Chapter 1
Introduction

> *"The real voyage of discovery consists not in seeking new landscapes but in having new eyes."*
> *- Proust*

Tea, a popular non-alcoholic beverage is consumed worldwide where the beverage is made from the infusion of the manufactured tea leaves. The world's tea consumption is highest for black tea, followed by green tea, oolong tea, and white tea (Khan and Mukhtar, 2019). The history of tea consumption dated back to the 5th century and by the end of the 6th century AD, tea was consumed not only for a therapeutic purpose but it became popular as a refreshing drink (Wilson and Clifford, 2012). Kakuzo Okakura (1863-1913), a Japanese cultural philosopher and the author of "The book of tea" (Okakura, 1964) stated, "Tea began as a medicine and grew into a beverage". In ancient China and Japan, tea was considered a ritual element and an essential part of daily life. The effective pharmaceutical activity of tea was very much known from the beginning thus ancient China consumed it as a medicinal beverage (Hilal, 2017). Tea in today's era is the most commonly consumed drink next to water (Hoffmann and Manning, 2005).

The tea plant is most suitable to cultivate in areas with increased humidity, moderate temperature, and acidic soils (Dufresne and Farnworth, 2001). *Camellia* is a genus with about 350 species available (Mukhopadhyay *et al.,* 2016). However, only two species, i.e., *Camellia sinensis* (L.) O. Kuntz and *Camellia assamica*, produce tea, for consumption in a variety of forms, including black, green, and oolong tea (Mondal *et al.,* 2004). However, in some parts of China, a different species, *C. taliensis*, is used to make tea (Rawal *et al.,* 2021). The variation in leaf size i.e., the small leaf tea plant and the large leaf tea plant has raised much speculation regarding the origin of tea hence providing many theories.

There are many theories and speculations about the origin of tea. Some report states that tea originated from Yunan and Sichuan in China based on studies in numerical taxonomy and multivariate analysis (Hasimoto, 2001). The history of tea dates back to China which states that“Tea” was first discovered around 2737 B.C. by the Emperor of China, Shen Nong when the tea leaves secretly blew into the pot of boiling water by a matter of chance (Patel, 2005). Tea was first originated in South Asia and slowly grown widely throughout Asia, Africa, and various parts of the Middle East (Chopade *et al.,* 2008).

Tea was originally introduced in India when the tea seeds were first brought from China and planted at the Botanical Garden of Calcutta in 1780 (Bezbaruah, 1999). Tea was planted in northeast India in 1836. However, reports say that Major Bruce in 1823 discovered wild tea plants growing in some parts of Sibsagar hills, which were then known as Rangpur hills, the capital of “Assam” (Ukers, 1935). Robert Bruce was a Scottish gentleman who introduced the tea plantations in Assam in the early 19th century. The first Indian tea from Assam was shipped to England for public sale in May 1823. After establishing a profitable industry in Assam's Brahmaputra valley, the viability of cultivating tea in the Himalayan foothills and elsewhere in India was investigated. In Kumaon, Dehra Dun, Garhwal, Kangra Valley, and Kulu, 78 plantations had been developed by 1863.

While attempting to introduce tea to India, British colonists discovered that tea plants with thicker leaves flourished in Assam as well and that these, when planted, thrived. The Singphos tribe of Assam had long grown the same plants, using tea chests provided by tribal chief Ningroola. In the past, botanists categorized the Assamese and Chinese types as separate species, but botanists now classify both as the same species, *Camellia sinensis*. The British East India Company began large-scale tea manufacturing in Assam, India, in the early 1820s, using a tea type traditionally prepared by the Singpho people.

Beginning in the 1850s, the tea business quickly grew, consuming vast tracts of land for tea plantations. By the turn of the century, Assam had surpassed China as the world's leading tea-producing region. Robert Fortune, who spent around two and a half years in China working for the Royal Horticultural Society of London from 1848 to 1851, is often credited with introducing Chinese tea plants, which are distinct from Indian tea. Fortune transported 20,000 tea plants and seedlings to the Darjeeling region of India, which is located on steep slopes in the Himalayan foothills and has the acid soil that *Camellia* plants prefer. He also brought a crew of Chinese tea workers who were taught to help in the production of tea leaves. Most of the Chinese tea plants failed to survive, except for few which remained in established in Indian gardens.

Tea plantation in the area of around 3.8 million hectares resulted in the worldwide production of 6.1 million metric tons of tea (Zhang *et al.,* 2020). The economically important tree crop is grown in over 52 countries (Mondal *et al.,* 2004; Chen and Chen, 2012). It is mostly cultivated in the region of China, India, Taiwan, Sri Lanka, Indonesia, Japan, and central African countries (Mukhtar and Ahmad, 2000). China and India are globally the two largest tea producers (Meegahakumbura *et al.,* 2018). As per the data given by FAOSTAT(http:// www.fao.org/faostat/en/#rankings/ countries_by_commodity), mainland China was the largest tea producer (2777200 tonnes) and India was the second-largest tea producer (1390080 tonnes).

Tea belongs to the genus *Camellia* and comes under the Theaceae family. Linnaeus previously named the tea plant *Thea sinensis*, which was published in Species Plantarum in the year 1753. However, Carl Ernst Otto Kuntze shifted the species to the genus *Camellia* later in 1887. It is now widely accepted in combination as *Camellia sinensis* (Linnaeus) O. Kuntze (Das and Ghosh, 2016).

The taxonomy of tea is still very confusing due to its plasticity of morphological traits that distinguishes tea taxa (Meegahakumbura *et al.,* 2018). Due to the environmental plasticity of morphological traits, previous attempts to classify tea germplasm by using morphological markers, gave unsuccessful results in revealing the taxonomic affinities between accessions (Balasaravanan *et al.,* 2003).

Classification of tea plants given by Sealy (1958) was based mainly on morphological and yield characters and subsequently, tea plants were grouped

Table 1.1 APG IV classification of tea updated by Angiosperm Phylogeny Group (Chase *et al.,* 2016)

Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Ericales
Family:	Theaceae
Genus:	*Camellia*
Species:	C. *sinensis*

in two different taxa i.e., dwarf, small leaf, and slow-growing variety, *Camellia sinensis* var. *sinensis* from China and tall, large leaf and quickly growing variety, *Camellia sinensis* var. *assamica* (Masters) Kitamura from Assam (India) i.e., Assam type. White (1962) revised the classification relying mainly on morphological features. He assigned a specific status to var. *sinensis* and var. *assamica* and recognized a Southern or Cambod form of *Camellia assamica* thus differentiating cultivated tea into three main species each with specific plant types viz. *Camellia sinensis* (China type), *Camellia assamica* (Assam type), and *Camellia assamica lasiocalyx* (Cambod type). However, according to Ming *et al.,* (2000), tea plant has broadly two varieties, *C.sinensis* var. *sinensis* (China type) and *C. sinensis* var. *assamica* (Assam type). Although previous reports have placed Cambod type tea as an alternative name for *C. sinensis* var. *assamica*, recent studies revealed it to be a hybrid of China and Assam teas (Wambulwa *et al.,* 2016). Nowadays, all tea is placed under *Camellia sinensis* irrespective of taxonomic differences. However, China, Assam, and Cambod varieties are still referred to as *Camellia sinensis*, *Camellia assamica*, and *Camellia assamica* ssp. *lasiocalyx* respectively (Kaundun *et al.,* 2000).

Sealy (1958) reported 82 species of *Camellia* native to southeast India. However, to date, The Plant List has included a total of 464 scientific names of the plant under the species rank for the genus *Camellia* and out of this a total of 248 are accepted species name (http://www.theplantlist.org/browse/A/Theaceae/*Camellia*/).The classification of tea updated by Angiosperm Phylogeny Group (Chase *et al.,* 2016) is provided in Table 1.1.

Tea is both commercially and taxonomically important among all the species of *Camellia* since other *Camellia* species do not harvest the world-famous brew (Banerjee 1992). Tea offers its contribution greatly to the country’s economy as well as job opportunities in countries like China, Kenya, India, Sri Lanka, etc.(Chen *et al.,* 2005). Other species of *Camellia* i.e., non-tea producers are used as ornamental plants. *Camellia sinensis* var. *sinensis* and C. *sinensis* var. *assamica* are the two main commercial taxa that are largely cultivated. However, some species like *C. taliensis, C. sinensis* var. *dehungensis, C. crassi columna*, have been used as tea in local parts of Asia, and particularly in Yunnan province (Chen *et al.,* 2005). The tea taxonomy is still a challenge and question of debate due to its complexity as well as a particular interest in only one taxon due to its commercial and economic demand. Studies related to taxonomic characteristics; genetic variation and biogeography would be of great benefit to identify genotypes with increased productivity, which can further be utilized to improve the cultivars (Banerjee, 1992).

Morphologically, the tea plant is an evergreen shrub or tree with numerous branches with leaves appearing dark, leathery, shiny, or shiny green, elongate ovate, roughly serrate, and alternate with the short petiole (Gruenwald, 2007). The cultivated plant attains a height of 0.6-1.5m whereas the height of wild tea plants goes up to 10 - 15 m. The leaves are usually alternate, lanceolate, and short-stalked; light green and leathery with serrated margin and hairs present beneath. It varies in length from 5 - 30 cm and about 4 cm in breadth. The mature leaves are usually smooth, coriaceous, and bright green, whereas young leaves are pubescent. The flowers of tree plants are white and fragrant having a diameter of 2.5-4cm and are usually found single or clustered in groups of two or four. The flowers produce brownish red capsules (Mahmood *et.al.,* 2005). The flattened fruit has a smooth, rounded, and trigonous three-celled capsule which bears solitary nut size seeds in each (Biswas, 2006).

The economic beverage crop is highly cross-pollinated and heterogeneous. The commercial pressure exerts improvement chances of high-yielding tea plants but it cannot help in broadening of genetic variability since the choices are confined to select elite mother plants within the usual hybrid populaces of tea (Hajra, 2001). The threat of losing numerous significant tea germplasm is expanding due to the quick uprooting of the old plantation. Therefore, it is very important to characterize the existing germplasm so that we can measure the plasticity shown in varying climatic conditions. Over time, the profundity of cross-pollination has led to the establishment of a broad genetic pool. Consecutively to study the genetic variation and maintain the gene pool, the germplasm of Indian tea is kept and maintained in three regions namely the UPASI (southern), TRA (northeastern), and IHBT (western Himalaya). These centers have not only maintained the germplasm but also developed and released biclonal seed stocks and varieties based on selection and selective breeding. Several accessions maintained in the gene banks may have few accessions with some specific and favorable dormant alleles, which might be very important for the improvement of cultivars. Overall, the preservation of tea germplasm is primarily of great importance since previous sectors of seed-grown tea are enormously being uprooted and their seed sources hardly exist, are no longer found, or are lost eventually (Karthigeyan and Sud, 2010).

The successful collection of tea genetic resources, its preservation, and utilization, at present or future breeding programs depend entirely on the knowledge of its genetic background relationship, diversity, and identification (Chen *et al.,* 2005).

Discrimination between archetypal China, Assam, and Cambod varieties is very difficult because of the excessive natural hybridization in tea (Visser, 1969). Widespread selection and cultivation of clonal tea based on yield, biotic and abiotic stress resistance, and quality, among the existing materials, can reduce genetic diversity. Therefore, utmost care should be taken to utilize clones of different origins and understand the genetic diversity at the molecular level (Mondal, 2002). Knowledge of the genetic variation is important for the breeding program since it provides the basis for the development of desirable genotypes (Kaundun *et al.,* 2000).

The practice of recurrent use of the same tea plants or popular cultivars as a parent along with the introduction of vegetative propagation for better productivity has rendered the tea plants limited genetic variation and also prone to diseases, pests, and abiotic stresses (Gunasekare *et al.,* 2012). The importance of germplasm collection and conservation is highlighted in many research articles. Core collection for Chinese tea germplasm based on cultivated region grouping and phenotypic data was established to enhance the utilization of genetic resources in improvement programs and simplify their management. Wang *et al.* (2011) has highlighted the importance of biotechnological approaches to strengthen germplasm activity for using them in a rational manner, assembling a functional core of germplasm to enhance practical applicability of tea genetic resources in tea breeding program and addressing different needs of growers and consumers (Gunasekare *et al.,* 2012).

Further characterization of germplasm based on different morphological characters and division accordingly based on traits has highlighted the importance of the phenetic study to utilize the germplasm to manage the gene bank effectively and efficiently (Piyasundara *et al.,* 2006). Marker-assisted breeding provides great advantages for varietal improvement by increasing the gene pool (Balasaravanan *et al.,* 2003). Precise assessment of the genetic relationships either among closely related species or among the cultivars belonging to different geographic areas is very much essential to give the source of information about desirable genes to the breeders and also establish core collections for improvement of the cultivar. Studying intraspecific relationships between the endemic varieties is likewise essential to the source of favored or specific genes.

Different molecular techniques like Restriction Fragment Length Polymorphism (RFLP), and Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD), provide a useful approach for studying the variability within plant germplasms (Kaundun *et al.,* 2000). The RFLP technique on the other hand requires a huge amount of pure DNA, which is quite time-consuming and robust. Whereas, the RAPD technique overcomes these limitations since this method can generate a large number of polymorphic bands relatively easily with a minimum amount of DNA. We can overcome the technical limitations of RFLP by the RAPD technique but the sensitivity of this technique to experimental conditions raises a question about its reproducibility (Mondal, 2002). The SSR (Simple sequence repeats) method reveals an extreme degree of allelic variation (Schlotterer *et al.,* 1991). Despite this advantage, this technique is laborious due to the requirement of complete sequence information, which is eventually needed for primer designing (Mondal, 2002). The Inter-SSR-PCR (ISSR-PCR) technique or PCR analysis employing anchored simple sequence repeats primers has grabbed the attention as an alternative method to characterize complex genomes. This technique employs SSR based oligonucleotides attached to either the 5’ or 3’ end consisting of 2-4 purine or pyrimidine residues, which aids in the initiation of PCR amplification of DNA segments flanked by microsatellite repeats that are inversely placed, closely spaced (Zietkiewicz *et al.,* 1994). This technique generates a PCR product, which shows multiple polymorphisms when resolved on an agarose gel. This has gained much attention due to its low cost, which avoids costly cloning and sequencing, unlike the microsatellite-based method.

Additionally, DNA barcoding is a very precise method for taxonomic identification. It uses a short standard sequence with abundant variation, which helps to analyze variation among the species. The various regions from the plastid genome have been used for DNA barcoding of land plants (Kress and Erickson, 2007; Singh *et al.,* 2012). However, the Consortium for the Barcode of Life (CboL) recommends rbcL and matK as standard. It is mostly suggested for DNA barcoding of land plants due to its higher variability between the species and its role for the phylogenetic re-establishment (Bafeel *et al.,* 2011; Kuzmina *et al.,* 2012). Information on variations in the chloroplast DNA (cpDNA) is being used widely in lineage and variation studies among the population. Inter and intra specific polymorphism can result from the variation of chloroplast DNA. Due to maternal transmission and lack of recombination, chances of detectable geographical subdivision of populations in a species increase (Katoh *et al.,* 2003). Previous work on tea clones established for Darjeeling and Dooars lacks a report on a study of genetic diversity using barcode primers. On the molecular front, there is very little knowledge regarding the study of genetic diversity employing various elite tea clones of Darjeeling using techniques like RAPD and ISSR since it is very important to record data regarding genetic fidelity. There is also a need to employ a barcode technique to study the variation within the chloroplast regions of elite tea clones.

Tea extracts are popular as ingredients in many dietary supplements and foods. Abundant scientific reports on the positive effect of tea on human health have aroused lots of interest among the researchers regarding chemical composition analysis of tea. Tea has a complex chemical composition, containing over 2000 components (Yashin *et al.,* 2015).The tea plant is the reservoir of phytochemical compounds of pharmaceutical importance. The chemical constituents of tea mainly include the polyphenols like catechins and flavonoids, alkaloids like caffeine, theobromine, theophylline, etc. It also consists of volatile oils, amino acids, polysaccharides, lipids, vitamins (vitamin C, Vitamin E, etc.), and inorganic elements like aluminum, fluorine, manganese, etc.(Sharangi, 2009). The polyphenols comprise mainly of the catechins and flavonoids which have mainly beneficial properties imparting good health. The flavonoids have antioxidant, antiallergic, anti-inflammatory, and anti-microbial properties. The primary catechin compounds mainly present in green tea are catechin, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (EGCG). Out of these, the epigallocatechin gallate (EGCG) is the most active component with enhanced bioactivity. Along with the assessment of tea quality, knowledge of the chemical composition of tea also helps to screen and manage the process of tea growing, manufacturing, and storage conditions. In particular, knowledge about the tea chemical constituents has enabled the establishment of the relationships between the chemical constituents and properties of tea owing to the identification of compounds imparting its aroma and taste. Therefore, assessment of tea quality should also focus on the extra determination of tea components beneficial to human health which will not only enhance the nutritional value of tea but also increase the quality of tea by optimization of the growth, manufacturing, and storage conditions (Yashin *et al.,* 2015).

Owing to the health benefits of polyphenols in tea different epidemiologic observations and laboratory studies have indicated that polyphenolic compounds present in tea may reduce the risk of a variety of illnesses, including cancer, coronary heart disease, atherosclerosis, high blood cholesterol concentrations, high blood pressure where most studies involved green tea limiting black tea evaluation also suggested combining tea polyphenols with other consumer products like food items and vitamin supplements for better utilization. Studies on the animal model have suggested the protective property of tea against certain cancers, cardiovascular diseases, and neurodegenerative diseases (Yang & Koo, 2000; Mandel & Youdim, 2004; Butt *et al.,* 2015).

The extracting solvent usually diffuses into solid plant tissue and solubilizes compounds with similar polarity (Chaitanya *et al.,* 2013). The successful extraction and determination of biologically active compounds from plants are mainly dependent on the type of solvent preferred and extraction techniques used in the pharmaceutical industry (Tiwari *et al.,* 2011), A varied range of research work has been performed on tea with the majority of work being carried on tea especially green tea and its pharmaceutical importance to prevent a various form of diseases like cardiovascular disease, cancer, neurodegenerative diseases, etc. Most of the research work has been focused on made tea or processed tea putting a limitation as such to tea plant (Mukhtar and Ahmad, 2000). Very little work has been done on phytochemical screening of fresh leaves (especially the mature ones) using a range of organic solvents with research being limited to standard solvents and processed tea.

Tea is a repository of phytochemicals and among different types of tea, green tea has gained much importance for its health benefits.

Various lifestyle diseases are caused by people's daily routines and habits (Tabish, 2017). The rising level of competitiveness, along with an unhealthy diet, has become a major source of stress. A variety of foods and beverages are becoming increasingly popular as stress-relieving foods and beverages. None, however, have been able to match the appeal of tea, an age-old useful beverage. Tea especially green tea and black tea is popular for its health benefits and various work has been accomplished to elucidate its role in reducing the risk of various diseases. However, there is still a lacuna and needs tea research to focus on other forms of tea. For instance, purple tea (PT) is unique of its kind due to its high level of anthocyanin coloring, which contributes to its powerful antioxidant capabilities (Sun *et al.,* 2016; Lv *et al.,* 2015). Apart from antioxidant function, anthocyanins have been shown to have cytotoxicity against cancer cells, induce apoptosis, and have an immunostimulatory effect (Joshi *et al.,* 2017). Although some of PT's health advantages have been investigated, its entire pharmacological potential has yet to be discovered. Almost no research has been done on the Indian PT, and most people are unaware of its existence, popularity, and health advantages. Furthermore, it is unknown how PT may operate against certain diseases or control many pathways in our bodies. PT has only a rudimentary medicinal understanding, and its mechanism of action is unknown. A thorough examination of Indian PT's secondary metabolites and pharmacological qualities is required to maximize its health benefits and appeal among customers.

Research trends in nanotechnology have also opened up in the green synthesis of various metal nanoparticle using green tea extracts (Gottimukkala *et al.,* 2017; Nakhjavani *et al.,* 2017; Senthilkumar *et al.,* 2014; Selvan *et al.,* 2018). Black tea has also been employed in the biogenic synthesis of various metal nanoparticles like Au and Ag (Begum *et al.,* 2009), Zinc Oxide (Taghavi *et al.,* 2017), palladium (Lebaschi *et al.,* 2017), iron copper, and silver (Asghar *et al.,* 2018). Green synthesis of metal nanoparticles has gained much attention over the years due to its simple yet large-scale production, less expensive, non-hazardous method, unlike the chemical route which is expensive and hazardous releasing various toxic byproducts (Senthilkumar and Sivakumar, 2014). Tea is enriched with polyphenols which act as both reducing and capping agents during the green synthesis of nanoparticles (Huang *et al.,* 2014). Various types of tea have been employed for the green synthesis but still there lack reports on other less popular or lesser-known types of tea.

Biological research has been improvised along with the tremendous progress of genome sequencing technology. The revolutionary advancement in genome sequencing has not only opened up the floodgates for whole-genome profiling but also has provided enormous scope for exciting investigations regarding the codon usage pattern of several genes and genomes and simultaneously address their evolutionary dynamics (Roy *et al.,* 2015). The patterns of codon usage differ significantly between organisms where genes and genomes exhibit diverse patterns of synonymous codon usage (Guo and Yuan, 2009; Sau and Deb, 2009). The three nucleotide bases building up the triplet codons act as basic coding components that code for a particular amino acid or cause protein chain initiation or termination. Except for Met and Trp, the 20 standard amino acids are coded by two to six synonymous codons which are used at frequencies that vary in different organisms (Feng *et al.,* 2013; Sablok *et al.,* 2011). extensive research has been focused on model plant genomes like *Arabidopsis* (Duret and Mouchiroud, 1999), important genes of monocot and dicot species (Murray *et al.,* 1989), monocot plastomes (Sablok *et al.,* 2011), plastomes of *Coffea arabica* (Nair *et al.,*2012), ESTs of *Citrus* species (Xu *et al.,* 2013), etc. However, detailed investigations relating to codon usage patterns in the whole genome of non-model yet, essential plants remain largely unaccomplished. The complexity of the tea genome made whole genome sequencing and assembly quite challenging but with the advancement of sequencing technology, we can now avail whole genome sequence of the tea plant. This opens up the door to a plethora of research and a new trend of analysis at the Bioinformatics level.

Hence, based on the critical appraisal on tea research as mentioned above, we selected the tea clones established for Darjeeling and Dooars region and laid the following objectives.

Objectives

1. Plant selection and maintenance of

germplasm.

2. Morphological documentation.
3. Molecular documentation

   To study the genetic diversity using different RAPD (Random Amplified Polymorphic DNA), ISSR (Inter simple sequence repeat), and RFLP (Restriction fragment length polymorphism) techniques.

4. DNA Barcode

   Explore the variation within the chloroplast region employing barcode primer, matK.

5. Phytochemical screening

   Qualitative and quantitative phytochemical assessment of different tea extracts prepared using various extracting solvents ranging from polar to nonpolar.

   Chemical Characterization and bioactivity study of selected tea extracts.

6. Green synthesis of the metal nanoparticles employing the lesser-known purple tea extracts.

   Synthesis of Silver nanoparticles

   Synthesis of ZnO nanoparticles

7. Tea Bioinformatics

   Exploring the different determinants governing the codon and amino acid usage of the tea plant (*Camellia sinensis*).

8. Tea polyphenols and human health

   Use of *in silico* polypharmacology to investigate the efficacy of Purple tea and mature tea leaf of clones endemic to the Darjeeling Hills against various lifestyle– diseases.■