

Abstract

Tea, *Camellia sinensis* (L.) Kuntze, is a widely consumed beverage with significant medicinal properties. Tea exhibits considerable genetic diversity, particularly in morphological traits. This study aims to analyse the morphological, micromorphological, anatomical, phytochemical, and molecular characteristics of various tea germplasm accessions in the Terai region of Darjeeling district, West Bengal to classify and understand the diversity of these accessions, facilitating tea improvement programs. Tea has notable benefits in protecting against diseases like cardiovascular disorders, cancer, and diabetes. India, particularly Darjeeling, is famed for its tea production. However, the characterization of tea germplasm in this region remains limited. Therefore, this study focuses on understanding the morphological and phytochemical diversity of tea germplasm in the Terai region of Darjeeling district.

The study used morphological, anatomical, phytochemical and molecular techniques to characterise different tea germplasm accessions. The Terai region of Darjeeling is geographically positioned at the foothills of Himalaya and co-ordinates from latitudes 25°31'05" to 26°13'10" N and longitudes 87°59'30" to 88°53' E with the elevations of 80 to 300 meters (260 to 980 feet). A survey was conducted across various tea-growing areas in the Terai region during different flushing seasons (First, Second, Monsoon, Autumn, and Winter) over three years (2020-2023). Out of 46 tea estates of this region, 39 were thoroughly surveyed. Seasonal variations were documented by analysing key growth stages—flushing, flowering, fruiting, and seedling. Thirty six (36) tea clones were collected from these estates, including Tocklai-released vegetative clones, TRA/Garden series clones and Tocklai Seed Stocks. Information about the estates, such as cultivation practices (organic or inorganic), clone names, and types of tea produced, were recorded.

Thirty-six tea germplasm accessions (TGAs) were characterized using 27 qualitative traits (leaf shape, apex habit, margin type, and edge undulation) and six quantitative vegetative morphological traits. Principal component analysis (PCA) showed that clone TV23 was distinct. Quantitative and qualitative floral morphological traits were used to characterize 36 TGAs. Twenty-four quantitative and 15 qualitative traits were analyzed, with pistil traits showing the most variation. Key qualitative traits included androecium and gynoecium height, ovary type, locule number, stigma position, style splitting, and stylar arm type. PCA revealed three distinct clusters, with TV16 and Tukdah383 forming separate clusters. Understanding these morphological variations is crucial for tea breeding and improvement programs.

This study also compared leaf epidermis micromorphology among 36 tea clones using light and scanning electron microscopy (SEM). Leaf epidermal cells were consistent in size and shape across clones, with polygonal and irregular shapes and varying anticlinal wall patterns. All clones had hypostomatic stomata (located on the lower leaf surface), and stomata types were anisocytic and anomocytic. Trichomes exhibited unicellular hair-like structures with variations in size and density, and immature leaves had higher trichome density than mature leaves. The epidermis analysis is significant for taxonomic and comparative studies.

Pollen morphology was studied using SEM, which is essential for taxonomic analysis, classification, and pollination studies. The pollen grains were tricolporate, varying in size, shape, germination pores, and wall ornamentation. TV29 had the smallest pollen (28.30 μ m), and TS520 had the largest (44.52 μ m). Pollen surface ornamentation helped to distinguish the clones, aiding in identifying and understanding the relationships. Further histology analysis showed variations in sclereids distribution, parenchyma thickness, and vascular bundle diameter. PCA of stem anatomy showed that PC1 and PC2 explained

65.92% of the variation, with cluster analysis revealing two major groups. PCA analysis with petiole traits showed TV1 as distinct. In terms of stoma, all clones had hypostomatic leaves with randomly distributed stomata on the lower surface. Sizes ranged from $18.91 \pm 2.07 \times 13.67 \pm 1.27 \text{ }\mu\text{m}$ (T3E3) to $36.10 \pm 0.74 \times 26.34 \pm 1.37 \text{ }\mu\text{m}$ (Sundaram), with stomatal index from 13.48 to 22.42 per mm^2 . TV19 and TS520 had the highest stomatal index, while TV29 and Heeleakah had the lowest. Younger leaves had more trichomes but fewer stomata than mature leaves. Stomatal characteristics are key for differentiating clones. The leaf architecture study showed that 33.33% of clones had lanceolate to elliptic shapes with variations in leaf margins, apex shapes, and venation patterns. PCA and cluster analysis identified key variations, and K-means clustering confirmed four major groups, with TV23 forming a distinct cluster.

TS520 and CP1 had the highest tannin levels (390 and 290 $\mu\text{g TAE/ml}$), while S3A3 and Heeleakah had the lowest. TS520 had the highest condensed tannin content (CTC) at 340 $\mu\text{g CE/ml}$, followed by TV17 (127 $\mu\text{g CE/ml}$). TS520 also exhibited the highest antioxidant activity (469.90 $\mu\text{g TAE/ml}$). Antioxidant assessments revealed that TS520 had the lowest IC_{50} values, indicating the highest antioxidant activity. The IC_{50} values for DPPH radical scavenging ranged from 5.16 to 54.55 μl , while ABTS radical scavenging ranged from 2.25 to 26.84 μl of tea extract. GC-MS analysis of TS520 revealed high levels of tannins, antioxidants, epigallocatechin, syclo-inositol, and other bioactive compounds like alpha and beta amyryn. Epigallocatechin has anti-diabetic, antioxidant, anticancer, and anti-obesity properties, while condensed tannins exhibit strong antioxidant and anticancer activities. TS520's lower caffeine content (25%) compared to other clones like TV1 and TV30 (82%) suggests that blending it with higher caffeine clones could produce a balanced tea with enhanced health benefits. Correlation analysis showed a moderate negative

association between antioxidant potential and leaf size and a strong negative association with the number of inter-secondary veins.

Phytochemicals from tea extracts, including scyllo inositol, epigallocatechin, gallic acid, and caffeine, were docked against the EGFR tyrosine kinase domain, a key player in cancer progression. Epigallocatechin showed the strongest binding (-6.25 kcal/mol) and inhibition constant (26.13 μ M). The TS520 clone, with the highest epigallocatechin content, emerged as a potential candidate for anticancer research.

Pollen germination studies revealed that TV1 had the highest pollen germination (96.07%), while TV26 had the lowest (0.55%). Clones like TV1, AV2, BB157, and Tukdah78 exhibited high germination rates (>80%). These accessions are conserved in the Department of Tea Science's ex-situ conservatory for further research.

In conclusion, this study comprehensively characterizes tea germplasm in the Terai region of Darjeeling district, focusing on their morphological, micromorphological, anatomical, and phytochemical traits. The findings highlight significant diversity among the tea clones, particularly in leaf architecture, stomatal characteristics, and bioactive compound content. The TS520 clone, with its high epigallocatechin and antioxidant content, stands out as a potential candidate for developing tea varieties with enhanced health benefits, such as anticancer and anti-diabetic properties. Furthermore, its lower caffeine content offers a balanced alternative for tea blends. Integrating morphological, anatomical, phytochemical and molecular data provides a valuable resource for tea breeding and improvement programs, ensuring the sustainable development of the tea industry in this region.