# SCREENING OF CAMELLIA SINENSIS (L.) KUNTZE GENOTYPES BASED ON

# MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION

Ghulam Abbas<sup>1</sup>, Erum Feroz<sup>1</sup>, Syeda Tatheer Fatima Naqvi<sup>1</sup>, Rehana Chaudhry<sup>1\*</sup> <sup>1</sup>Department of Botany, Chatter Klass Campus, University of Azad Jammu and Kashmir, Muzaffarabad-13100, Pakistan \*Corressponding author: **rehana\_ajku@yahoo.com** 

# ABSTRACT

Camellia sinensis (L.) Kuntze commonly known as tea beverage is frequently drunk liquid refreshment all over the world. It belongs to the family Theaceae. Tea leaves constituted different secondary metabolites, essential amino acids, minerals, lipids and vitamins which carried out different types of bioactivities and have a vital function for human health and nutrition. In Pakistan, there are many areas where tea plant is cultivated. In this research work, four different varieties of tea including Chuye, Indonesian, Japanese and P-7 were selected from Pakistan Agriculture Research Council - National Tea and High Value Crops Research Institute (PARC-NTHRI), Shinkiari, Mansehra, Khyber Pakhtunkhwa, Pakistan to characterize some morphological and biochemical parameters. Standard techniques were carried out to determine the proximate compositions of ash, crude fiber and measurement of moisture content in all prepared samples of tea. To check the availability of subsidiary products in aqueous, chloroformed and ethanolic extract of tea leaves of all varieties, their color intensity was recognized using standard chemical tests. Atomic Absorption Spectrophotometer, Flame photometer digital explorer and UV Spectrometer were used to determine the mineral accumulation in different samples of tea varieties. Proportion of ash constituents, crude or raw fiber and moisture contents in all selected tea specimens from four varieties was noted that ranged between 6.3% to 7.37%, 2% to 3.5% and 3% to 7.5% respectively. Different chemical tests of secondary metabolites were performed for alkaloids, carotenoids, flavonoids, glycosides, phenol, phlobatannins, saponins, steroids, tannins and terpenoids in all samples of selected tea varieties. Alkaloids and saponins were found absent in both aqueous and ethanolic extracts of tea samples, while present in chloroformed extracts. Carotenoids and phlobatannins were totally absent in all tea extracts. Minerals contents in selected tea samples were determined in following quantity order: K (149.1 to 159.1 mg/L > Ca (21.46 to 30.85 mg/L) > Mg (10.07 to 12.40 mg/L) > Na (6.364 to 29 mg/L) > Co (4.882 to 9.482 mg/L > Mn (1.007 to 1.441 mg/L) > Fe (0.786 to 2.842 mg/L) > Pb (0.673 to 3.797 mg/L) > Zn (0.440 to 1.609 mg/L > Ni (0.200 to 2.27 mg/L) > Cu (0.053 to 0.384 mg/L). Present study screened out morphological parameters of four tea varieties, phytochemical trait analysis, and minerals constitutions with qualitative and quantitative variations. Statistical analysis of collected data against selected tea varieties revealed the suitable candidate genotypes and their morphological potential for future cultivation to get better results and maximum yield and also enabled us to choose that genotype for research purposes.

#### Key words: Camellia sinensis, characterization, morpho-physiological screening

#### **INTRODUCTION**

*Camellia sinensis* (L.) O. Kuntze. generally known as tea, belongs to (Family: Theaceae) is one of the most abundantly cultivated plant in all over the world. Theaceae family includes twenty-three genera, majority of them scattered in the humid and semitropical parts of south East Asia and United States of America (Bezbaruah, 1971). Camellia is the only economically beneficial genus in the family, with approximately 280 species. Just several species, such as *C. japonica, C. sasanqua, and C. reticulata,* are mainly cultivated all over the community for their majestic ornamental values, whereas one specie, *C. oleiferea,* is grown in China and Japan for cooking oils obtained out from seed. The family's most important species is *C. sinensis* which yields commercial tea (Hoi et al., 2021) and indeed the liquid that is taken the most throughout the globe Tuxill (1999). *C. sinensis* is an evergreen tree or shrub that grows to a height of 10 - 15 m in the wild and 0.6 - 1.5 m in cultivation. The leaves of plants are light green, short stalked, evergreen, alternate, ovate, serrate margin, orbicular or pubescent underneath , and differ in length from 5 to 30 cm. Leaves usually are pubescent, while mature leaves are bright green, smooth, and fleshy. Flowers are usually white as well as fragrant, 2.5 - 4 cm in diameter, and can be reported alone or in groups of two or four. Flower heads have several carpels and a yellow anther, and they actually create brownish red granules. Fruit is a hollowed, soft, round and trigonous three chambered capsule with a single seed, the size of a small nut (Tariq et al., 2010).

The region of origin for tea seems to be southwestern China Xia et al. (2020). The world largest tropical and subtropical areas are where industrial wild tea is planted in contrast to the rainforests in different segments of China (Jahangirzadeh et al., 2021a). More than 50 percent of the world's tea is grown by Asian nations, primarily China, India, and Sri Lanka. It has recently been planted in plenty of other locations around the world with slightly mild and hot in summer climate regions (Rout, 2006). *C. sinensis* (L.) Kuntze is the source including all tea varieties (Arachchi et al., 2019a).

Tea is currently the most widely consumed better and healthier drink around the world (Butt and Suleman, 2009). *C. sinensis* from each subtype of tea, which varies in a wide range of tastes, fragrances, properties, and patterns, offers us a wonderful selection of tea varieties. Several types of tea, including black, white, greenish, pu-erh (also known as red tea in certain regions), orthodoxy, oolong (also known as blue coloured tea in some regions), and matcha, are produced using foliage from a single species. (Hilal and

Engelhardt, 2007).

Tea drinking has a long and storied history in Pakistan and is currently a significant factor of the country's community interaction. So under supervision of the Pakistan Tea Board, the first tea experiments were carried out in 1958 in village Baffa (district Mansehra, Khyber Pakhtunkhwa). The inbreeding depression of tea is among the most important resources for genetic improvement, nanotechnology research, and has valuable possibilities for the entire tea industry going forward. In China, Tokyo, India, Kenyan, etc., as well as more recently in both Pakistan and Iran, a sizable number of biological components for tea, as well as the *C. sinensis* and its belongs to the genus and variants, have been gathered and maintained (Arachchi et al., 2019b). Mature tea leaves often include antioxidants, flavanonols, anthocyanin, phenylpropanoids, and free amino acids, caffeine, aminophylline, polysaccharides, soluble reduced sugars, carbohydrate, polypeptide, fats, pigment (chloroplast, carotene, and xanthophyll), minerals (Na, K, Pb, Co, Ni, Zn, Cu, Ca, Mg, Mn, and Fe) and volatile compounds (Oliveira et al., 2016; Gul. Sahib, 2011). *C. taliensis and C. sinensis var. assamica* had relatively high levels of phytochemicals and aqueous extracts (Chen et al., 2005). Primary phytochemicals of tea includes catechins and quercetin, chemicals (caffeine, theo-bromine, theophylline), volatile chemicals, carbohydrates, amino acids, triglycerides, and minerals Li et al. (2017).

Tea is renowned for its cytotoxic activity, which work against spontaneous malignant stimulation, DNA damage and instability, proliferation and differentiation, cancerous development, and dissemination in addition to being a stimulation and an antioxidant. Tea leaves extraction is known to lower the risk of a number of disorders Monobe et al. (2008). Studies have indicated that drinking tea, particularly green tea, can lower your risk of developing malignancies of the mouth, stomach, small intestine, pancreatic, chest, breast, skin, ureter, and ovaries. Natural antioxidants (Mukhtar and Ahmad, 2000) can prevent revascularization and tumour cell metastatic spread, whilst phytochemicals can stop tumour cell growth and trigger apoptosis (Mukhopadhyay et al., 2016). Reactive oxygen species, ROS contributes to a wide range of diseases, such as cancers, tends to maintain, alzheimer, renal problems, gastritis, atherosclerosis, and acquired immune deficiency syndrome (AIDS). The changing the chemical identity aid in tea lowered these harmful free radicals.

It is essential to know and understand the biological variations, relationships, and identifications of all types of *Camellia sinensis* (L.), Kuntze for the acquisition, maintenance, extraction, usage, current, and long-

term genetic improvement for tea. In order to increase a nation's economic commercial importance, it is also necessary to research the many types of tea (Jahangirzadeh et al., 2021b). The following aims and objectives guided the conduct of the current research (1) To characterize the morphological and biochemical parameters of tea genotypes. (2) To check the quantitative and qualitative value of Caffeine, Polyphenol Amino acid and other phytochemicals in tea germplasm. (3) Minerals analysis and to point out those tea genotypes which are high yielding?

#### MATERIAL AND METHODS

### **Study Area**

All of the studies for this assessment were completed in Pakistan Agriculture Research Council-National Tea and High value crops Research Institute (PARC-NTHRI), Shinkiari Mansehra KPK Pakistan, from CAMSATS University Abbottabad and also some of work was completed from University of Azad Jammu and Kashmir Muzaffarabad.

#### **Measurement of Morphological Parameters**

Morphological parameters of four tea varieties and clone (Chuye, Indonesian, Japanese and P-7) were studied. Morphological data of qualitative and quantitative parameters were analysed in the field such as height of plants, distance between nodes of plants, diameter of main stem, leaf length, leaf width with the help of measuring scale in cm, while the number of branches, leaves on each plant, bush canopy, leaf colour, leaf margin, leaf shape, leaves types, stem colour, stem type were recorded visually.

### **Phytochemical Analysis**

One bud and two leaves of four tea varieties such as Chuye, Indonesian, Japanese and P-7 were collected separately, shadow dried, pulverized and stored in fume hood. Extracts made from aqueous, chloroformic as well as ethanolic solvent and analyzed through spectrometric tests. Tannins, Steroids, Alkaloids, Phenols, Phlobatanin, Caretenoids, Glycosides, Terpenoids, Flavonoides and Saponins were done various systematic techniques for qualitative identification.

### **Qualitative analysis**

Flavonoids: Detected using H2SO4 and ammonia<sub>(aq.)</sub>, indicated by loss of yellow colour of extract
Saponins: Detected by forming a lathered solution with distilled water
Tannins: Detected using 2ml FeCl<sub>2</sub> in leaf extract, forming yellowish-brown precipitate
Terpenoids: Using chloroform and HCL, identified by forming reddish-brown color
Alkaloids: Detected using methanolic supernatant and HCL, with six drops of chemicals like Mayour's, Wagner's, or Drangendroff's added, identified by orange color
Glycosides: Detected using H2L, acetic acid and ferric chloride, indicated by brown ring
Steroids: Detected using H2SO<sub>4</sub>, indicated by reddish brown color
Carotenoids: Detected using FeCl<sub>3</sub>, indicated by blue color
Phenols: Detected using FeCl<sub>3</sub>, indicated by blue-green color
Phlobatannins: Detected by presence of red precipitate in phyto solvent extracts
Quantitative analysis

### **Determination of moisture**

Following AACC's method 44-01, the moisture contents of several types of tea were assessed. The percentage of wetness was determined using the formula

% moisture = Weight of Sample x 100 Total weight of sample

### **Determination of ash**

Ash contents were calculated using the procedure described in AACC (2000) method 08-01. Percentage of ash was determined using the formula

% Ash = weight of ash X 100 Weight of sample

#### **Determination of crude fiber**

Crude fiber was evaluated in accordance with the process specified in AACC (2000) method 32-10.

#### **Polyphenol test**

Measured using iron titrate solution and spectrometery method

|                          | E x 3.914 | L1     |       |
|--------------------------|-----------|--------|-------|
| The tea phenolic content | X         |        | x 100 |
|                          | 100       | L2 x M |       |

# Amino acid test

Measured using ninhydrin solution and spectrophotometry

# **Caffeine content**

Measured using basic lead acetate and spectrophotometry

### Minerals analysis

# Samples Preparation and determination for Mineral Analysis

Fresh tree leaves were collected from four varieties and crushed into powder and stored in a desiccator. Afterwards, samples were digested using a combination of acids (HCL, H2SO4, HNO3), and minerals were analyzed using various methods such as Spectrophotometry for phosphorous, Flame photometry for sodium and potassium, and Atomic absorption spectroscopy (AAS) for magnesium (Mg), manganese (Mn), Calcium (Ca), iron (Fe), Zinc (Zn), Cobalt (Co), Copper (Cu), Lead (Pb), and Nickel (Ni).

# STATISTICAL ANALYSIS

Analysis of Variance (ANOVA) for Statistical Analysis of data.

Principal component analysis (PCA) was used to reduce the dimensionality of the data and identify patterns.

# **RESULTS AND DISCUSSION**

## **Quantitative (numerical) Parameters**

Numerical data of selected tea varieties (Chuye, Indonesian, Japanese and P-7) were recorded as follows.

## Plants height (cm)

Height of different plants were measured randomly showed a significant difference (Table 1.).

Maximum plant height was 100 cm and minimum was 66 cm from different repeats.

| Source of |        |    |             |             |             |             |
|-----------|--------|----|-------------|-------------|-------------|-------------|
| Variation | SS     | df | MS          | F           | P-value     | F crit      |
| Rows      | 297.75 | 3  | 99.25       | 3.354929577 | 0.055340175 | 3.490294819 |
| Columns   | 96.2   | 4  | 24.05       | 0.812957746 | 0.540690264 | 3.259166727 |
| Error     | 355    | 12 | 29.58333333 |             |             |             |
|           |        |    |             |             |             |             |
|           |        |    |             |             |             |             |
| Total     | 748.95 | 19 |             |             |             |             |

# **Diameter of main stem (cm)**

Significant differences were observed in different plants among selected tea varieties (Table 2.) while diameters were being recorded. Maximum diameter was 19 cm and minimum was 7 cm among all tea varieties.

| Source of |        |    |             |             |             |             |
|-----------|--------|----|-------------|-------------|-------------|-------------|
| Variation | SS     | Df | MS          | F           | P-value     | F crit      |
| Rows      | 64.15  | 3  | 21.38333333 | 1.85807386  | 0.190504896 | 3.490294819 |
| Columns   | 27.5   | 4  | 6.875       | 0.597393193 | 0.671494707 | 3.259166727 |
| Error     | 138.1  | 12 | 11.50833333 |             |             |             |
|           |        |    |             |             |             |             |
|           |        |    |             |             |             |             |
| Total     | 229.75 | 19 |             |             |             |             |

## Number of branches/plant

The number of branches among selected varities ranged from a minimum of 9 to a maximum of 25, with significant variability (Table 3).

Table 3. Table of Analysis of variance for number of branches on mature plant

| Source of |        |    |       |             |             |             |
|-----------|--------|----|-------|-------------|-------------|-------------|
| Variation | SS     | Df | MS    | F           | P-value     | F crit      |
| Rows      | 142.95 | 3  | 47.65 | 3.153888582 | 0.064532138 | 3.490294819 |
| Columns   | 26.3   | 4  | 6.575 | 0.435190292 | 0.780843363 | 3.259166727 |

| Error | 181.3  | 12 15.10833333 |  |
|-------|--------|----------------|--|
|       |        |                |  |
|       |        |                |  |
|       |        |                |  |
| Total | 350.55 | 19             |  |

# Leaf length (cm)

Leaf length amongst tea varieties oscillated from 4cm (P-7) to 12cm (Indonesian), showing significant variations (Table 4).

| Table 4. Table of Analysis of | variance for | leaves le | ength (cm) |
|-------------------------------|--------------|-----------|------------|
|-------------------------------|--------------|-----------|------------|

| Source of |        |    |             |             |             |             |
|-----------|--------|----|-------------|-------------|-------------|-------------|
| Variation | SS     | df | MS          | F           | P-value     | F crit      |
| Rows      | 92.95  | 3  | 30.98333333 | 32.90265487 | 4.52985E-06 | 3.490294819 |
| Columns   | 2.3    | 4  | 0.575       | 0.610619469 | 0.662901291 | 3.259166727 |
| Error     | 11.3   | 12 | 0.941666667 |             |             |             |
|           |        |    |             |             |             |             |
|           |        |    |             |             |             |             |
| Total     | 106.55 | 19 |             |             |             |             |

# Leaf width (cm)

Width of leaves between varieties of tea showed significant variations (Table 5), while maximum width was recorded 1.4 cm (P-7) and minimum width was 5 cm (Indonesian)

# Table 5. Table of Analysis of variance for width of leaves (cm)

| Source of |       |    |             |             |             |             |
|-----------|-------|----|-------------|-------------|-------------|-------------|
| Variation | SS    | df | MS          | F           | P-value     | F crit      |
| Rows      | 8.95  | 3  | 2.983333333 | 4.904109589 | 0.018882875 | 3.490294819 |
| Columns   | 2.3   | 4  | 0.575       | 0.945205479 | 0.471253281 | 3.259166727 |
| Error     | 7.3   | 12 | 0.608333333 |             |             |             |
|           |       |    |             |             |             |             |
|           |       |    |             |             |             |             |
| Total     | 18.55 | 19 |             |             |             |             |

## **Total number of leaves /plant**

The total number of leaves per plant varied significantly between varieties (Table 6), ranging from 220 (Japanese) to 347 (P-7). Green leaves only observed in Indonesian while other three varieties displayed dark green leaves. Leaf shape showed clear variations. Indonesian exhibited broad shape leaves, P-7 showed ovate, Japanese was elliptical and Chuye indicated ovate and elliptical leaf shapes. Leaf margins of all varieties were serrate. Moreover, the leaf apex of only Indonesian was downturned while all three varieties were straight. Chuye and P-7 showed medium size, Japanese small while Indonesian showed large size of leaves with significant difference.

| Source of |         |    |             |             |             |             |
|-----------|---------|----|-------------|-------------|-------------|-------------|
| Variation | SS      | df | MS          | F           | P-value     | F crit      |
| Rows      | 19343.2 | 3  | 6447.733333 | 11.73951569 | 0.000698605 | 3.490294819 |
| Columns   | 609.2   | 4  | 152.3       | 0.277295624 | 0.887031725 | 3.259166727 |
| Error     | 6590.8  | 12 | 549.2333333 |             |             |             |
| Total     | 26543.2 | 19 |             |             |             |             |

 Table 6. Table of Analysis of variance for total number of leaves per plant

## Leaf area (cm<sup>2</sup>)

A significant difference was observed (Table 7), the maximum of  $47 \text{cm}^2$  (Indonesian) followed by minimum of 24 cm<sup>2</sup> (Chuye).

| Source of |        |    |             |             |             |             |
|-----------|--------|----|-------------|-------------|-------------|-------------|
| Variation | SS     | df | MS          | F           | P-value     | F crit      |
| Rows      | 297.75 | 3  | 99.25       | 3.354929577 | 0.055340175 | 3.490294819 |
| Columns   | 96.2   | 4  | 24.05       | 0.812957746 | 0.540690264 | 3.259166727 |
| Error     | 355    | 12 | 29.58333333 |             |             |             |
| Total     | 748.95 | 19 |             |             |             |             |

Table 7. Table of Analysis of variance for leaf area (cm<sup>2</sup>)

#### **Phytochemical Analysis:**

Phytochemical analysis was carried out. Differences and similarities were noted. Data were analyzed both qualitatively and quantitatively.

# **Qualitative Analysis**

Phytochemical analysis of different tea varieties revealed variations in secondary metabolites. Three solvent extracts i.e., aqueous, chloroformic and ethyl alcohol solvent were used for analysis. All extracts

contained flavonoids, glycosides, corticosteroids, phenylic mixture, and tannins while phlobatannins were totally absent. Alkaloids and terpenoids were present in aqueous and chloroformic extract, absent in ethanolic extract. Carotenoids and saponins were only present in chloroformic extracts

#### **Quantitative Analysis**

Following types of quantitative data were analyzed during research (Fig. 4) for four varieties. Moisture content ranged from 6.3% to 7.37% among selected tea varieties, with highest in Indonesian followed by P-7 with lowest moisture content. Mean values and standard deviations can be seen in (Table 8). The results in line with Atalay and Erge (2017).

#### Ash content

Ash content was found to be ranged from 2% to 3.5%. Less moisture and large amount of ash was found in P- 7 which indicated that tea variety is a good source of minerals (Rehman et al., 2012). Mean values and standard deviations can be seen in (Table 8).

#### Crude fiber

Crude fiber varied from 3% to 7.5%, with highest in P-7. Mean values and standard deviations can be seen in (Table 8). Fiber sometime might be affected during process of curling and tearing reported by Waheed et al. (2019).

| Table 8. Examinations evaluation | aluated for | four different | types of tea | leaves extract, in |
|----------------------------------|-------------|----------------|--------------|--------------------|
| close proximity                  |             |                |              |                    |

| Tea Specimen/<br>Varieties | Water percentage<br>(%) ± SD | Residue of ash<br>content (%) ± SD | Raw Fiber or<br>cellulose (%) ± SD |
|----------------------------|------------------------------|------------------------------------|------------------------------------|
| 1. Chuye                   | 2.85 ± 0.0611                | 0.216 ± 0.005                      | 0.62 ± 0.16                        |
| 2. Indonesian              | 2.88 ± 0.0529                | 0.194 ± 0.006                      | 0.66 ± 0.10                        |
| 3. Japanese                | 2.94 ± 0.0650                | 0.199 ± 0.011                      | 0.55 ± 0.19                        |

| 4. P-7 | $2.66 \pm 0.0721$ | $0.207 \pm 0.012$ | $0.55 \pm 0.11$ |
|--------|-------------------|-------------------|-----------------|
|        |                   |                   |                 |

Results indicates percentage mean values  $\pm$  standard deviations

## **Determination of polyphenols**

Explored values showed highly significant variations (Fig. 1). The maximum value was obtained 6.4 mg/ml in P-7 variety while minimum values were explored in Japanese as 3.1 mg/ml.

### **Determination of amino acids**

Maximum amino acid was determined 1.581 mg/ml in Indonesian tea variety. Minimum amino acid was found 0.801 mg/ml in Japanese tea samples (Fig. 2).

# **Determination of caffeine**

Maximum value was found 0.928 mg/ml in P-7 while minimum was 0.51 mg/ml in Chuye (Fig. 3).

### Macro minerals concentration

Macro minerals were studied using Atomic Absorption Photo Spectrometer. Following macro minerals were found i.e. Na, K, Ca and Mg (Table 9). The ratios of minerals were 6.36 to 129.0 mgL<sup>-1</sup>, 149.1 to 159.1 mgL<sup>-1</sup>, 21.46 to 30.85 mgL<sup>-1</sup> and 10.07 to 12.40 mgL<sup>-1</sup> respectively.

| Names      | Na                | К               | Ca                 | Mg                |
|------------|-------------------|-----------------|--------------------|-------------------|
| Chuye      | 17.037 ± 9.60     | 146.275 ± 3.777 | $27.0675 \pm 4.00$ | 11.30 ± 0.95      |
| Indonesian | $15.40 \pm 11.7$  | 113.99 ± 4.666  | 20. 30 ± 4.45      | $12.34 \pm 0.787$ |
| Japanese   | $19.00 \pm 10.30$ | 134.33 ± 3.77   | 32.23 ± 4.65       | 12.342 ± 1.896    |
| P-7        | 15.55 ± 13.111    | 156. 234 ± 5.2  | 25. 34 ± 3.688     | 9.2421± 1.896     |

Table 9. Concentration of Macro Minerals in selected tea varieties (mgL<sup>-1</sup>)

#### Micro minerals concentration

Micro minerals were studied using Atomic Absorption Photo Spectrometer using selected tea samples. Following macro minerals were found i.e. Pb, Co, Ni, Zn, Cu, Mn and Fe (Table 10). The ratios of minerals were from 0.673 to 3.797 mgL<sup>-1</sup>, 4.88 to 9.482 mgL<sup>-1</sup>, 0.200 to27.70 mgL<sup>-1</sup>, 0.440 to 1.609 mgL<sup>-1</sup>, 0.053 to 0.38 mgL<sup>-1</sup>, 1.007 to 1.441 mgL<sup>-1</sup> and 0.786 to 2. 842 mgL<sup>-1</sup> respectively.

| Name       | Pb                   | Со                  | Ni                  | Zn                 |
|------------|----------------------|---------------------|---------------------|--------------------|
|            |                      |                     |                     |                    |
| Chuye      | $1.65 \pm 1.446$     | $6.3717 \pm 2.1423$ | $1.059 \pm 0.872$   | 0.9270±0.550750    |
|            |                      |                     |                     |                    |
| Indonesian | $1.43 \pm 1.4235$    | 7.766 ± 3. 3543     | $1.435 \pm 1.0787$  | $0.242 \pm 0.332$  |
|            |                      |                     |                     |                    |
| Japanese   | $1.2242 \pm 2.01232$ | $6.122 \pm 3.6345$  | $1.32532 \pm 1.242$ | $0.4412 \pm 0.312$ |
|            |                      |                     |                     |                    |
| P-7        | $2.000 \pm 1.353$    | 5.5353 ± 4.3242     | $1.421 \pm 0.124$   | $1.092 \pm 1.234$  |
|            |                      |                     |                     |                    |

Table 10. Concentration of Micro Minerals in selected tea varieties (mgL<sup>-1</sup>)

#### CONCLUSION

Present study on four selected tea varieties (Chuye, Indonesian, Japanese and P-7) revealed that different varieties exhibit different morphological and physiological characteristics. Significant variations were observed in plant height, diameter, leaf length, leaf width and number of branches. Indonesian variety was dominant among all in height of plant, leaf width and length, while Japanese variety was excelled in diameter. Slight differences were noted in quantitative parameters. The selected tea varities showed considerable variations regarding to secondary metabolites. For analysing the secondary metabolites, tea varieties were treated with three different solvent extracts i.e., aqueous, chloroformic and ethyl alcohol extract. Exploration of these three solvents revealed the existence of flavonoids, glycosides, phenol or phenylic acid, corticosteroids and tannins amongst all extracts. Phlobatannins was totally absent in all extracts.

Alkaloids and terpenoids were absent in ethanolic solution while present in other two extracts. Carotenoids and saponins were present in chloroformic extracts but absent in aqueous and ethanolic extracts. Polyphenol was also analyzed, with the maximum value 6.4 mg/ml in P-7 variety while minimum value was 3.1 mg/ml in Japanese. Amino acids was determined to be high in the selected varieties. Maximum amino acid was determined 1.581 mg/ml in Indonesian tea variety. Minimum amino acid was found 0.801 mg/ml in Japanese tea samples. Caffeine is also major content in tea which was explored in selected varieties. Maximum value was found 0.928 mg/ml in P-7 while minimum was 0.51 mg/ml in Chuye. Minerals concentration in selected tea varieties selected from Pakistan Agriculture Research Council - National Tea and High value crops Research Institute (PARC- NTHRI), Shinkiari, Mansehran, and Khyber Pakhtunkhwa Pakistan were screened out in COMSATS University Abbotabad and results were recorded. Minerals contents in selected tea samples were determined in following quantity order: K (149.1 to 159.1 mgL<sup>-1</sup>) > Ca (21.46 to 30.85  $mgL^{-1}$ ) > Mg (10.07 to 12.40 mgL<sup>-1</sup>) > Na (6.364 to 29 mgL<sup>-1</sup>) > Co (4.882 to 9.482 mgL<sup>-1</sup>) > Mn (1.007 to  $1.441 \text{ mgL}^{-1}$  > Fe (0.786 to 2.842 mgL<sup>-1</sup>) > Pb (0.673 to 3.797 mgL<sup>-1</sup>) > Zn (0.440 to 1.609 mgL<sup>-1</sup>) > Ni  $(0.200 \text{ to } 2.27 \text{ mgL}^{-1}) > \text{Cu} (0.053 \text{ to } 0.384 \text{ mgL}^{-1})$ . Both macro and micro minerals studied. All minerals were enriched in tea varieties. Further studies can reveal and explore why morphological and physiological parameters vary among tea varieties, and how to harness the benefits of minerals present in these varieties.

#### REFERENCES

- Arachchi R SS W. (2019a). The Effect of Green Practices on Tourists' Behavioral Intention: A Study of Hoteliers in Central Province of Sri Lanka. *Peradeniya Management Review*, 1(2).
- Arachchi N SS M., Ganegama R., Husna AWF., Chandima D L., Hettigama N., Premadasa J and Agampodi
   S B. (2019b). Suicidal ideation and intentional self-harm in pregnancy as a neglected agenda in maternal health; an experience from rural Sri Lanka. *Reproductive health*, 16; 1-7.
- Atalay D and Erge H. S. (2017). Determination of some physical and chemical properties of white, green and black teas (*Camellia sinensis*). *GIDA*, 42(5); 494-504.

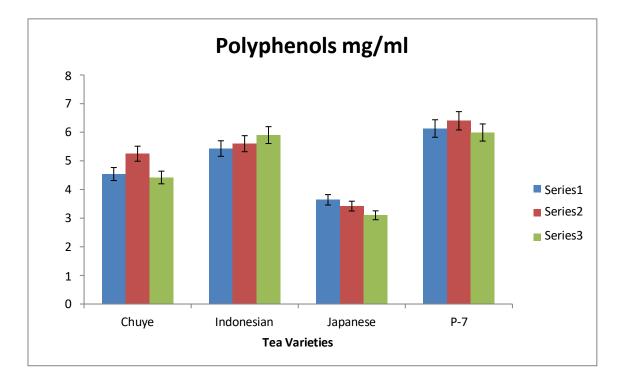
- Bezbaruah, H. P. (1971). Cytological investigations in the family Theaceae—I. Chromosome numbers in some Camellia species and allied genera. *Caryologia*, 24(4), 421-426.
- Butt M S and Sultan M T. (2009). Green tea: nature's defense against malignancies. *Critical reviews in food science and nutrition*, 49(5); 463-473.
- Chen L., Gao Q. K., Chen D M and Xu C J. (2005). The use of RAPD markers for detecting genetic diversity, relationship and molecular identification of Chinese elite tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] preserved in a tea germplasm repository. *Biodiversity and Conservation*, 14(6); 1433-1444.
- Gul S. (2011). Molecular and Phytochemical Elaboration of Tea Genotypes Grown in Pakistan (Doctoral dissertation, Hazara University, Mansehra).
- Hilal Y and Engelhardt U. (2007). Characterisation of white tea–Comparison to green and black tea. Journal für Verbraucherschutz und Lebensmittelsicherheit, 2(4); 414-421.
- Hoi Q V., Thin D B and Thinh B B. (2021). Some research on the genus Camellia l.(Theaceae) with representatives in Vietnam. *Вестник Нижневартовского государственного университета*, (2 (54));5-11.
- Olivera A P., Junior R G., Quintans J and da Silva Almeida J R G. (2016). *Camellia sinensis* (L.) Kuntze: A review of chemical and nutraceutical properties. *Natural products: Research reviews*, *4*;21-62.
- Jahangirzadeh Khiavi S., Falakro K and Safaei Chaeikar S. (2021a). PCR-RFLP analyses of chloroplast DNA in some cultivated tea (Camellia sp.) genotypes. *Journal of Horticulture and Postharvest Research*, 4(1-March 2021); 25-36.
- Jahangirzadeh Khiavi S., Falakro K and Safaei Chaeikar S. (2021b). PCR-RFLP analyses of chloroplast DNA in some cultivated tea (Camellia sp.) genotypes. *Journal of Horticulture and Postharvest*

Research, 4(1-March 2021); 25-36.

- Li Y F., Ouyang S H., Chang Y Q., Wang T M., Li W X., Tian H Y and He R. (2017). A comparative analysis of chemical compositions in Camellia sinensis var. puanensis Kurihara, a novel Chinese tea, by HPLC and UFLC-Q-TOF- MS/MS. *Food Chemistry*, *216*; 282-288.
- Monobe M., Ema K., Kato F and Maeda-Yamamoto M. (2008). Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *Journal of Agricultural and Food Chemistry*, 56(4); 1423-1427.
- Mukhopadhyay M., Mondal T K and Chand P K. (2016). Biotechnological advances in tea (*Camellia sinensis* [L.] O. Kuntze): a review. *Plant cell reports*, 35(2); 255-287.
- Mukhtar H and Ahmad N. (2000). Tea polyphenols: prevention of cancer and optimizing health. *The American journal of clinical nutrition*, *71*(6); 1698S-1702S.
- Oliveira A P., Guimarães A L., de Oliveira-Júnior R G., Quintans J de Medeiros F A., Barbosa-Filho J.
  M., da Silva Almeida J RG. (2016). *Camellia sinensis* (L.) Kuntze: A review of chemical and nutraceutical properties. *Natural products: Research reviews*, 4; 21-62.
- Rehman B., Akmal N., Khan M A and Rani S. (2012). Economics of tea production in Pakistan. *Asian Journal of Agriculture and Rural Development*, 2(393-2016-23828); 506-513.
- Rout G R. (2006). Effect of auxins on adventitious root development from single node cuttings of *Camellia sinensis* (L.) Kuntze and associated biochemical changes. *Plant growth regulation*, 48(2);111-117.
- Tariq M., Naveed A and Barkat A K. (2010). The morphology, characteristics, and medicinal properties of Camellia sinensisâ€<sup>TM</sup> tea. Journal of Medicinal Plants Research, 4(19); 2028-2033.
- Tuxill J. (1999). Appreciating the benefits of plant biodiversity. State of the World, 96-114.

Waheed A., Bashir A., Hamid F S., Bashir M., Ali S., Ahmed N and Gul H. (2019). Evaluation of Secondary Metabolites and Proximate Composition in Different Varieties of Green Tea. *Journal of Advanced Research in Biochemistry and Pharmacology*, 2(2); 14-19.

Xia E H., Tong W., Wu Q., Wei S., Zhao J., Zhang Z. Z and Wan X C. (2020). Tea plant genomics: achievements, challenges and perspectives. *Horticulture research*, 7(1); 1-19.



# **FIGURES**

Fig 1. Polyphenols for selected four tea varieties

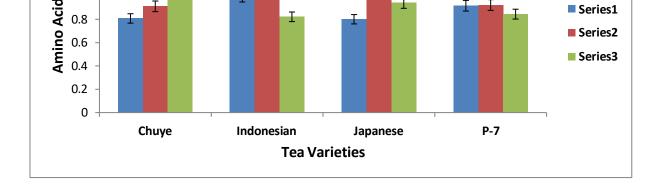


Fig. 2. Amino acids for selected four tea varieties

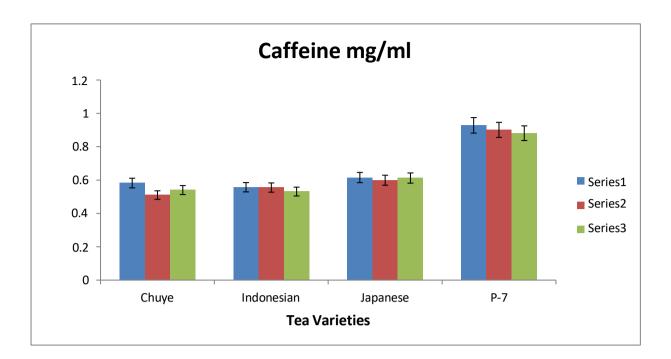


Fig. 3 Caffeine in selected four tea varieties

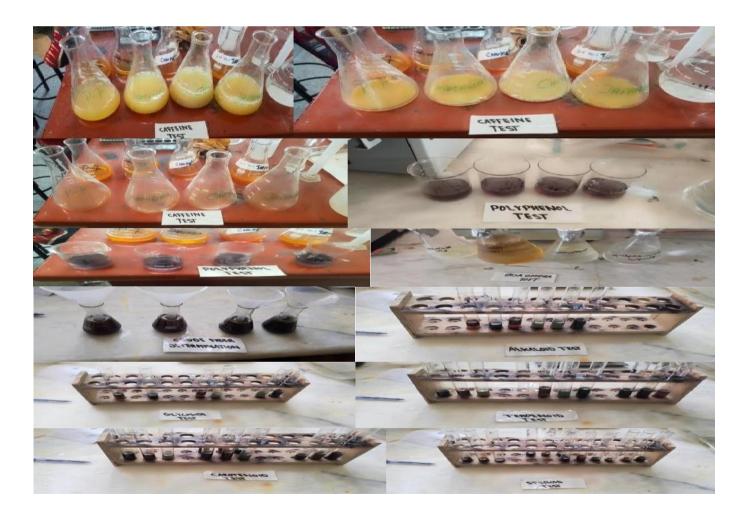


Fig 4. Quantitative analysis of four varieties of *Camellia sinensis* from selected area.



Fig 5. Tea varieties from Pakistan Agriculture Research Council-National Tea and High value crops Research Institute (PARC-NTHRI), Shinkiari Mansehra KPK Pakistan