights, and new shoot weight. Both the clone TV-23 and H/B/2/3 had the greater biochemical compounds like theaflavins (TF), thearubigins (TR), the ratio of TF and TR, high polymerized substances, caffeine content with a good liquor colour index. Considering both morphological and biochemical parameters, clone H/B/2/3 and TV-23 can be considered in quality tea production in the Bangladesh. The information generated from this study can be used broadly in tea research and also facilitate its utilization in the future breeding programme and quality production of tea.

## AUTHOR CONTRIBUTION

HMRC performed the experiments, analyzed and interpreted data. AFMSI, JKS and MAA conceptualized and designed the study. FIM and RT performed the statistical analysis and drafted the manuscript.

**Table 4: Correlations among the traits of different tea genotypes**

| | LL | LB | LLR | LVP | LMSP | LLP | LLPFS | INL | LA | FSW | DSW | FDR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TR** | -0.09 | -0.48** | 0.43** | 0.45** | -0.004 | -0.14 | 0.17* | -0.05 | -0.22** | -0.25** | -0.35** | 0.12 |
| **TF** | -0.38** | -0.62** | 0.36** | 0.28** | -0.64** | 0.30** | 0.35** | -0.34** | -0.07 | -0.56** | -0.48** | -0.04 |
| **RFR** | 0.12 | -0.18* | 0.28** | 0.36** | 0.39** | -0.36** | -0.01 | 0.14 | -0.20* | 0.05 | -0.11 | 0.17* |
| **HPS** | 0.08 | -0.18* | 0.21 | 0.47** | 0.18* | -0.16* | 0.04 | 0.15 | -0.13 | -0.14 | -0.21** | 0.09 |
| **TLC** | -0.09 | -0.40** | 0.34** | 0.42** | -0.004 | -0.11 | 0.18* | -0.03 | -0.14 | -0.24** | -0.34** | 0.12 |
| **CI** | -0.28** | -0.04 | -0.16* | -0.35** | -0.59** | 0.42** | 0.16* | 0-.31** | 0.21** | -0.18* | -0.05 | -0.12 |
| **CC** | 0.24** | 0.33** | -0.22** | 0.002 | 0.55** | -0.29** | -0.01 | 0.34** | 0.21** | 0.30** | 0.13 | 0.17* |

LL-leaf length in cm, LB-leaf breadth in cm, LLR-LL and LB ratio, LVP-number of leaf veins in pair, LMSP-number of pair of serratulation on leaf margin serrulated form in pair, LLP-length of leaf pedicel in cm, LLPFS-length of leaf pedicel to first serration in cm, INL-internodal length in cm, LA-leaf angle in degree, FSW-fresh shoot weight in gram, DSW-dry shoot weight in gram and FDR-FSW/DSW, TF-theaflavin, TR-thearubigin, FRR-TF and TR ratio, HPS-hipolymerized substances, TLC-total liquor colour, CI-colour index, CC-caffeine content (mg/100gm of tea). * Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

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