

Chapter 1

Introduction

Introduction

Tea, scientifically known as *Camellia sinensis* (L.) Kuntze is an evergreen small tree belonging to the family Theaceae Mirb. and ranks as the second most consumed non-alcoholic beverage worldwide (Ahmed & Stepp, 2013). Tea beverages are primarily prepared from the young, undeveloped leaves and shoots of the tea plant and are reported to have medicinal benefits against various pathological conditions, including cardiovascular diseases, cancer, diabetes, and arthritis (Khan & Mukhtar, 2013). Though tea is grown in diverse regions globally, it is predominantly cultivated in Southeast Asian countries, including India, China, and Sri Lanka (Bezbaruah, 1999; Rajanna & Ramakrishnan, 2010). *Camellia* L. is the largest genus of the family Theaceae, and it has many economic and medicinal values (Ho, 1999; Hoi et al., 2021). Initially, in China, *Camellia sinensis* was used as a beverage and as a medicine important for human health. Genus *Camellia* L., which includes a total of 229 accepted species (Powo, 2023). Linnaeus gave the name “*Camellia*” in honor of Joseph Kamel, a Maravian Jesuit who collected tea plants in the Philippines and sent them to Ray and Petiver between the years 1683 to 1700. An alternative perspective suggests that the genus *Camellia* is named after an Italian priest, Camelli, who is credited with bringing *Camellia japonica* L., a decorative plant from Japan, to Europe in 1739. Linnaeus first named the tea plant *Thea sinensis* in 1753 in “Species Plantarum” (Van et al., 2021). Author Linnaeus first described two genera, *Thea* and *Camellia*, where *Thea sinensis* belongs to the genus *Thea* (Class Polyandria Monogynia) and *Camellia japonica* belongs to the genus *Camellia* (Class Monadelphia Polyandria), was recorded in “Species Plantarum” (Zhao et al., 2017). Subsequently, in 1887, Carl Ernst Otto Kuntze reclassified the species,

resulting in the new combined name *Camellia sinensis*. *Camellia sinensis* (L.) Kuntze includes 5 accepted intraspecific and has 2 Synonyms. Its important synonyms include *Camellia theifera* Griff. and *Thea chinensis* Seem. But, in 1895, Engler and Prantle merged the genus *Thea* with *Camellia* (Baruah, 2015; Powo, 2023). Many studies recorded and classified the genus *Camellia* based on morphological characters (Airy-Shaw, 1936; Keng, 1962; Sealy, 1958). Tender leaves and young shoots of *Camellia sinensis* are primarily used for tea production (Chen et al., 2012). Commercial tea cultivars are recognized for three varieties of tea, viz. China variety, *Camellia sinensis* var. *sinensis* (L.) Kuntze, Assam variety, *Camellia sinensis* var. *assamica* (Royle ex Hook.) Steenis, Cambod variety, *Camellia assamica* subsp. *lasiocalyx* (G. Watt) W. Wight (Ariyaratna et al., 2011; Mondal, 2014).

The native range of the species *Camellia sinensis* (L.) Kuntze are Assam, China South-Central, China Southeast, East Himalaya, Hainan, Laos, Myanmar, Thailand, and Vietnam. It was introduced into Andaman Is., Argentina Northeast, Bangladesh, Bolivia, Borneo, Cambodia, China North-Central, Ethiopia, Guinea, Gulf of Guinea Is., Japan, Korea, Mauritius, Nepal, Réunion, Seychelles, Sri Lanka, Tibet, Trinidad-Tobago, Turkey (Powo, 2023). In the case of *Camellia sinensis* var. *assamica* (Royle ex Hook.) Steenis is native to Assam, China South-Central, China Southeast, East Himalaya, Hainan, Laos, Myanmar, Thailand, and Vietnam and introduced into Bangladesh, Bolivia, Nepal (Powo, 2023).

Although China, India, Burma, and Thailand are all native regions for *Camellia sinensis*, China began the first to utilize tea as medicine and then used it as a beverage (Martin, 2011). From China, tea has spread globally, yet a significant variety is still predominantly found in mainland Southeast Asia (Bezbaruah, 1999; Mohanan & Sharma, 1982;

Sharma & Venkataramani, 1974). In India, tea seeds were brought from China and sown at the Botanical Garden, Calcutta in 1780 (Bezbaruah, 1999). India ranks as the world's second-largest producer of tea, following China. This includes renowned varieties such as Assam tea and Darjeeling tea (Hazra et al., 2019). India's tea industry is one of the country's oldest and most efficiently organized agricultural enterprises (Borborah & Gogoi, 2007).

India has sixteen tea-growing states, with Assam, West Bengal, Tamil Nadu, and Kerala contributing approximately 95% of the nation's total tea production (Hazarika & Borah, 2013). In India, there are three distinctly different tea-growing regions. These regions are geographically separated and produce different tea types, which are separated according to their flavour. The three tea-growing regions of India are Darjeeling (North Eastern India), Assam (far North-East India), and Nilgiri (South India)(Willson & Clifford, 2012).

Darjeeling is situated in the central part of the IUCN-recognized “Himalaya Hotspot,” with around 30% of the endemic higher group of plants. Darjeeling district is located in West Bengal, India, at the extreme north of an elevation between 80 and 2200 m above mean sea level (AMSL). In 1839, Dr. Campbell, a civil surgeon of the Indian Medical Service, was transferred to Darjeeling, and he collected tea seeds from China and then started a tea plantation in Darjeeling. During this period, the government also established tea nurseries in Darjeeling (Singh et al., 2016). Tea is the main cultivated crop in this district, and most of this district’s land is used for tea cultivation. Darjeeling tea has a distinctive and naturally occurring quality and flavour, which has won patronage and has been famous worldwide for over a century. In 2004, Darjeeling tea became the first product in India to be registered under the Geographical Indications of Goods (Registration and Protection) Act of 1999 (Singh et al., 2016).

The tea plant is cross-pollinated, resulting in highly heterogeneous populations beneficial for natural hybridization methods (Kindom-Ward 1950; Banerjee 1992). Therefore, it is important to characterize different tea germplasm accessions (tea clones) for proper identification and improvement programmes utilizing available genetic resources (Ranatunga et al., 2017). Proper identification of germplasm accessions depends mainly on proper characterization and evaluation. So, germplasm accessions need to be characterized using different phenotypical sets of descriptors. Different sets of descriptors are analyzed according to “The International Plant Genetic Resources Institute” (IPGRI), which has published a standardized descriptor list for tea (IPGRI, 1997) and DUS guidelines (Parker & Namuth-Covert, 2017).

Morphological characters are useful descriptors for primarily characterizing genetic resources and identifying different tea Germplasm accessions. Among the morphological descriptors, floral traits are important and can be used to identify proper accessions and give an idea about the relationship between species of the same genus (Lu et al., 2012). Mainly leaf and floral morphology have been used to characterise tea germplasm in many previous studies (Jayaweera et al., 2013; Wachira et al., 2013). The characterization of tea Germplasm collections is important to plant breeders for its utilization and the efficient and effective management of gene banks. Morphological and anatomical characters are used as genetic markers for proper identification and characterization of different tea Germplasm accessions in many countries (Gunasekare et al., 2001, 2012; Piyasundra et al., 2008; Ranatunga et al., 2017; Su et al., 2007). In some cases, several metric and nonparametric morphological characters were also used to study genetic diversity between tea germplasm accessions (Wickramaratna, 1981; Toyao & Takeda, 1999).

A detailed and comprehensive investigation of the leaf and stem anatomy of different tea germplasm accessions is required to shed light on future taxonomic studies and the correct scientific identification of commercial tea species and varieties (Das et al., 2023). The leaf and stem micromorphology or anatomy established a better systematic construction and helped in the proper identification and classification of different tea germplasm accessions (Das et al., 2023). In this study, the anatomical features of the stem and leaf of different tea clones are analyzed and highlighted to show the important diagnostic features that can be decisive for their taxonomic significance and show variation among studied tea clones. Plant leaves vary widely in shape, size, and structure due to environmental factors like climate, light, rainfall, soil, terrain, and altitude (Boeger et al., 2004; Givnish, 1984; Sosnovsky, 2015). Leaf plays a vital role in facilitating interactions between living organisms and their environment, including processes like gas exchange (Agrawal et al., 2009; Russo et al., 2010).

On the other hand, leaf epidermis plays a pivotal role in plant-pathogen interactions (Fuchs et al., 2016). Leaf features such as the quantity and quality of wax and cuticle, the size, position, and shape of stomata and lenticels, along with the presence of thick cell walls, play a crucial role in protecting against pathogen infections (Araujo et al., 2014). Previous studies have shown that trichomes and the cuticular wax layer act as protective barriers against pathogen attacks, while stomata play an active role in bacterial invasion (Ferdinand et al., 2000; Xu et al., 2011). Cuticular waxes are complex mixtures of long-chain fatty acids, alcohols, aldehydes, esters, and n-alkanes. The cuticle is a physical barrier and a dynamic host defence system (Kunst & Samuels, 2003; Pollard et al., 2008). A previous study showed that the presence of higher cuticular wax may be more resistant to the pathogen attack than a lower cuticular wax load (Wang et al., 2020). Leaf epidermal characteristics, such as the

morphology of trichomes and stomata, showed taxonomically important traits for plant species identification and the exact identification of some medicinally important plants (Choi et al., 2024; Rehman et al., 2022). The morphology and density of epidermal trichomes and stomata were affected by leaf age, environmental stress, and salinity (Bertolino et al., 2019). The density of trichomes and stomata showed more in the young leaves compared to the old leaves (Adebooye et al., 2012). Some plant species are identified by leaf trichome micromorphology from entire, fragmented, or powder leaf materials (Machado et al., 2021). Also, stomatal density and trichome shape change even in the same species, which are grown in different environmental conditions influenced by different environmental conditions (Ichie et al., 2016).

Trichomes are uni- or multicellular surface structures having cell walls and lumen. They originate from epidermal cells and develop on the outer surface of various plant organs (Wagner et al., 2004; Werker, 2000). Trichomes exhibit a wide range of sizes, spanning from just a few microns to several centimeters, and demonstrate impressive species-specific diversity in their shapes (Payne, 1978). Trichomes are primarily located on leaves and stems, but depending on the species, they can also be found on petals, petioles, peduncles, and seeds (Wagner et al., 2004). Their direct interaction with the environment dictates their protective role, absorption, secretion activities, and influence on pollination and seed dispersal (Ehleringer, 1984; Evert et al., 2006). Regarding morphology, trichomes can vary in cell number (unicellular or multicellular), arrangement (uniseriate, biseriate, or multiseriate), shape (e.g., branched, unbranched, symmetrical, asymmetrical), and length (Werker, 2000). Trichome types are very significant characters for identifying different plant species at the generic or species level (Hoot, 1991; Krak & Mráz, 2008; Reis et al., 2002;

Sahu, 1984; Stebbins, 1953). Glandular trichomes share the ability to produce, store, and secrete substantial quantities of various secondary metabolites which are resistant to herbivores (Agrawal et al., 2009; Fahn, 2000; Glas et al., 2012; Levin, 1973; Schilmiller et al., 2008; Wagner, 1991). Specialized metabolites from glandular trichomes are commercially important as natural pesticides, food additives, and pharmaceuticals (Adeneye, 2016; Aharoni et al., 2006).

Stomata are the main channels for gaseous exchange and the loss of water vapour between the leaf and the surrounding atmosphere (Bowman, 2011). Stomatal control of water loss helps plants adapt to their environments and expand their range, suggesting that stomata play a key role in speciation and evolution (Raven, 2002). The density and morphology of stomata in plant leaves may vary between species to adapt to the different environmental conditions in their habitats (Sack et al., 2006; Salisbury et al., 1928).

In vascular plants, leaves are crucial in various physiological functions, including water transport, gas exchange, and photosynthesis. These physiological functions rely on the leaf's architectural structure (Ash et al., 1999; Kenrick & Crane, 1997). Previous research indicated that leaf morphological traits were one of the simplest and most effective methods for diversity analysis of tea clones (Wachira et al., 2013). Leaves typically display polymorphism, showcasing various morphological and anatomical characteristics. Among these characteristics, the venation pattern stands out as a crucial aspect of leaf structure, reflecting the organization of veins and veinlets throughout the leaf lamina. Apart from this, major and minor venation patterns can be distinguished by different timing of development and differences in gene expression during formation, as well as by the sizes and branching patterns of veins (Haritatos et al., 2000; Meyer & Esau, 1960). It was reported that all tea

clones showed reticulate venation, as veins originate laterally from the midrib of the leaf, forming a net-like structure (Ellis et al., 2009; McKown et al., 2010). Therefore, leaf architectural study, which includes different venation patterns, classification of venation patterns, the shape of the leaf, and leaf marginal type, was mentioned to be an important taxonomic and systematic study to compare between genus and species of both living and fossil plants (Al-Hadeethi et al., 2019; Dilcher, 1974; Hickey, 1973; Hickey & Wolfe, 1975; Melville, 1976; Pole, 1991). Previously, different venation patterns were employed for their identification, comparative analysis, and taxonomic study of many dicotyledonous (Banerjee & Deshpande 1973; Inamdar & Murthy 1978; Rao & Narmada 1994; Wang et al. 2001) and monocotyledonous families (Inamdar et al., 1983a; Narawatthana et al., 2023).

The mode of arrangement of veins and the veinlets in the lamina of leaves is known as venation, which is important for leaf architectural study (Metcalf & Chalk, 1979). The terminology of leaf venation patterns showed taxonomic significance and identification of plants, which have already been discussed by (Levin, 1929; Melville, 1976; Pole, 1991). Morphological characterization and classification of the leaf-architectural study of dicotyledonous plants have been well studied in taxonomical significance in many studies (Hickey, 1973; Hickey and Wolfe, 1975). Different degrees of leaf venation pattern, leaf marginal configuration, and leaf shape are included in the leaf-architecture study (Metcalf & Chalk, 1979). Venation pattern includes the different degrees of vein category, the interrelation between vein, vein islets, and leaf margin, and different shapes of areoles, which are essential for describing leaf architectural study and provide a taxonomic clue which is described previously (Hickey, 1973). Many plants showed dichotomous branching and open-type venation patterns, which gradually evolved complex structures and varied genus

to species level using different venation patterns and vein terminology (Dilcher, 1974). The venation patterns of some species are reticulate, and some are parallel; mostly dicotyledonous plants have reticulate venation patterns. Angiosperms have the greatest vein structure diversity, identified using a leaf venation architecture study (Ellis et al., 2009; Hickey, 1973; McKown et al., 2010). Most angiosperm leaves have four and seven orders of venation. Generally, the primary and secondary veins are the major structural veins of the leaf, while the tertiary veins are the largest veins that fill the field of the leaf (Hickey & Wolfe, 1975). Therefore, all of these studies evidenced that leaf architectural study is very important for leaf identification, determining the types of leaves belonging to which plants or families, and is also important for taxonomic study and evolutionary clues (Zarinkamar & Sharifsadat, 2016), and also, the terminology of venation pattern is crucial for leaf architecture because veins are associated with the development and shape of leaf lamina (Al-Hadeethi et al., 2019).

Apart from the leaves, flowers are another important part of the plants having significance in taxonomic studies. The flower is the primary reproductive structure of angiosperms. In plants, the male reproductive part is known as the stamen, and the female reproductive part is called the pistil. The stamen comprises two parts: the anther and the filament (Mallick, 2020). Pollen grains, the male gametes contained within the anther, are tiny reproductive cells essential for double fertilization in plant sexual reproduction (Hornick et al., 2022; Kumarihami et al., 2015). Pollen grains have three parts: an outer wall called the exine, an inner wall called the intine, and a central cytoplasmic portion. They possess distinct morphological features that differ in size, shape, aperture number, and exine ornamentation among various angiosperm plant species (Shi et al., 2022; Zhang et al., 2016). The

morphology of pollen is crucial for plant taxonomy and species identification, as well as for the study of phylogeny (Fuchs, 1967; Motyleva et al., 2017; Ragho, 2020). Pollen morphological characteristics are crucial in various fields, including plant taxonomy, species identification, evolution, phylogenetics, plant breeding programs, and biotechnology (Lu et al., 2012; Luna & Ochoterena, 2004; Sathyapala et al., 1998; Song et al., 2020; Zhang et al., 2021). Pollen traits like size, shape, surface ornamentation, and furrow number are stable and genetically conserved, unaffected by environmental factors. These traits aid in species identification and classification. Moreover, a relationship between floral reproductive traits and fruit yield in tea can also be observed (Lo et al., 2023; López-Orozco et al., 2023; Santiago & Sharkey, 2019).

Pollen germination and pollen tube growth studies are important for plant fertilization and seed development. Pollen germination study of different plant species provided the physiology and biochemical characteristics of pollen germination and pollen tube growth formation (Heslop-Harrison, 1987). Pollen germination and pollen tube development are generally classified into four stages: the imbibition phase, lag phase, tube initiation phase, and rapid elongation phase (Derksen et al. 1995). The time taken for every phase of pollen germination varies from species to species depending upon the internal physiology of pollen and external environmental factors (Zaman, 2006). Tea plants are cross-pollinated plants, where the pollen grains are transferred from an anther of one flower to the stigma of another flower borne on different plants of the same species, showing many overlapping morphological and physiological characteristic (Mondal, 2020). One of the advantages of cross-pollination is that after fertilization, seeds are produced in greater quantities and are more viable, and as a result, produce healthier plants (Ahrent & Caviness, 1994). In this

context, pollen germination tests are necessary to observe the pollen viability as described previously (Radičević, et al. 2013). Morphological, anatomical, chemical, and molecular characterization of tea plants have been discussed previously, but no studies have been conducted on pollen morphology and germination of tea plants. So, another objective of this study is the pollen germination of different tea clones maintained under similar climatic conditions.

Tea, the second most-consumed nonalcoholic beverage after water, is rich in phytochemicals and is an antioxidant. Compounds in tea inhibit free radicals associated with diseases like cancer and diabetes (Lobo et al., 2010), and tea leaves are used for their antioxidant properties in treating various ailments (Ostrowska et al., 2001). Fresh tea leaves contain different polyphenolic compounds, such as catechins, theaflavin, and tannin, which have antioxidant potential (Drynan et al., 2010). A previous study reported a high genetic diversity among different tea clones of southern regions of the Caspian Sea (Dargah et al. 2023b). Moreover, genetic variations and diverse origins contributed to variations in phytochemicals among different tea clones, ultimately influencing the overall quality of the (Dargah et al. 2023a). Phytochemicals content also varied from tea clone to clone, which led to the variation in their antioxidant activity (Kamal et al., 2022; Sarkar et al., 2022). Therefore, it is essential to characterize different tea germplasm accessions (tea clones) by using available genetic resources to identify different tea clones properly, and it is also crucial to determine which tea clone contains more antioxidant potential.

Various statistical techniques, including cluster and Principal Component Analysis (PCA), were used to analyze various taxonomic structures and classifications. These techniques are helpful in understanding the relationship between different morphological traits among

germplasm accessions and categorizing them into different groups (Rajanna et al., 2011).

A significant number of researchers have conducted extensive work across diverse areas related to tea globally. The foothills i.e. Terai and Duars regions, along with the mountain slopes of Darjeeling, are the prime areas for tea cultivation. A diverse range of tea clones is cultivated across a 22,000-hectare area in the Terai region of the Darjeeling district of West Bengal, spanning elevations from 80 to 300 meters above mean sea level (Misra et al., 2018). Characterization of different tea Germplasm has been done in different regions of different countries like Sri Lanka, China, Bangladesh, Indonesia, etc. But, till now, no proper characterization of tea germplasm has been done in the Darjeeling district. Despite the cultivation of numerous tea clones in this area, there needs to be more comprehensive studies on different tea germplasm accessions for taxonomic significance, and limited understanding exists regarding the clone with the highest tannin and antioxidant content. Characterization of different tea germplasm in the Darjeeling district of West Bengal will give an idea about the exact variation between the tea germplasm accessions. Morphological, anatomical, molecular and biochemical characterization of different tea germplasm accessions will give an idea for their utilization in tea crop improvement programs and plant breeding methods.

Hence, it is urgent to characterize and analyse different tea germplasm accessions for the development of tea breeding and tea conservation. Despite the agronomic interest in tea cultivars since their commercial release, the main objective of the present work is the comprehensive study of the diversity of different tea germplasm accessions and relations among them in the Terai region of Darjeeling district based on the morphological, anatomical, molecular and phytochemical analysis by different statistical methods. Another goal of this study is to determine genetic diversity's nature and extent and categorizing

accessions into major groups. However, in the Darjeeling district, numerous clones of tea (*Camellia sinensis*) have been cultivated, all exhibiting fairly similar phenotypes. This similarity has made it challenging to distinguish and accurately identify them. Thus, a thorough examination of both macro and micro morphological characteristics is necessary for their differentiation and proper classification. Surprisingly, no previous studies have been conducted on the macro and micromorphology of the various tea clones. In *Camellia sinensis* clones, detailed descriptions of leaf epidermal micro morphology are lacking. Accordingly, a comprehensive analysis of the leaf epidermis across different tea clones is crucial for advancing the systematic framework and understanding the diverse variations and relationships among them. This study also aimed to analyse the total tannins, condensed tannins, and antioxidant activity thoroughly among different tea cultivars available in the sub-Himalayan Terai region of the Darjeeling district.

For the chemical analysis, the ultimate goal was to identify the clone with the highest antioxidant content and total antioxidant activity. In this study, we analyzed the total tannin, condensed tannin content and antioxidant activity of the aqueous extract (hot water extraction) of the leaves of each clone. Further, multivariate analysis, such as Principal Component Analysis (PCA) and cluster analysis, were employed to understand the distribution and clustering patterns of the clones. These methods are essential for understanding the relationships between different leaf morphological characteristics in the germplasm accessions and categorizing them, as mentioned by (Rajanna et al., 2011a). Additionally, in-depth antioxidant activity and gas chromatography-mass spectrometry (GC-MS) based phytochemicals analysis were performed with the aqueous extract of the tea clones.

Overall, the study emphasizes differences in morphological, anatomical, molecular, phytochemical and antioxidant potential, enabling growers to identify different tea clones, genetically diverse tea clones, phytochemical-rich tea clones, high caffeine-rich tea clones and antioxidant-rich tea clones based on their morphological anatomical, molecular, phytochemical and antioxidant analysis. Also, it enhances our understanding of the importance of characterization and other correlated leaf morphological traits within the domains of taxonomic and evolutionary research.