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Tea: Genome and Genetics

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Tapan Kumar Mondal
ICAR—National Institute for Plant Biotechnology
Indian Council of Agricultural Research
PUSA, Delhi, India

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Dedicated to my beloved father Dr. Sankardas Mondal and mother late Smt. Gauri Mondal

Foreword

Several million people of different cultures around the world start the day by drinking tea in the morning. Tea is an important plantation crop that provides morning favorite warm drinks. The tea industry not only generates employment but is an eco-friendly industry that conserves the nature. The increasing world population and reduction of land demand the vertical growth of tea productivity. With all great effort, the yield achieved has been 16,000 kg green leaf/ha but being an agro-based industry it is also vulnerable to climate change. Thus, the need of the hour of this industry is to develop high yielding, quality climate resilient tea clone. Thus altogether there is a need to apply the scientific endeavor to increase the yield at lower cost of production, which can only be possible with the application of modern genomics tools along with conventional breeding.

Botanically tea is mainly of two types, i.e., *Camellia sinensis* or china type with short leaf as well as smaller bush size and *Camellia assamica* or Assam type with bigger leaf as well as larger bush type. A special high-quality tea is also well known as “Darjeeling tea” with GI tag grown in Darjeeling, West Bengal. However though they are different species but they bred so freely that distinct morphological; classes are missing and some people also consider the third category *Camellia assamica* subspp. Lasiocalyx. Due to several botanical constrains such as cross-pollination with longer gestation period and nonavailability of mutants, conventional breeding is slow which is restricted to selection of elite bushes only. Systematic plant breeding approaches to harvest the genetic gain such as transgressive breeding or targeted backcross breeding have not been applied in tea for varietal improvement. On the other hand, among more than 300 species in the genus *Camellia* only these two have been domesticated for drinking purpose though *Camellia taliensis* in a limited scale is also used for producing tea in some countries such as China. So far pre-breeding or using wild species as pollen donor in tea breeding has not been attempted seriously, which has the potential to develop climate resilient, high yielding quality tea clones. However among many, one reason could be the non-availability or use of the genomics resource for improvement of this genus.

Nevertheless, since 2018, two Chinese groups and one Indian group simultaneously decoded the genomes of both the species of tea which have made a quantum jump for generation of data across the world in various fields such as transcriptomics, proteomics, and metabolomics which resulted in detailed insights about the genomic resources such as various DNA markers which have been displayed by various data bases for end users. This will surely have an impact for a better understanding of tea quality trait and marker assistant breeding for QTL identification through association mapping approaches.

I am pleased and happy to say that Dr. Tapan Kumar Mondal, Principal Scientist, ICAR-National Institute for Plant Biotechnology, New Delhi, has made significant contributions in the field of tea biotechnology research including its genome sequence. His own research and contributions in the area of chromosome research, breeding, tissue culture, physiology, and structural/functional genomics are synthesized in this book. I am sure this book will be very useful to researchers and planners not only working in the tea industry but also in similar woody species in addition to having utility among policy makers and science managers. Thus, I feel humbled and extremely happy to write the foreword for this book.

Prof. Swapan Kumar Datta

Distinguished Biotechnological Research
University of Calcutta
Kolkatta, India

Ex Vice Chancellor
Visva-Bharati University
Santiniketan, India

Ex-Deputy Director General (Crop Science)
Indian Council of Agricultural research
Government of India
New Delhi, India
30 August 2020

Preface

Tea is an important industrial crop that supports the life of several millions of plantation workers globally mostly in disadvantageous areas. At the same time, it is also the morning drink of several million people worldwide. Interestingly, several wild species such as *C. japonica* are also important due to its elegant flower color. Due to perennial nature with a life span more than 100 years, breeding to improve the cultivars is difficult and limited to few aspects only. During the last four decades, initially as a student, later as a teacher and humble science worker, I was and surely will remain fascinated by this beautiful plant whose not only test but also scenic beauty of plantation always refresh the mind. While working with this plant, at various tea research institutes in nearly four 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 cytogenetic, breeding, physiology, biochemistry, and functional genomics of tea and its wild relatives has been taken place. Although several topical reviews, scientific articles, and few books have been published on tea and *Camellia* species, I have felt the need to have an update on this area.

In 2014, my first book *Breeding and Biotechnology of Tea and Wild Relatives* was published, which documented all the research findings till that time. After that 3 genomes of tea were decoded by different groups. Thus, while surveying the literatures, two important things I could notice. First, there are several discoveries of new species of Camellia particularly from Vietnam and some parts of Louse and Eastern China, indicating that speciation under this genus is still very active. Second, massive works have been done on functional genomics followed by metabolomics and proteomics of various kinds mainly targeting some useful traits relevant to tea as well as Camellia or to characterize the mutants that are available in the natural germplasm. Gene expression during manufacturing has also been studied. I have tried to capture all these findings in this book. Besides I have also updated the information related to respective chapters of my previous books.

I owe my sincere debt of gratitude towards 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, late Prof. P.S. Ahuja, Ex-Director General, CSIR, Government of India and other Scientists of

the Institute of Himalayan Bioresource Technology, India, Prof. Pradip Kumar Chand, Vice Chancellor, North Orissa University, India, Scientists of UPASI, Tamil Nadu, Tea Board, Kolkata, Tocklai Tea Research Institute, Assam, and Prof. P. C. Deka, Vice Chancellor, Sir Padampat Singhania University, Rajasthan. Few people also inspired me further to work on tea breeding, and they are the late Prof. N. K. Jain, Mr. P. Haridas, Tata Tea Ltd., Munnar, and some of my planter friends of Southern India, Dooars, West Bengal as well as in Assam. I would also like to give my special thanks to Dr. Tilak Raj Sharma, Deputy Director General (Crop Science), Indian Council of Agricultural Research, New Delhi for his guidance as a mentor.

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, i.e., Priya and Shyamlendu, elder brother Prof. Swapan Kumar Mondal and his family, i.e., Simadi and Joy, Kaku, and his family, little niece Stuti are also gratefully acknowledged. I am also thankful to the noted tea scientists, Prof. C.R. Park of USA, Prof. A.M. Vieitez of Spain, Prof. S Yamaguchi of Japan, Prof. Z. Chen of China, Prof. I.D. Singh of India, late Dr. S.C. Das, and Prof. 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 detailed search worldwide.

I am also grateful to my Ph.D. students Dr. Pranay Bantawa, Dr. Olivia Saha Roy, Dr. Akan Das, Dr. Pratap Subba, Dr. Mainaak Mukhopadhyay, Dr. Showkat Ahmed Ganie, Miss Soni Chowrasia, Miss Jyoti Nishad, Miss Megha Rohila, Alok kumar Panda, Mr. Abhishek Mazumder, Mr. Hukum C Rawal as I was enriched 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, and research on breeding and biotechnology of tea and other *Camellia* species with an intension of serving science and society.

New Delhi, India — Tapan Kumar Mondal

About the Book

Tea is an important non-alcoholic beverage plant of the world. The cultivation of tea is very important as it earns revenue for the tea growing nations specially developing countries such as India. To bring down the cost of production, cultivation practices have been standardized over decades of research. Although several improved geographically adopted clones have been developed by conventional breeding which has contributed significantly to industries of various counties, for varietal improvement of tea and other Camellia species with ornamental value. However, genetic gain through systematic breeding with control cross has not been explored. It is slow for several decades due to the constraint of botany of the plant, non-availability of mutant and sufficient wild tea but the main reason was non-availability of genetic and genomic information of tea. But in the past few decades several works related to genetics, genomics, and biotechnology have been done including cytogenetic, traditional breeding, tissue culture, as well as DNA-based markers. With the recent introduction of the genome sequencing of tea, the scope of application of marker-assistant breeding and biotechnology has been increased manyfold. Thus in this book, the state of the art on various aspects of breeding and biotechnology work of tea and other wild *Camellia* species has been compiled in eight chapters. They are (1) Introduction that deals with the origin and descriptions of health benefits as well as morphological classification as first chapter, (2) Genetics and breeding that comprise cytogenetic effects, taxonomy of the genus Camellia, and various conventional breeding techniques of tea along with their genetic resources, (3) Micropropagation that deals with an in-depth study of clonal propagation, (4) Somatic embryogenesis along with alternative techniques such as suspension culture, cryo-preservation, etc., (5) Molecular breeding that deals with the application of various DNA-based markers, QTL discovery, population genetics, linkage map, etc., (6) Genetic transformation and associated factors, (7) Stress physiology that compiles various works done in tea along with its wild relatives on quality, abiotic as well as biotic stress, and (8) Functional genomics that describes the various works of molecular cloning and characterizations, differential gene expression, high-throughput transcriptome sequencing, etc., transcriptomics study that describes the application of next generation sequencing to discover various genes that are related to various traits of tea, and noncoding RNA which describes the discovery of various noncoding RNA in tea and related genera. Sufficient work in the last few years also forced me to decide to have three independent topics. They are metabolomics which deals with different metabolomic study, proteomics which describes the discovery of different proteins, genome sequence which also deals with the work related to three published genome sequences, database management, gene family study, and resequencing of tea genome. Importantly, the author has included exclusive tables in most of the chapters that include a summary of the works in a particular topic. In a nutshell, the book compiles the work that has been done, identifies the problems, analyses the gaps in breeding and biotechnological works of tea as well as its wild species, and discusses the future scope as conclusion. Every effort has been made to include all the published works till June, 2020.

The book will be a useful resource for postgraduate, doctoral as well postdoctoral students working in the field of tea as well as other woody plants. This will also be useful for scientists working in the areas of life sciences, genomics, biotechnology, and molecular biology.

Contents

About the Author

Tapan Kumar Mondal Dr. Tapan Kumar Mondal joined the 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 Tetley 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. From 2016, he is working as Principal Scientist at ICAR-National Institute for Plant Biotechnology, New Delhi. He carried out his postdoctoral training with Prof. J. K. Zhu of the University of California, Riverside, USA, on "Regulation of small RNA under cold stress of Arabidopsis" and later worked at the University of Illinois, Urbana-Champaign, USA, on "Identification of nitrogen use efficient genes of maize by RNAseq."

Dr. Mondal has significantly contributed to various areas of biotechnology and genetic resource management of tea. His work leads several maiden findings in tea such as in vivo somatic embryogenesis, discovery of miRNA, lncRNA, and cirRNA of tea for the first time, and the development of first transgenic tea plants. His team decoded the mitochondrial genome of tea for the first time. Recently his team did the genome sequence of Indian Tea cultivar TV-1. He has also submitted several gene sequences of tea at NCBI and also published more than 60 research papers 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, and CSIR fellowship and is a life member of several professional societies. He has written one book on tea biotechnology and edited one book on wild species of rice. His team has developed several databases on tea and developed software for gene expression study. He also bagged the "Young Scientist Award" by Korean Society of Tea Science in 2003 and Japan Tea Science Society in 2004.

Abbreviations

ABA	Abscisic acid
AS	Acetosyringone
AMT	Ammonium transporters
ANN	Artificial neural network
BAC	Bacterial artificial chromosome
6-BAP	6-Benzylaminopurine
dCAPS	Derived cleaved amplified polymorphism marker
cM	Centimorgan
CTAB	Cetyl trimethyl ammonium bromide
cv	Cultivar
°C	Degree Celsius
4-CL	4-Coumaroyl-CoA
CM	Coconut milk
2,4-D	2,4-Dichlorophenoxy acetic acid
DMSO	Dimethyl sulfoxide
DDRT	Differential display reverse transcriptase
d	Day(s)
EST	Expressed sequence tag
g	Gram(s)
g/L	Gram(s) per liter
GA_3	Gibberellic acid
GC-MS	Gas chromatography mass spectroscopy
gus	β-glucuronidase gene
gusint	β-glucuronidase gene with an intron
h	Hour(s)
ha	Hectare(s)
hpt	Hygromycin phosphotransferase gene
HPLC	High performance liquid chromatography
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kn	Kinetin
KPa	Kilopascal
kb	Kilobase pair

lncRNA	Long noncoding RNA
MAS	Marker-assisted selection
M	Molar
min	Minute(s)
m	Meter(s)
7-NMT	7-N-methyltransferase
mL	Milliliter(s)
miRNA	MicroRNA
mM	Micromolar
MPSS	Massive parallel signature sequencing
MS	Murashige and Skoog's (1962) medium
MYA	Million years ago
μL	Microliter
NAA	Naphthalene acetic acid
NCBI	National Centre for Biotechnology Information
NGS	Next generation sequencing
nM	Nano mole
npt-II	Neomycin phosphotransferase gene
O.D.	Optical density
PA	Proanthocyanidin
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
pM	Pico mole
%	Percent
QTL	Quantitative trait loci
Q-PCR	Quantitative-PCR
rpm	Revolution per minute
RNAseq	RNA sequencing
RACE	Rapid amplification of cDNA end
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
sdH_2O	Sterile distilled water
s	Second(s)
SE	Somatic embryogenesis
SSR	Simple sequence repeat
SSH	Suppression subtractive hybridization
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 Planters' Association of Southern India
v/v	Volume by volume
WPM	Woody plant medium of Lloyd and McCown (1982)
w/v	Weight by volume
mg	Microgram
ng	Nanogram
YE	Yeast extracts
YMB	Yeast mannitol broth

1 Introduction

1.1 Tea and *Camellia*: An Overview

Tea [*Camellia sinensis* (L.) O. Kuntze] belongs to the family Theaceae. It is the oldest non-alcoholic caffeine-containing beverage in the world. Chinese were the first to use tea as medicinal drink and later as beverage and have been doing so for the past 3000 years (Eden 1958). The cultivated taxa comprise 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. However, two species *C. irrawadiensis* and *C. taliensis* have great morphological resemble with cultivated taxa and are used to make tea in some part of China though they have very low content of caffeine and though both these species might have contributed in the cultivated gene pool of tea. 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 color and born singly or in pairs at the axils. The fruits are green in color 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 the biggest leaves, (2) China type with the smallest leaves, and (3) Cambod type with leaf sizes in between those of Assam and China types.

Tea thrives well within the latitudinal ranges between 45°N and 34°S though extended beyond 60°N to nearly reaching 52°S covering about 61 countries. Tea occupies about 3.94 million ha of cultivable land of the world with an annual production of about 4 million t (Basu Majumder et al. 2010). Despite occupying only 16.4% of the total tea-growing areas of the world, India ranks second as the producer, consumer, and exporter. Hence tea plays a pivotal role in the national economy of India with an annual turnover of 860 million US dollars.

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Fig. 1.1 Tea plantation of India. (**a**) Palampur, Himachal Pradesh, India, where tea plant is grown in hilly terrain; (**b**) Valparai, Tamil Nadu, South India, where tea plant is grown mainly in hilly terrain; (**c**) Assam, the "tea bowl" of India. Plants are grown under shade trees and grown mostly in plain area

1.2 History

Tea plants are believed to have been discovered accidentally by the Chinese legendary Emperor Shennong 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 the cultivation of tea plant in Sichuan province about 3000 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, 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 the British Parliament. This was the beginning of the present-day tea industry of India.

1.3 Origin and Distribution

Southeast Asia is the original home for tea. According to Wight (1959), the primary center 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 centers of origin were considered to be located in the 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).

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 [*Camellia sinensis* (L) O. 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, and marginal veins that are indistinct and appear sunken in the lamina. Blade is elliptic with obtuse or broadly obtuse apex, base is cuneate, and margin is bluntly serrulate to sinuate-serrulate with more or less incurved teeth. The leaf is 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 color. Petiole is short, 3–7 mm long, and stout, usually giving the leaf an erect pose. Flowers are borne singly or in pairs in the cataphyllary axils. Pedicel is short, 6–10 mm long, clavate, and glabrous with 2–3 sub-opposite 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, and glabrous green. Petals are 7–8, shallowly cup-shaped, 1.5–2 cm long, broad oval to sub-orbicular, and generally white sometimes with pale pink pigmentation. Stamen is numerous; arranged in two whorls, inner ones shorter and fewer in number and outer longer and more numerous; 8–13 mm long; and united at the

base for a few mm with the corolla lobes. Ovary is white and densely hairy, three locular ovules are present 3–5 in each loculus, and placentation is axial. Style is generally 3, sometimes up to 5, free for the greater part of their length, occasionally free up to the base of the ovary. Stigma is apical. The number of capsules varied from 1, 2, or 3, 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 and 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 third of its height, possessing a robust branching system. In typical plants, leaf is thin and glossy with more or less acuminate apex with distinct marginal veins. Leaf blade is usually broad elliptic and 8–20 cm long and 3.5–7.5 cm wide, base cuniate, and 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 and pedicels with scars of three caducous bracteoles, smooth and green. Sepals are 5–6 unequal, leathery, and 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 Sub species*. 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 and 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 and broadly elliptic; marginal veins are not very prominent. Ovary is 3–4 in number with 5-locular. Styles are 3–5 in number, free nearly up to half the length, and 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; other two such as *C. irrawadiensis* and *C. taliensis* are occasionally used in China for making 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,” “non-fermented” or “green tea” and lesser known “semi-fermented” or “oolong tea” are

Fig. 1.2 Taxonomic position of tea

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.

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 traditionally consumed in China, Japan, and Taiwan, but its consumption is increasing gradually across the world due to health benefit properties. 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.

For black tea, the young tender leaves are completely fermented after withering. The fermentation results oxidation and polymerization of polyphenols, changing the nature of the chemical constituents of tea leaves and forming theaflavin and thearubigin. These polyphenols are responsible for the briskness, strength, color, taste, aroma, and pungency associated with black tea. The infusion of black tea has a bright red or copper color, 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 color in the finish product. Green tea infusion has a smell of fresh vegetables and low caffeine content. In oolong tea, primary polyphenols are allowed to partly oxidize. 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 color of black tea and flavor of green tea.

Tea was used initially as medicine and later as beverage and has now been 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 cinnamic 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 tumorigenesis by protecting cellular components from oxidative damage through free radical scavenging. Indeed many of the studies had confirmed the free radical 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 vitamins C and E and carotene. The polyphenols block free radical 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 tumor 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 aging. The antioxidant activity of tea polyphenols was due to their ability not only to scavenge superoxides but also 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. 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.
2. Cardiovascular activity: Tea polyphenols and flavonoids had been reported to inhibit either enzymatic or non-enzymatic lipid peroxidation, an oxidative process implicated in several pathological conditions including atherosclerosis. Specifically, it had been suggested that tea polyphenols lower the oxidation of LDL cholesterol, with a consequent decreased risk of heart diseases. It had been observed that green tea polyphenols significantly reduced the levels of serum LDL, very-low-density lipoproteins (VLDL), and triglycerides. At the same time, they increased the levels of high-density lipoproteins (HDL). This observation had been strengthen by the finding that in hypercholesterolemic rats, green tea polyphenols lowered blood cholesterol levels and reduced blood pressure in spontaneously hypertensive animals. Tea polyphenols also inhibited the absorption of dietary fats and cholesterols (Chen et al. 2000).
3. Anticancer activity: It is the most thoroughly studied function of tea polyphenols. It can protect the cells against cancer at several stages of carcinogenesis including cancer prevention, endogenous carcinogenic activation, DNA damage and destabilization, cell proliferation, neoplastic growth, and metastasis. Tea especially green tea reduced the incidence of cancers of the stomach, small intestine, pancreas, lung, breast, skin, urinary bladder, prostrate, esophagus, and mouth (Vasisht et al. 2003). Also it had been shown to reduce tumor size and growth in cancer-bearing animals. Green tea polyphenols directly inhibited the cytochrome P-450 enzyme systems (phase I enzyme) that played a pivotal role in carcinogenic activation. Concurrently, they boosted the activity

of phase II enzyme (e.g., glutathione transferase) that made xenobiotics hydrophilic for clearance of the body. The process was crucial for carcinogenic detoxification. Recently, it was proposed to associate the anticancer activity of EGCG with the inhibition of urokinase, one of the most frequently expressed enzymes in human cancers. Green tea showed a protective effect against damage produced by UV radiation and reactive oxygen species to the dermis through apostasies and inhibiting lipid peroxidation.

4. Antidiabetic effect: Tea drinking was shown to possess antidiabetic activity and was effective in the prevention and treatment of diabetes. Tea polyphenols lowered the serum glucose by inhibiting the activity of the starch-digesting enzyme, amylase. Polyphenol inhibits both salivary and intestinal amylase. As a result, the starch was broken down more slowly, and the sudden rise in serum glucose was minimized. In addition, tea polyphenols also reduced the intestinal absorption of glucose (Gomes et al. 1995).
5. Antiarthritic activity: Tea polyphenol demonstrated an exceptional protection against arthritis. The major polyphenols showing antiarthritic effect include epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate. It has been postulated that the antioxidant property of polyphenol might be useful in the prevention and severity of arthritis (Tapiero et al. 2002).
6. Antiplaque activity: Tea polyphenols acted in two different ways to inhibit the growth and adherence of oral bacteria. Firstly, it inhibited the growth of periodontal disease-producing bacterium, *Porphyromonas gingivalis*, and decay-causing bacteria such as *Streptococcus mutans*. Therefore, green tea as mouth rinse resulted in less plaque and periodontal diseases. Secondly, it inhibited the enzyme amylase present in the saliva, and the starch in the mouth did not get converted into glucose and maltose. Less nutrition was thus available to decay-causing bacteria (Yu et al. 1995).
7. Antiviral activity: Tea extract had been shown to have virucidal activity against polio, influenza, and herpes simplex virus (Okubo and Juneja 1997).
8. Anti-AIDS activity: Green tea polyphenols are antimutagenic and act as effective adjuvant to drug therapy. It had been discovered that polyphenols from green tea and their oxidation products could inhibit the reverse transcriptase or polymerase of several types of viruses, including HIV-1 and herpes simplex-1 (Hashimoto et al. 1996). However, research in this area is still in its initial stages.
9. Anorectic effect: The tea polyphenols inhibited catechol-O-methyl transferase, and caffeine inhibited transcellular phosphodiesterase, thus stimulated thermogenesis, and helped to manage obesity. The release of glucose was slowed down by tea, and thus harmful spiking of insulin was prevented (Kwanashie et al. 1989).
10. Antimicrobial activity: The crude catechins and theaflavins had been found to have an antibacterial activity. They were believed to damage bacterial cell membranes. Tea had been used in the treatment of diarrheal infections and cholera. Polyphenols killed the spores of *Clostridium botulinum* and thus displayed antibacterial activity against foodborne diseases and were also effective against heat-resistant bacilli like *Bacillus subtilis*, *Bacillus cereus*, and *Vibrio parahaemolyticus*. Green tea also had protozoacidal properties (Hamilton-Miller 1995).

11. Other biological effects: It had also been reported that green tea polyphenols exhibited neuromuscular, antiangiogenic, antihepatotoxic, antiproliferative/apoptotic, and immunomodulatory effects (Sueoka et al. 2001).

1.7 Landmarks of Biotechnological Works

An overview of various landmark works of tea is depicted in Fig. 1.3. Caffeine (1,3,7-trimethylxanthine) is the main alkaloid for which tea is valued. It was purified from tea leaves of field-grown plants during 1821 (Spedding and Wilson 1964). However with the advancement of cell culture techniques, attentions were paid to

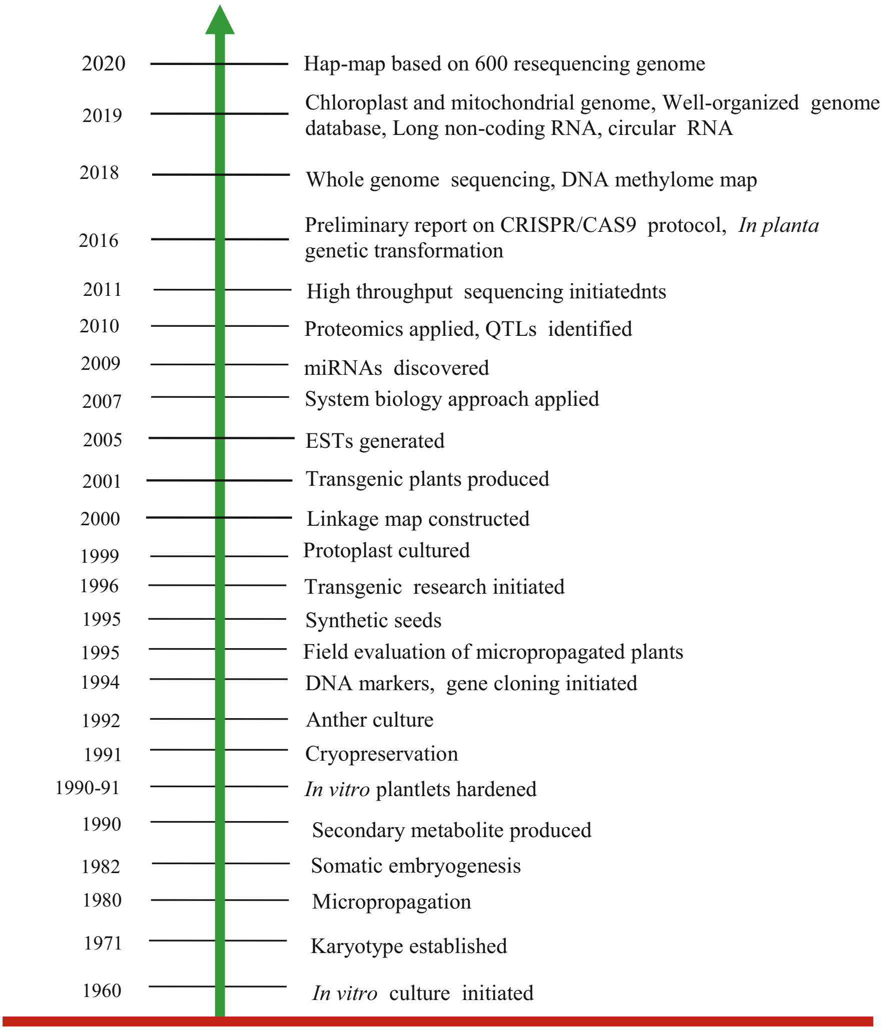

Fig. 1.3 Time-frame flow of landmark discoveries of tea

produce in higher quantity caffeine from the in vitro callus tissue (Ogutuga and Northcote 1970). Simultaneously, cytogenetic works were also initiated. Accordingly chromosome number had been established for the most available taxa of *Camellia* including tea (Bezbaruah 1971) at Tea Experimental Station, Jorhat, India, and elsewhere. However, it was evident from the literature that while Forrest (1969) was pioneer to establish the in vitro culture of tea, Kato (1985) did a systematic study on micropropagation. Since then a significant amount of work has been done in tea and its wild relatives on various aspect of in vitro culture (Fig. 1.4). In tea, somatic embryogenesis had been fully exploited for clonal

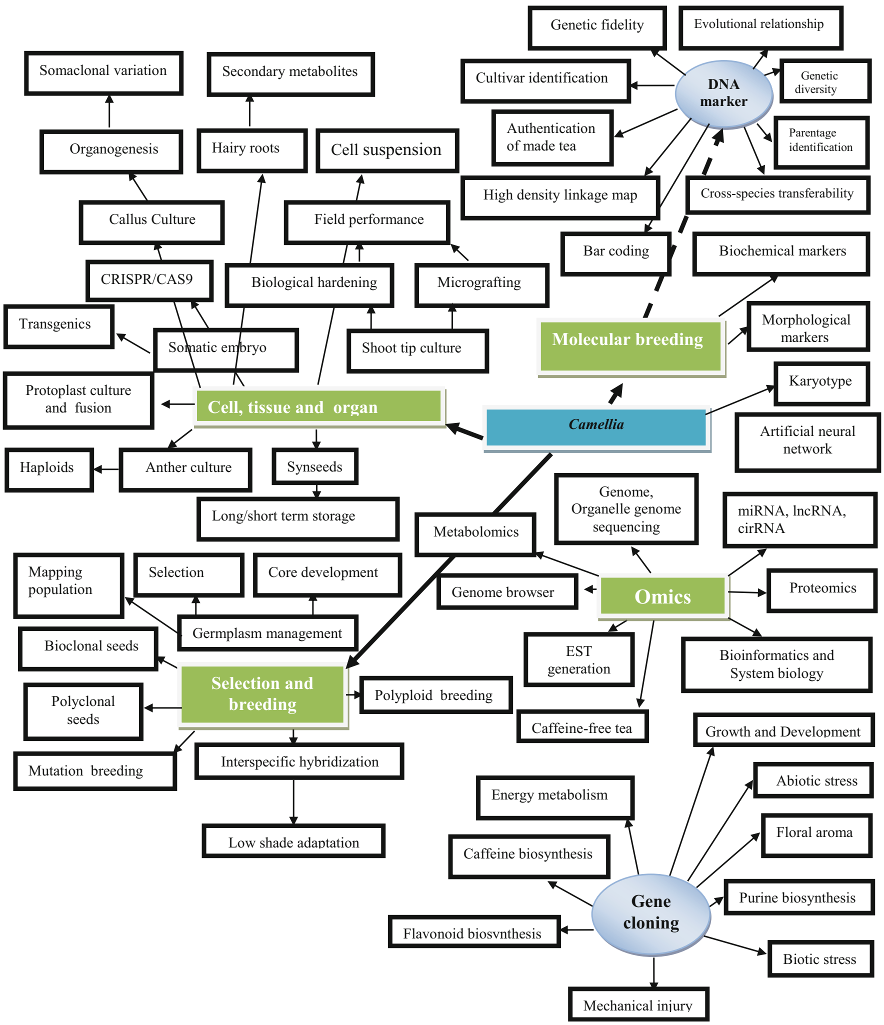

Fig. 1.4 Schematic explanation of *Camellia* improvement. The bold arrows are the major areas of research. Dotted arrows are the sub-areas. Thin arrows are the different applications with a major or sub-major area