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Dedicated to
my beloved parents

Preface

Tea is an important industrial crop that supports the life of several million plantation workers working globally. It is the morning drink of several million people worldwide. Interestingly, several wild species such as *C. japonica* are important due to its elegant flower colour. Because of its perennial nature with a life span of more than 100 years, breeding of tea and its wild species to improve the cultivars is difficult and limited to only few aspects. During the past 2 decades, as a student, teacher and humble science worker, I was, am, and surely will remain fascinated by this beautiful plant whose not only taste but also scenic beauty of plantation always refreshes my mind. While working with this plant, at various tea research institutes in the last 2 decades, I have experienced the present practices, gaps and scope of varietal improvement works and felt the need of *in vitro* culture, molecular breeding, and genomics to supplement the conventional breeding works. With the initiation of cell culture technique in 1968, a significant amount of work on various aspects of breeding and biotechnology of tea and its wild relatives has been done. Although several topical reviews and scientific articles have been published on tea and *Camellia* species, yet they are not codified in a single document.

I am deeply indebted to my teachers who blessed me to learn about this crop and plant biotechnology as a whole. Therefore, I sincerely acknowledge my thanks to my beloved teachers of Assam Agricultural University, Prof. P. S. Ahuja, Director and other Scientists of Institute of Himalayan Bioresource Technology, India, Prof. P. K. Chand of Utkal University, Scientists of UPASI, Tamil Nadu, Tocklai Experimental Station, Assam and Prof. P. C. Deka, Vice Chancellor, Sir Padampat Singhania University, Udaipur. Few people also inspired me to work further on tea breeding and they are Prof. N. K. Jain, Mr P. Haridas, and some of my planter friends of Southern India, Dooars, West Bengal as well as Assam.

I would also like to thank my wife, Dr. Bipasa Sarkar who helped me to improve the manuscript in several ways. Lastly, my son, Vaibhav, my younger sister, Tia and her family, elder brother, Prof. Swapan Kumar Mondal and his family, Kaku and his family are also gratefully acknowledged. I am also thankful to Profs. C. R. Park of USA, A. M. Vieitez of Spain, S. Matsumoto of Japan, Z. Apostolides of South Africa, Z. Chen of China, I. D. Singh of Sri Lanka, S. C. Das, T.R. Sharma, and L. M. S. Palni, India for my personal interactions with them since my student days. I apologize for those works, if any, which did not appear in this book despite a detail search worldwide.

I am also grateful to my PhD students Dr. Pranay, Olivia, Akan, Pratap, Mainaak and Showkat as I was enriched with knowledge while working with them. It is my sincere belief that this book will serve the requirement of students, scientists and industries involved in studies, teaching, research on breeding and biotechnology of tea and other *Camellia* species with an intension of serving science and society.

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Contents

About the Author

Dr. Tapan Kumar Mondal joined at Institute of Himalayan Bioresource Technology (CSIR), Himachal Pradesh, India, for his Ph.D degree which he completed in 1998. After that, he served as Deputy Manager, Research and Development Department, Tata Tatley Ltd, Munnar, Kerala till March, 2002. Since then up to 2010, he further served as Assistant Professor at North Bengal Agricultural University, Cooch Behar, West Bengal. Later in February 2010, he joined as Senior Scientist (Plant Biotechnology) at National Bureau of Plant Genetic Resource (ICAR), New Delhi. He carried out his Post Doctoral training with Prof. J. K. Zhu of University of California, Riverside, USA, on 'Regulation of small RNA under cold stress of Arabidopsis' and later worked at University of Illinois, Urbana-Champaign, USA, on 'Identification of nitrogen use efficient genes of maize by RNAseq'.

Dr. Mondal has significantly contributed in various areas of biotechnology and genetic resource management of tea. His work leads to develop the first transgenic tea plants. He has also submitted several gene sequences of tea at NCBI and also published more than 60 publications in this area. He was PI of various projects of tea biotechnology funded by DBT, DST, ICAR and Tea Board, India.

He is the recipient of University merit scholarship, scholarship from Indian Tea association, ICAR JRF, DBT fellowship, CSIR fellowship and life member of several professional societies. He also bagged 'Young scientist award' by Korean Society of Tea Science in 2003 and Japan Tea Science Society in 2004.

Abbreviations

ABA	Abscisic acid
AS	Acetosyringone
BAC	Bacterial artificial chromosome
ANN	Artificial neural network
6-BAP	6-Benzylaminopurine
cM	Centimorgan
CTAB	Cetyl trimethyl ammonium bromide
cv	Cultivar
°C	Degree celsius
CM	Coconut milk
2,4-D	2,4-Dichlorophenoxy acetic acid
DMSO	Dimethyl sulfoxide
d	Day(s)
g	Gram(s)
g/l	Gram(s) per litre
GA3	Gibberellic acid
GUS	*β*-glucuronidase
gus	*β*-glucuronidase gene
gusint	*β*-glucuronidase gene with an intron
h	Hour(s)
ha	Hectare(s)
hpt	Hygromygcin phophotransferase gene
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kn	Kinetin
KPa	Kilo pascal
kb	Kilo base pair
M	Molar
min	Minute(s)
m	Meter(s)
ml	Millilitre(s)
mM	Micromolar
MS	Murashige and Skoog's (1962) medium
µl	Microlitre
NAA	Napthalene acetic acid
NCBI	National Centre for Biotechnology Information

nM	Nano mole
npt-II	Neomycin phosphotransferase gene
O.D.	Optical density
PCR	Polymerase chain reaction
PVP	Polyvinyl pyrrolidone
pM	Pico mole
%	Percent
QTL	Quantitative trait loci
Q-PCR	Quantitative-PCR
rpm	Revolution per minute
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
sdH_2O	Sterile distilled water
Sec	Second(s)
SE	Somatic embryogenesis
SSR	Suppression subtractive hybridization
SSH	Simple sequence repeat
SNP	Single nucleotide polymorphism
t	Tonnes
Taq Pol.	*Taq* DNA polymerase
TBA	Tertiary butyl alcohol
TBE	Tris borate EDTA
TES	Tocklai experimental station
TDZ	Thidiazuron
TE	Tris-EDTA
UV	Ultra-violet
UPGMA	Unweighted pair group method with arithmetic mean
UPASI	United Planter Association of South-India
v/v	Volume by volume
WPM	Woody plant medium of Lloyd and McCown (1982)
w/v	Weight by volume
mg	Micro gram
ng	Nano gram
YE	Yeast extracts
YMB	Yeast mannitol broth

Introduction

1

1.1 Tea and Camellia: An Overview

Tea (*Camellia sinensis* (L.) O Kuntze) belongs to the family Theacea. It is the oldest non-alcoholic caffeine-containing beverage in the world. Chinese were the first to use tea as a medicinal drink, later as a beverage and have been doing so for the past 3,000 years (Eden 1958). The cultivated taxa comprise of three main natural hybrids. They are: *C. sinensis* (L.) O. Kuntze or China type, *C. assamica* (Masters) or Assam type and *C. assamica* subspecies *lasiocalyx* (Planchon ex Watt.) or Cambod or southern type. Tea is an evergreen, perennial, cross-pollinated plant and grows naturally as tall as 15 m. However, under cultivated condition, the bush height of 60–100 cm is maintained (Fig. 1.1) for harvesting the tender leaves to be processed for making the beverages. The flowers are white in colour and born singly or pairs at the axils. The fruits are green in colour with 2–3 seeds and start bearing within 5–6 years after planting. Leaf is the main criterion by which three types of tea are classified. Briefly, they are: (1) Assam type with biggest leaves, (2) China type with smallest leaves and (3) Cambod, leaves size are in-between of Assam and China type.

Tea thrives well within the latitudinal ranges between 45°N and 34°S that cross about 52 countries. Tea occupies about 2.7 million ha of cultivable land of the world with an annual production of about 2.2 million t. Despite occupying only 16.4% of the total tea growing areas of the world, India ranks first as the producer, consumer and exporter. Hence, tea plays a pivotal role in the national economy of India with an annual turnover of US$ 660 million.

1.2 History

Tea plants are believed to have discovered accidentally by the Chinese legendry Emperor Sheng Nong around 2737 BC. As soon as medicinal value began to be attributed to tea by Chinese, a demand for supplies of tea sprang up which results in cultivation of tea plant in Sichuan province about 3,000 years ago. Subsequently, the knowledge of tea cultivation was spread everywhere by the fine arts of Buddhism. Though, in India, wild tea plant was discovered by C. A. Bruce in Assam during 1823 but seeds were also brought by G. J. Gordon from China in 1836 for establishing a commercial garden in India. Later C. A. Bruce was appointed as the superintendent of tea plantation who took active interest to cultivate the indigenous tea plant. Soon commercial interests moved in and the world's first privately owned Tea Company, the Assam Tea Company, Assam, India was established on 12 February, 1839 with the directives from British Parliament. This was the beginning of the present day Tea Industry of India.

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Fig. 1.1 Tea plantation at Palampur, Himachal Pradesh, India. Plants are growing under shade trees

1.3 Origin and Distribution

Southeast Asia is the original home for tea. According to Wight (1959), the primary centre of origin of tea was considered around the point of intersection of latitude 29°N and longitude 98°E near the source of the river, Irrawaddy, the point of confluence where lands of Assam, North Burma, Southwest China and Tibet met. Secondary centres of origin were considered to be located in Southeast China, Indochina, Mizoram and Meghalaya (Kingdon-Ward 1950). The above areas were, therefore, considered to be the zone of origin and dispersion of the genus *Camellia* as a whole (Sealy 1958). However, presently tea is grown within the latitudinal range of 45°N to 34°S.

Tea was introduced to Japan from China in the early part of the eighth century. From Japan, tea cultivation extended to Indonesia during the seventeenth century. In Sri Lanka, tea was first planted in 1839 when seeds were taken from Calcutta, India. In USSR, tea cultivation started when seeds were imported from China towards the end of last century. Later, from USSR, seeds were exported to Turkey in the year 1939–1940. In Europe, tea was introduced in 1740 by the East India Company's Captain Goff but those plants which were planted in the Royal Botanic Garden at Kew in England, could not survive (Sealy 1958) and the first successful introduction was achieved by a British merchant cum naturalist, John Ellis during 1768 (Aiton 1789; Booth 1830). From there, tea cultivation was extended to the African countries at the end of the nineteenth century.

1.4 Morphological Descriptions

A summary of the morphological characters of the three races of tea plants as described by Wight (1962), Barua (1963) and Bezbaruah (1971) is given below:

1. The China type {*C. sinensis* (L.) Kuntze}: It is a big shrub, 1–2 m tall with many virgate stems arising from the base of the plant near the ground with hard, thick and leathery leaf, matty surface, marginal veins indistinct and appears sunken in lamina. Blade elliptic with obtuse or broadly obtuse apex, base cuneate, margin bluntly serrulate to sinuate-serrulate with more or less incurved teeth, glabrous above and villose below when young, becoming sparsely villose as the leaf ages, ultimately becoming glabrous. Young leaves are garnet-

brown through ox-blood to purple in colour. Petiole is short, 3–7 mm long, stout, usually giving the leaf an erect pose. Flowers are borne singly or in pairs in the cataphyllary axils. Pedicel short, 6–10 mm long, clavate, glabrous with 2–3 subopposite scars little below the middle, marking the position of caducous bractioles 2–5 mm long. Sepals are 5–6 in number, imbricate, persistent, leathery, ovate or orbicular, 3–6 mm long, glabrous green. Petals are 7–8, shallowly cup-shaped, 1.5–2 cm long, broad oval to suborbicular, generally white sometimes with pale pink pigmentation. Stamen numerous, arranged in two whorls, inner ones shorter and fewer in number, outer longer and more numerous, 8–13 mm long, united at the base for a few millimetres with the corolla lobes. Ovary is white and densely hairy; three locular ovules are present 3–5 in each loculous, placentation axial. Style generally three, sometimes up to five, free for the greater part of their length, occasionally free up to the base of the ovary. Stigma is apical. The number of capsules is one, two or three coccate, containing 1–3 nearly spherical seeds with 10–15 mm in diameter. Based on leaf sizes, Sealy (1958) recognized two forms of *C. sinensis* (a) f. macrophylla (sieb.) Kitamura, with wild leaves 4–14 cm long, 2–2.5 cm wide and (b) f. parvifolia (Miq.) Sealy, with leaves 1.5–1.6 cm long and 1–1.2 cm wide.

2. The Assam type {*C. assamica* (Masters)}: It is a small tree, 10–15 m tall with a trunk sometimes up to one-thirds of its height, possesses a robust branch system. In typical plants, leaf is thin, glossy with more or less acuminate apex and distinct marginal veins. Leaf blade is usually broadly elliptic, 8–20 cm long and 3.5–7.5 cm wide, base cuneate, margin obscurely denticulate to bluntly wide serrulate, glabrous or persistently hairy on the midrib below. Flowers are single or in pairs on the cataphyllary axils, pedicels with scars of three caducous bracteoles, smooth and green. Sepals are 5–6 unequal, leathery, persistent. Petals are white 7–8 in number, occasionally with pale yellow pigmentation at the base of the petals. Stamens are numerous as in *C. sinensis*.
3. The southern form or Cambod type {*C. assamica* subspecies. lasiocalyx (Planch. MS)}: It is a small fastigiate tree, 6–10 m tall, with several upright, almost equally developed branches. Leaf is more or less erect, glossy and yellowish-green when young, light green at maturity changing to coppery-yellow or pinkish-red from autumn till the end of the season. Petioles are pinkish-red at the base. Leaf size is intermediate between China and Assam type, broadly elliptic, marginal veins not very prominent. Ovaries are 3–4 in number with five locular. Styles are 3–5 in number, free nearly up to half the length, straight with apical or linear stigma. On the other floral characters, it resembles the Assam plant, with the difference that four or more bracteoles are found on the pedicel of flowers.

1.5 Taxonomy and Nomenclature

The taxonomic position of tea is given below (Fig. 1.2). It is noteworthy to mention that the family comprises 11 genus and the genus *Camellia* has more than 325 species. Out of that, only two are commercially cultivated for producing the tea.

1.6 Economic Importance and Health Benefits

The economic importance of the genus *Camellia* is primarily due to the tea. Though tea is mainly consumed in the form of ‘fermented tea’ or ‘black tea’, yet ‘non-fermented’ or ‘green tea’ and lesser known ‘semi-fermented’ or ‘oolong tea’ is also available. They differ in their method of manufacture, chemical constituent, appearance and organoleptic taste. While black tea is widely used in India and other European countries, green tea is popular in China, Japan and Taiwan. Oolong tea is mainly consumed in some parts of China as well as Taiwan. Worldwide, 80 % black tea, 18 % green tea and 2 % oolong tea are being produced.

Kingdom	Plantae - Plants
Subkingdom	Tracheobionta - Vascular plants
Superdivision	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons
Subclass	Dilleniidae
Order	Theales
Family	Theaceae
Genus	*Camellia* L.
Species	*Camellia sinensis* (L.) O. Kuntze - tea
Types	Assam, China, Cambod
Cultivar	600 recognized worldwide.

Fig. 1.2 Taxonomic position of tea

For black tea, the young tender leaves are completely fermented after withering. The fermentation results in oxidation and polymerization of polyphenols, changing the nature of the chemicals constituents of tea leaves and forming theaflavin and thearubigin. These polyphenols are responsible for the briskness, strength, colour, taste, aroma and pungency associated with black tea. The infusion of black tea has a bright red or copper colour, astringent taste and characteristic aroma. On the other hand, green tea is unfermented and is the least processed among the three types. The plucked leaves are harvested and steamed immediately to inactivate the enzymes to prevent oxidation and polymerization of primary polyphenols which result in retaining of green colour in the finish product. Green tea infusion has a smell of fresh vegetables, low caffeine content. In oolong tea, primary polyphenols are allowed to oxidize partly. Oolong tea is not common and is intermediate in characteristic between green and black tea. Immediately after plucking, the tea leaves are partially fermented for about half the time of black tea. It has the colour of black tea and flavour of green tea.

Tea was used initially as a medicine, later as a beverage and now proven well as future potential of becoming an important industrial and pharmaceutical raw material. Scientific reports in the last two decades have validated many beneficial claims for tea. The majority of the beneficial effects have been attributed to the polyphenolic constituents. Several studies suggest that phenolics may be of importance in reducing the incidence of degenerative diseases such as cancer and arteriosclerosis. The most relevant compounds in dietary regime are cinamic acid derivatives and flavonoids. As natural polyphenols remain unchanged in green tea, it can be said that green tea is more beneficial than black tea. The various health benefits in relation to cancer, arthritis, cardiovascular diseases, diabetes and obesity are described below:

1. Antioxidant activity: Most beneficial effects of tea catechins were attributed to their antioxidant properties that sequester metal ions and scavenge oxygen species and free radicals (Wiseman et al. 1977). Among the different components of catechin, (-)-Epigallocatechin 3-O-gallate (EGCG), was the most potent chemical of the epicatechin derivatives tested for biological activity. It was thought to prevent tumourigenesis by protecting cellular components from oxidative damage through free radical scavenging. Indeed many of the studies had confirmed the free radicals scavenging activity of EGCG as well as its antimutagenic, antiangiogenic, antiproliferating and /or pro-apoptotic effects on mammalian cells both *in vitro* and *in vivo* (Allemain 1999). Tea catechins had been found to be better antioxidants than vitamin C, E, tocopherol and carotene. The polyphenols blocked free radicals damage to lipids (found in cell membranes and serum lipids), nucleic acids and proteins (like those found as cellular enzymes and structural proteins). Damage to these cell components can lead to tumour formation. The oxidative damage by oxygen free radicals of low density lipoproteins (LDL) in serum led to arteriosclerosis and coronary heart diseases. The oxidation of cell membranes and other cell components led to ageing. The antioxidants activity of tea polyphenols was not only due to their ability to scavenge superoxides but also due to increase the activity of some detoxifying enzymes such as glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase and quinine reductase in the small intestine, liver and lungs which are the of the body. However, the antioxidant activity of tea is diminished by the addition of milk to the infusion due to binding of tea polyphenols to milk proteins.