Research Article



Morphologial and Anatomical Studies of Tea Varieties and Clones Grown at Nthri, Shinkiari, Mansehra, Pakistan

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Abstract | The primary knowledge gap and problem statement for all tea varieties and clones in my study field relate to their morphological and anatomical features. Tea belongs to family Theaceae and Camellaceace. The experiment was carried out for the morphological, anatomical studies of tea varieties and clones grown at National Tea and High Value Crops Research Institute, Shinkari Mansehra. The field experiments were conduct in Randomized Complete Block Design. Morphological and anatomical character of different tea varieties and clone were analyzed by standard techniques. Morphological character of various tea varieties and clones were record both qualitative and quantitative data such as stem colour stem, stem length, stem girth, number of leaves plant⁻¹ leaf length, leaf width, leaf type, leaf apex, leaf base, leaf shape, distance between nodes, pedicel length, flower colour, and flower diameter. Tea samples were analyzed for anatomical parameters such as epidermis cells, mesophyll cells and epidermal anatomy of leaves. The stem length, stem girth, number of leaves plant⁻¹, leaf width, leaf length, distance between node, pedicel length and flower in tea samples ranged between 9.66-71.80cm, 1.40-2.54cm, 7.66-15.66cm, 4.83-1.66cm, 11.50 3.86cm 4.76-1.50cm, 2.03-1.06 and 4.56-2.66cm. In anatomical study transverse section of tea varieties and clone showed similar features in epidermis, mesophyll tissues and vascular bundles. Stomatal index of different tea varieties and clones was array from 33.9 to 45.4 respectively. Highest was mentioned in clone P-7 because high number of stomata found in lower epidermis.

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Keywords | Tea, Theaceae, NTHRI, Shinkiari, Morphology character, Anatomical feature, Stomata, Transverses section

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Introduction

Tea belongs to the family Theaceae and genus Camellia, which has 82 species. It grows in tropical and subtropical regions. The tea (*Camellia*) sinensis L) plant basically originated in China, and drinking began in the 16th century. The tea plant is grown in tropical and subtropical areas. It is cultivated in 30 different countries around the world. Tea is the second most popular beverage in

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the world after water (Akhlas et al., 2003). Tea is the second most popular beverage in the world after water (Akhlas et al., 2003). The plant is a mediumsized, evergreen shrub or tree having branches in common condition. The leaves are light green with a short pediculate, coriaceous, elliptical, alternate, and serrated margin. Having pearl-white flowers that are actinomorphic, 2.5-4 cm in diameter, and found singly or in clusters of two or six. Fruit is a flattened, smooth, rounded, trigonous capsule with one seed solitary in each, about the size of a small nut (Biswas, 2006). There are three different types of tea, i.e., green, black, and oblong, which depend on quality and chemical composition. The quality of tea (Camellia sinensis L.) is best for aroma, volatile compounds, taste, and colour (Cabrera et al., 2006). Pakistan has a long tradition of tea drinking, which has become an integral part of social life. The quality and chemical composition of tea flush vary under varying climatic conditions (Madiha et al., 2017). Some varieties have been grown at NTHRI Shinkiari Mansehra according to their capacity and habitat on the basis of their physical characteristics. The value of tea is also affected by cultural practises, the environment, climatic conditions, and tea processing techniques (Waheed et al., 2017). Tea crops has a medicinal and industrial value as well for different purposes. Morphology is a branch of biology that deals with study of external structure of plant body. In morphology both qualitative and quantitative characters are very importance like leaf shape, leaf length, stem girth and flower colour etc. (Gostin, 2011). Anatomy is also branch of science biology that explain the internal structure and source of identification of plant (Perxu et al., 2009). Anatomy deals with interior cell and tissue of plant. Epidermal anatomy of plant includes stomata type, stomata length and epidermal shape etc. Epidermis character have been considered to be of great use in studying relationship between taxa and there are few descriptions of epidermal micromorphology but just limited number of species were included (Ao et al., 2007).

Leaf morphology and anatomy have always played an important role in plant taxonomy and identification of taxa (Parnell and Meade, 2003).

The present trial was carried out to find-out morphological characters and anatomical features of different lines and varieties tea.

Materials and Methods

Experimental site and plant material

During 2021-2022 at National Tea and high Value Crops Research Institute, Shinkari, Mansehra (KPK), morphological, physicochemical and other activities were conducted. The plant material was collected from National Tea and High Value Crops Research Institute, Shinkiari, Mansehra.

Experimental deign and treatment combination

Three replications of a randomised complete block design were used to set up the experiment.

Climatic factor of NTHRI, Shinkiari

The tea crop grows in Shinkari's climate, which is ideal.

Collection of tea varieties and clones from NTHRI

There some tea varieties and clones grown at National Tea and High Crops Research Institute, Shinkari, Mansehra, such as Qi-Men, Sri-Lanka, Roupi, Chuye, Japanese, Indonesian, Turkish, Jue king, P-3, P-5, P-7, P-8 and P-9 were selected for experimentation. Tea samples were collected form National Tea and high value crops Shinkari, Mansehra, Pakistan. The sample was washed, dried under the sun, crushed, and powdered with an electric grinder. All these samples were used for anatomical and morphologic studies.

Research work in laboratory of NTHRI, Shinkiari

The study was conducted in two phases: the morphological aspect was the first, and the anatomical, physical, and chemical variations in several tea varieties and clones grown at NTHRI Shinkiari, Mansehra, were the second. Main portion of experiment was conducted in open field while another part was carried out in laboratory of NTHRI, shinkari and Hazara University Mansehra.

Investigation of morphological characteristics

Morphological characters of different tea varieties and clones according to standard method of Ashrad *et al.* (2002) were recorded. Morphological data, both qualitative and quantitative characters such as leaf length, leaf width, leaf colour, leaf margin, leaf shape, stem colour, stem girth, flower colour, flower diameter etc. were analysed in field.

Protocol used for anatomical studies

Anatomical studies of leaves and stem (tea plant)



were used by the protocol Poornima *et al.* (2009) with slight modification.

Transverse section of tea leaves

Apparatus/equipment: Some material were used for transverse section of tea leaves such as beaker, glass slide, watch glass, cover slip, dropper, needle, blade, slide box, nail polish, heat Lamp, Forceps, Staining, light microscope and photography, potatoes, paper tape, permanent marker, brush.

Preservation

The plant material (tea leaf) were preserved in three solvents for about 48 hours to study the anatomical parameters. The composition of preservative as follows:

- Ethyl Alcohol (C2H5OH) = 10ml
- Acetic acid (CH3COOH) = 10ml
- Formalin (HCHO)= 10ml

Section cutting

Transverse section of leaves were taken following the procedure of Poornima *et al.* (2009). To observe the anatomical feature of tea leaf thin transverse sections were made with the help of sharp blade. During section cutting both blade and section was kept in water. The section was removed from razor with help of brush and place onto the slide. Transverse sections were prepared in large number and only thin section was select and stained. After making permanent slide, it seen under microscopic and photograph and micrometry.

Morphological attributes

Qualitative parameters: Qualitative parameter are those which cannot be measured and countable. Data was recorded on various parameter such as:

- Leaf colour: Fresh and mature leaves colour was recorded.
- Leaf shape: Shape of first leaf was observed and noted.
- Leaf margin: Leaf margins were observed as well as recorded.
- Leaf base and apex: Leave base and apex was observed as well as recorded.
- Stem colour and type: Stem colour and surface was also observed.
- Flower colour: Flower colour were observed.

Quantitative parameters

Quantitative parameter are those which can measurable. Following quantitative parameter were

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recorded.

- Leaf width and length (cm): Leaf width and length was measured by using graduated scales
- Shoot length (cm): Stem lengths were measured with help of scale and noted.
- Distance between nodes (cm): Internode distance were measured by using graduated scale and observed.
- Stem thickness: Stem thickness was measured by Vernier caliper and data was noted in centimeter
- Pedicel length (cm): Pedicel of length was measured with help of scale and was noted in centimeter
- Flower diameter (cm): Flower diameter were also measured by using graduated scale.

Anatomical attributes

- No of stomata per plant: The number of per plant were count in the specific area through compound microscopic.
- Stomatal index: The stomata index were determine by the specific formula are given below
- Stomatal Index = $S/(E+S) \times 100$
- Stomata length and width: The stomata length of tea varieties and clones were determine in specific area through measured by compound microscopic.
- Number epidermal cell: The number of epidermal cell were measured in specific area through microscopic.
- Epidermis length and width : The epidermis length of tea varieties and clone were measured in specific site through microspic

Statistical software

Data were analysed using statistical software 10.1 for morphological data. Table were design in MS. Word 2013 version. Anatomical data of means values were analysed in MS. Excel.

Results and Discussion

Morphological characteristics

Morphological data of several tea varieties and clone was based on their leaf, stem and flower character but differed from variety to variety. Both qualitative and quantitative boundary of morphology data of different tea varieties and clone were recorded.

Qualitative characters of morphological data

The qualitative characters of morphological data of various tea varieties and clones are shown in Table 1. Stem colour was observed green and dark green in

various tea varieties and clones. Leaf base was acute in all tea varieties and clones. The leaf apex were recorded acuminate in Turkish, Sir Lanka, clone P-3, clone P-5, and clone P-9 whereas other tea samples were having acute leaf apex. Leaf margin were observed serrulate in clone P-8, P-7, P-3, Turkish, Sir Lanka and Qi-men variety. Flower colour was reported pearl white in all tea varieties and clones.

Quantitative parameters of morphological data Quantitative parameters of different tea varieties

Table 1: Qualitative characters of morphological data of tea varieties and clones.

and clones were varying from one to other and data was reported in Table 2.

Stem length (cm)

Data regarded stem length is present in (Table 2). Analysis of variance showed non-significant variation in stem length and ranged from 9.66 to 17.66 (unit?). The maximum stem girth was observed in Sir Lanka (17.80cm) variety but in case of minimum stem length was found in Clone P-7 (9.66cm).

Varieties/Camellia				Qualitative character					
sinensis L	Stem colour	Leaf base	Leaf apex	Leaf shape	Leaf position	Leaf margin	Flower colour		
Roupi	Light green	Acute	Acute	Elliptical	Alternate	Serrate	Pearl white		
Qi-men	Light green	Acute	Acute	Ovate	Alternate	Serrulate	Pearl white		
Chuye	Dark green	Acute	Acute	Elliptical	Alternate	Serrate	Pearl white		
Jue king	Dark green	Acute	Acute	Elliptical	Alternate	Serrate	Pearl white		
Japanese	Dark green	Acute	Acute	Narrow elliptical	Alternate	Serrate	Pearl white		
Turkish	Dark green	Acute	Acuminate	Elliptical	Alternate	Serrulate	Pearl white		
Sir lanka	Light green	Acute	Acuminate	Ovate	Alternate	Serrulate	Pearl white		
Indonesian	Light green	Acute	Acute	Broadly elliptical	Alternate	Serrate	Pearl white		
Tea clones									
P-3	Dark green	Acute	Acuminate	Elliptical	Alternate	Serrulate	Pearl white		
P-5	Dark green	Acute	Acuminate	Elliptical	Alternate	Serrate	Pearl white		
P-7	Dark green	Acute	Acute	Ovate	Alternate	Serrulate	Pearl white		
P-8	Dark green	Acute	Acute	Elliptical	Alternate	Serrulate	Pearl white		
P-9	Dark green	Acute	Acuminate	Elliptical	Alternate	Serrate	Pearl white		

Table 2: Quantitative character of morphological data of tea varieties and clones (Mean value of stem height, stem girth, leaf length, leaf width, pedicel length, flower diameter of tea varieties and clones).

Varieties	Stem length (cm)	Stem girth (cm)	Number of leaves/plant	Leaf length (cm)	Leaf width (cm)	Distance between nodes (cm)	Pedicel length (cm)	Flower di- ameter (cm)
Roupi	16.16a	1.68cd	15.66a	6.46b	2.43bc	3.36bc	1.20bc	4.00a
Qi-men	14.13ab	2.54a	14.33ab	6.80b	2.66bc	2.16de	1.10c	4.46a
Chuye	15.66ab	1.80bcd	11.33abcd	6.70b	2.30bcd	1.73de	1.56abc	4.06a
Jue king	12.33	1.92bcd	13.33abc	7.60b	2.73bc	1.83de	1.60abc	3.66ab
Japanese	17.66a	1.86bcd	9.66bcd	7.13b	2.70bc	3.56bc	1.76ab	4.56a
Turkish	16.33a	1.58cd	8.66cd	6.50b	2.93b	3.80ab	1.66abc	4.43a
Sir Lanka	17.80a	2.27ab	12.33abcd	3.86c	1.66d	2.73cd	1.76ab	3.83a
Indonesian	14.33ab	2.54a	7.66d	11.50a	4.83a	4.76a	1.63abc	2.66b
P-3	15.33ab	1.77bcd	13.33abc	5.53bc	2.06cd	1.50e	1.23bc	3.60ab
P-5	13.66ab	1.40d	9.33cd	7.16b	2.26bcd	2.16de	2.03a	4.23a
P-7	9.66b	1.75bcd	14.66a	5.63bc	2.16cd	2.66cd	1.63abc	3.46ab
P-8	13.33ab	2.04abc	12.66abc	7.23b	2.66bc	1.86de	1.53abc	3.46ab
P-9	11.66ab	1.52cd	11.00abcd	6.73b	2.26bcd	1.80de	1.06c	3.50ab
LSD Value	6.4345	0.5823	4.8158	2.0845	0.7510	1.0632	0.6494	1.1357

Note: each value is a mean of three replications.

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Stem girth (cm)

Statistical analysis of data showed significant variation in stem girth at different tea varieties and clones (Table 2). The data varied from 1.40 cm-2.54 cm. Maximum stem girth was observed in Qi-men and Indonesian (2.54cm) and minimum stem girth were recorded in P-5 (1.40cm).

Number of leaves plant⁻¹

Number of leaves per plant of each tea varieties and clones were ranged from 15.66 to 7.66 (Table 2). Maximum increase (15.66 cm) in number of leaves was observed in Roupi variety while minimum decrease in number of leaves was recorded in Indonesian variety (7.66cm).

Leaf width (cm)

Data of leaf width were ranged from 1.66 to 4.83 cm (Table 2). Statistical analysis of data showed highly significant variation in leaf width at different tea assortment and clone. The highest leaf width was observed in Indonesian (4.83cm) whereas minimum leaf width were found in Sir Lanka (1.66cm).

Leaf length (cm)

Length of different tea verities and clones was were ranged from 11.50cm to 3.86cm (Table 2). Highest leaf length was reported in Indonesian (11.50cm) while lowest leaf length were showed in Sir Lanka tea variety (3.86cm).

Distance between nodes (cm)

Analysis for distance between nodes showed highly significant variation at various tea assortment and clones (Table 2). The data range from 1.50cm to 4.76cm. Highest (4.76) distance between nodes was recorded in Indonesian variety but in case of lowest (1.50cm) distance between node was found in clone P-3.

Pedicel length (cm)

The pedicel length varied form 1.06 cm to 2.03cm (Table 2). Maximum pedicel length was noted in Clone P-5 (2.03 cm) while minimum value was (1.06) recorded in clone P-9. Analysis of variance showed significant variation at various tea assortment and clones.

Flower diameter (cm)

The flower diameter varied from 2.66cm to 4.56 cm (Table 2). Highest flowers diameter was present in

Japanese (4.56 cm) tea variety whereas lowest flower diameter was observed in Indonesian (2.66cm) tea variety.

Anatomical studies of tea leaves

The transverse section of tea varieties and clones showed the following features in Figure 1.



Figure 1: Transverses section of various part of tea leaf: A: cuticle, B: Upper epidermis, C: Palisade parenchyma, D: Spongy parenchyma, E: Vascular bundle, F: Lower epidermis, G: Parenchyma, H: stoma.

Stomata index of tea varieties and clones

Stomata number, number of epidermis cell was measured in all tea varieties and clones and data was recorded taken by specific formula. Results of stomata index was shown in the Table 3.

Table 3: Stomatal index of tea leaves.

S. No Varieties and clones Stomatal index = $S/(E+S) \times 100$

~~~~			5/(1 . 5) ~ 100
1.	Roupi	33.3	
2.	Qi- men	45.4	
3.	Chuye	42.8	
4.	Jue king	35.7	
5.	Japanese	42.8	
6.	Turkish	40.2	
7.	Sir lanka	36.5	
8.	Indonesian	40	
9.	P-3	38.0	
10.	P-5	33.9	
11.	P-7	47.5	
12.	P-8	37.9	

#### Epidermal anatomy

Anatomy of epidermis of different tea varieties and clones was carried out i.e., upper and lower surface. (Figures 2 and 3).

#### Qualitative characters

The epidermis cell of tea varieties and clones are triangular, sinuos and repand. Trichome and stomata are also present in lower epidermis but present in upper epidermis both tea varieties and clones.



Morphologial and anatomical studies of tea varieties

**Table 4:** Epidermal anatomy of tea leaves based on qualitative character qualitative character of tea varieties and clone.

Qualitative character of tea varieties and clone													
Roupi Qi-m	en Chuye	Jue king	Japanese	Turkish	Sir lanka	Indonesian	p-3	P-5	P-7	P-8	P-9		
Shape of epide	Triangular, sinuose or repand												
Layer of epider	nis			Single layer									
Epidermis cell margin					Smooth								
Absent and pre	Present ( lower surface)												
Type of Trichome				Non-granular/unbranched									
Stomata types				Anomocytic									
Shape of guard cell Bean shape													

#### Table 5: Quantitative characters of tea leaf based on the epidermal anatomy.

Varieties		Qualitative character									
and clones	No. of epidermal Cells /area	Epidermal length	Epidermal width	No of stomata/ area	Stomata length	Stomata width					
Roupi	100	15 µm	12 µm	50	15µm	13µm					
Qi- men	120	16µm	14µm	95	13µm	14µm					
Chuye	110	20 µm	17 µm	85	14µm	14µm					
Jue king	160	15 µm	22 µm	89	14µm	16µm					
Japanese	200	22µm	13µm	150	13µm	15µm					
Turkish	89	12µm	16µm	60	16µm	14µm					
Sir lanka	78	13µm	15µm	45	13µm	13µm					
Indonesian	105	17 µm	18 µm	70	17µm	14µm					
P-3	130	10 µm	20 µm	80	15µm	13µm					
P-5	150	19 µm	12 µm	77	15µm	14 µm					
P-7	210	40 µm	20 µm	190	14µm	12µm					
P-8	90	11 µm	15 µm	55	12µm	14µm					
P-9	144	13µm	15µm	102	15µm	14µm					



Figure 2: Upper epidermis of tea sample.

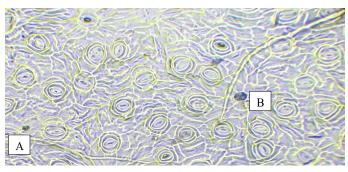


Figure 3: Lower epidermis cell of tea sample: A, Stomata; B, trichome.

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Trichome may be non-granular and type of stomata are anomocytic in all tea varieties and clones. The shape of guard cells are bean like. All these qualitative characters were shown in Table 4.

#### Quantitative characters

In the leaves of different tea samples varied from stomata length and width. The number of stomata and number of epidermis cell per area is also determined in Table 5. All readings were taken on 10 X magnification of microscope. These results were presented in Table 5.

#### Morphological characteristics of tea samples

Morphological analyses both qualitative and quantitative character were examined in various tea varieties and clones. Qualitative character of all tea varieties and clones showed minor differences to each other. The stem colour, type and surface in all tea varieties and clone were observed light green



colour and minute hair present as well as woody and erect. Leaf base was recorded acute in all tea varieties and clone but there was no difference. Leaf apex of some tea sample was recorded acuminate in Turkish and Sir Lanka, P-5, P-3, P-9 while acute leaf apex were found in other tea varieties and clone. Leaf margin were found serrulate in tea varieties and clone of Qi-men, Turkish, Sir Lanka, P-3, P-7 and P-8 whereas serrate margin were observed in few tea varieties and clone. These results are similar with finding of Luo et al. (2004) who reported that light green and dark leaves type only depend upon leaves colour and high serration margin depend on leaf margin. Elliptical shape of leaves were found in Roupi, Chuye, Jue king, Turkish (varieties), P-3, P-5, P-8 and P-9 (clone) while ovate leaf shape were found Qi-men, Sir lanka (varieties), P-3 (clone) etc. These result also dissimilarities with pervious work of Jala (2011) who observed that some shape of leaves of become Lanceolate and some morphological parameter in wishdone flower (Torenia fourmerie). Data of stem length, stem girth, leaf width, leaf length, internodes distance, pedicel length and flower diameter were found to be in the range from 9.66 - 17.66, 1.40-2.54, 1.62-4.83, 1.50-4.76, 1.06-2.03 and 2.66-4.56cm respectively. Highest stem length were found in Sir Lanka variety (11.80) and lowest in clone of P-7. Present work showed dissimilar with previous work of Wei et al. (2005) who observed that stem length range from 5.6 cm to 13.2 cm. Maximum stem girth data were indicated in Indonesian and Qi-men variety but least were observed in clone P-5). Present results agree with pervious work of Shanmugarajah (1986) who studied that at nursery stage tea clone differ in stem girth. Peak leaf length (11.50) and width (4.83) were showed in Indonesian variety while lowest value were reported in Sir Lanka (3.86cm, 1.66cm). Our results showed dissimilarities with previous work of Jin et al. (2005) that leaf length in Camellia sinensis L. varied from 3.2 to 6.4 cm. leaf length and width ratio is dependent upon the length and width of leaf. Highest length of pedicel and flower were found in clone P-5 (2.03cm), Japanese (4.56cm) variety whereas lowest value were recorded in clone P-9 (1.66cm) and Indonesian variety (2.66). Lowest distance between nodes (1.50cm) were shown in Clone P-3 and highest internodes distance were in Indonesian variety (4.76cm). Current study showed resemblance with result of pervious work of Sha and Guo (2005) who found that distance between

nodes range from 1.7 cm to 6.6 cm, respectively.

#### Anatomical studies of tea samples

The cross section of different tea varieties and clones were showed the presence of cuticle, upper epidermis, mesophyll cell, vascular bundle and lower epidermis in Figure 1. Lower epidermis larger than upper epidermis. Lu *et al.* (2008) who reported single layer of epidermis in Camellia rhytidophylla.

Stomata index of different tea varieties and clones were found in Table 3 and range from 33.3 to 45.4, respectively. Anatomical features of different tea varieties and clones were found similar in leaf epidermal anatomy such as epidermis shape, stomata types, trichome shape and guard cell shape. Epidermal anatomy were observed both qualitative and quantitative character but quantitative feature were differ in all tea sample and clone. In qualitative character, the epidermal shape of tea leaves were showed triangular, sinuous and repand whereas shape of stomata and guard cell were observed anomocytic and bean shape in tea sample (Table 4). The type of trichomes were found non granular or unbranched in some tea varieties and clone. These results are similar with pervious work of Perveen et al. (2007) who recorded that stomata type is anomocytic. In Epidermal anatomy, the major important in taxonomy and classification are stomata character but it's no easy to observe similar in same. Present work were dissimilar with previous work of Chengqi et al. (2002) who observed that stomata type in all tea varieties is anioscytic and paracytic (Table 4). Stomata length of different tea and clone were range from 13 to 17 µm. The stomata width of tea sample were varied between 12 to 16 µm. Number of epidermis cell more than number of stomata. Number of stomata and number of epidermis were found numerous in all tea varieties and clone but randomly count in area.

#### **Conclusions and Recommendations**

In present study morphological analysis of both qualitative and quantitative characters of various tea varieties and clones were done. Morphology characteristics is initial step of tea varieties and clones because each parameter was different such stem colour, type, surface, leaf shape, leaf apex and base etc. Anatomical studied are more essential than morphological characteristics. Transverse section

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of tea varieties and clones showed similar feature in cuticle, lower and upper epidermis, mesophyll tissue and vascular bundle but major different in stomata index and epidermal anatomy were found. In my point of view Indonesian tea variety is very essential in future because it leave was board and great important in medicine. Therefore, present study was helpful for students in future and provide important source for the identification of research plant as well as beneficial for medicinal purposes.

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#### **Novelty Statement**

As far as our knowledge, this kind of research has been carried out for the first time on tea crops after the climate change in the area. The research presents novel ideas about morphology and anatomy of the tea which will tell us about the characterization of morphological variability, it will allow breeders to identify varieties with desirable characteristics for future research and will improve the quality of tea.

#### Author's Contribution

**Danish Kamal:** Designed the experiment, managed, collected and analysed the data.

**Muhammad Abbass Khan:** Technical guidance at each and every step of the research process.

**Ghulam Mujtaba-Shah:** Technically review the whole paper.

Naveed Ahmed: Helped in data collection and data analysis.

Maryam Iqbal: Designed the experiment, managed, collected and analyzed te data.

**Basharat Hussain Shah:** Technical guidance regarding layout of the experiment.

**Imtiaz Ahmed:** Helped in draft improvement and English language of the manuscript.

#### Conflict of interest

The authors have declared no conflict of interest.

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