Genetics and Breeding

2

2.1 Introduction

Although applications of breeding in tea are difficult, entire varietal development in tea and other *Camellia* species have been done through conventional breeding which started way back in 1939. Since then, several developments of genetics and breeding have taken place, which are discussed in this chapter.

2.2 Genome Size

The genome size of a tea plant was initially estimated to be 4.0 G bases (Hanson et al. 2001; Tanaka et al. 2005); however, intraspecific and interspecific variations of 2C DNA content were also observed in the genus *Camellia*. It had been found that while intrapopulation variations of 2C DNA content of tea were 5.87 to 6.4 pg of DNA, the interpopulation variations of 2C DNA (pg) content of different species varied from 2.5 to 25 (Huang et al. 2013). The higher DNA content was due to higher levels of ploidy. For example, *C. oleifera* and *C. sasanqua* had 2C DNA (pg) 17.47 and 18.79 as they were found to be octaploids (Huang et al. 2013)**.** Tea chromosomes were found to be small and had a tendency to clump together due to 'stickiness'. Tea is diploid ($2n=30$; basic chromosome number, $x=15$) and karyotype ranges from 1.28 to 3.44 μm (Bezbaruah 1971). The *r* value (ratio of long arm to short arm) for all the 15 pairs of chromosomes ranged from 1.00 to 1.91. This consistency in diploid chromosome number suggested a monophyletic origin for all *Camellia* species. However, a few higher ploidy levels, such as triploids ($2n=45$), tetraploids ($2n=60$), pentaploids ($2n=75$), hexapolids ($2n=90$), octaploids ($2n=120$) and aneuploids ($2n\pm1$ to 29) had also been identified (Singh 1980; Huang et al. 2013).

2.3 Diversity of the Genus

The genus of *Camellia* had 40 species in 1920. The number of species was increased to 87 in 1958 (Sealy 1958), and more than 267 species were registered in 1982 (Chang and Bartholomew 1984). Presently, this genus is believed to comprise more than 300 species Mondal et al. 2004 with the latest discovery of *C. cherryana* in 2012 (Orel and Wilson 2012), that indicated genetically instable and high outbreeding nature of the genus. By a conservative estimation, there are more than 30,000 cultivated varieties of ornamental Camellia worldwide. The *Camellia* is the largest genus of the family 'Theaceae'. The genus is valued for tea due to the presence of caffeine, a purine alkaloid, which acts as a stimulus for the central nervous system of human beings. Nagata and Sakai (1984) reported the distribution of caffeine in 23 species of *Camellia*. The caffeine content on a dry weight basis in some of them was as follows: *C. sinensis* var. *sinensis* (3.5 %), *C. sinensis* var. assamica (4 %), *C. taliensis* (2.54 %), and *C. kissi* (0.02 %).

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Among these, *C. kissi* belongs to the section *Paracamellia* and the other genera belong to the section *Thea*. The other three genera in the family are *Eurya* with 140 species, *Ternstroemia* with 130 species and *Adinandra* with 100 species. Apart from having caffeine in the three species, around 50 species of the genus *Camellia* were known to produce oil for industrial uses (Mondal 2011).

The classification of genus had been revisited many times by several workers (Chen et al. 2000), but Chang and Bartholomew (1984) remained the most popular who divided the whole *Camellia* genus into 4 subgenera and 20 sections in total, which are depicted below with the example of some prominent species in each section.

I Subgenus*	Protocamellia
Section I	Archecamellia
	C. granthamiana
	C. albogigas
	C. pleurocarpa
Section II	Stereocarpus
	C. krempfii
	C. dormoyana
	C. yunnanensis
	C. liberistyla
	C. liberistyloides
Section III	Piquetia
	C. piquetiana
II Subgenus	Camellia
Section IV	Olifera
	C. gauchowensis
	C. sasanqua
	C. vietnamensis
	C. oleifera
Section V	Furfuracea
	C. integerrima
	C. polypetala
	C. latipetiolata
	C. crapnelliana
	C. furfuracea
	C. oblata
	C. gaudichaudii
	C. parafurfuracea
Section VI	Paracamellia
	C. grijsii
	C. confusa
	C. kissii
	C. lutescens
	C. fluviatilis
	C. brevistyla
	C. obtusifolia
	C. maliflora
	C. miyagii
	C. shensiensis
	C. brevissima
	C. puniceiflora
	C. tenii
	C. microphylla
	C. phaeoclada
	C. weiningensis
Section VII	Pseudocamellia
	C. szechuanensis
	C. chungkingensis
	C. trichocarpa
	C. ilicifolia
	C. henryana
Section VIII	Tuberculata
	C. tuberculata
	C. anlungensis
	C. obovatifolia
	C. rhytidocarpa
	C. litchii
	C. parvimuricata
Section IX	Luteoflora
	C. luteoflora
Section X	Camellia
	C. omeiensis
	C. polyodonta
	C. lapidea
	C. mairei
	C. villosa
	C. kweichowensis
	C. albovillosa
	C. albescens
	C. tunganica
	C. trichosperma
	C. phellocapsa
	C. semiserrata
	C. multiperulata
	C. lungshenensis
	C. reticulata
	C. pitardii
	C. hiemalis
	C. uraku
	C. edithae
	C. xylocarpa

I Subgenus*	Protocamellia
	C. hongkongensis
	C. cryptoneura
	C. oviformis
	C. compressa
	C. setiperulata
	C. saluenensis
	C. boreali-yunnanica
	C. lucidissima
	C. magnocarpa
	C. japonica
	C. subintegra
	C. longicaudata
III Subgenus	Thea
Section XI	Corallina
	C. coralline
	C. tonkinensis
	C. wardii
	C. pilosperma
	C. fleuryi
	C. nitidissimia
	C. paucipunctata
	C. lienshanensis
	C. pentamera
	C. scariosisepala
	C. acutiserrata
Section XII	Brachyandra
	C. muricatula
	C. szemaoensis
	C. pachyandra
	C. xanthochroma
	C. amplexifolia
	C. brachyandra
	C. nervosa
	C. nematodea
	C. gilbertii
	C. crassipetala
	C. yangkiangensis
	C. parviflora
Section XIII	Longipedicellata
	C. amplexicaulis
	C. petelotii
	C. longipedicellata
	C. indochinensis
Section XIV	Chrysantha
	C. flava
	C. aurea
	C. chrysantha
	C. flavida
	C. impressinervis
	C. euphlebia
	C. chrysanthoides
	C. tunghinensis
	C. pingguoensis
	C. pubipetala
Section XV	Calpandria
	C. lanceolata
	C. connata
Section XVI	Thea
	C. quinquelocularis
	C. tachangensis
	C. crassicolumna
	C. pentastyla
	C. taliensis
	C. irrawadiensis
	C. crispula
	C. gymogyna
	C. costata
	C. yunkiangensis
	C. leptophylla
	C. pubicosta
	C. angustifolia
	C. sinensis
	C. assamica
	C. pubilimba
	C. waldenae
	C. fangchensis
	C. ptilophylla
	C. parvisepala
Section XVII	Longissima
	C. longissima
	C. gracilipes
Section XVIII	Glaberrima
	C. glaberrima
	C. kwangtungensis
IV Subgenus	Metacamellia
Section XIX	Theopsis
	C. macrosepala
	C. cuspidata
	C. grandiflora
	C. chekiangensis
	C. longicuspis
	C. crassipes
	C. longicalyx
	C. forrestii
	C. acutisepala
	C. buxifolia
	C. minutiflora
	C. parvicuspidata
	C. acutissima
	C. subacutissima

I Subgenus*	Protocamellia
	C. callidonta
	C. handelii
	C. triantha
	C. costei
	C. tsaii
	C. synaptica
	C. transnokoensis
	C. rosthorniana
	C. lutchuensis
	C. euryoides
	C. trichoclada
	C. parvilimba
	C. brevipes
	C. elongata
	C. longicarpa
	C. parvilapidea
	C. stuartiana
	C. transarisanensis
	C. fraterna
	C. dubia
	C. percuspidata
	C. membranacea
	C. rosaeflora
	C. campanisepala
	C. lancilimba
	C. tsingpienensis
	C. pubisepala
	C. parviovata
	C. viridicalyx
	C. lancicalyx
	C. parvicaudata
	C. subglabra
	C. nokoensis
	C. tsofuii
	C. trichandra
Section XX	Eriandra
	C. villicarpa
	C. cratera
	C. punctata
	C. lawaii
	C. trigonocarpa
	C. cordifolia
	C. wenshanensis
	C. melliana
	C. candida
	C. caudata
	C. assimiloides
	C. assimilis
	C. edentata
	C. salicifolia

* adopted with modification from Mondal 2011

Owing to extensive internal hybridization between different *Camellia* taxa, several intergrades, introgressants and putative hybrids had been found. They were arranged in a gradient based on morphological characters that extended from China types through intermediates to those of Assam types. Indeed, because of the extreme homogenization, existence of the pure archetypes of tea was doubtful (Visser 1969). Numerous hybrids, therefore, were referred to as China, Assam or Cambod tea depending on the morphological proximity to the main taxon (Banerjee 1992a). Tea breeds well with some of the wild relatives, and thus taxonomists had always been interested to identify such hybrids due to suspected involvements in tea genetic pool. Two particularly interesting taxa were *C. irrawadiensis* and *C. taliensis* whose morphological distributions overlapped with that of tea (Banerjee 1992a). It had also been postulated that some desirable traits such as anthocyanin pigmentation or special quality characters of Darjeeling tea might have been introduced from those wild species (Wood and Barua 1958).

Other *Camellia* species, which were suspected to have contributed to the tea genetic pool by hybridization, include *C. flava* (Pifard) Sealy, *C. petelotii* (Merrill) Sealy (Wight 1962) and possibly *C. lutescens* Dyer (Sharma and Venkataramani 1974). The role of *C. taliensis* was, however, not clear because the species itself was considered to be a hybrid between *C. sinensis* and *C. irrawadiensis* (Wood and Barua 1958; Visser 1969). Therefore, it was generally agreed that at least three taxa, i.e. *C. assamica, C. sinensis, C. assamica* sub sp. *lasiocalyx* and to an extent *C. irrawadiensis* had mainly contributed to the genome of tea. The term 'tea' should therefore cover progenies of these taxa and the hybrids thereof or between them.

Apart from this natural diversity, the different tea research institutes and dedicated planters had further developed a number of varieties with better yield, quality and traits such as tolerance to drought, diseases, etc. In my estimation, more than 1,200 such commercial cultivars of tea have been developed and released for cultivation worldwide, and many of them have special characters (Table 2.1).

Table 2.1 Different tea cultivars with special characters. (Adopted from Mondal 2009)

Special characters	Clone	Originator	Reference
Wind tolerance	UPASI-2, UPASI-10	UPASI-TRF, India	Sharma and Satyanarayana (1987)
Drought resistance	UPASI-9	UPASI-TRF, India	Sharma and Satyanarayana (1987)
Frost resistance/tolerance	BS 53	HPKV-TES, India	Deka et al. (2006)
Small leaf	CH-1, Vimtal	IHBT and TES, Assam, India	Mondal et al. (2004)
Biggest leaf	Betjan	Betjan T.E, India	Singh (1980)
Blister blight tolerance	TRI-2043, DT-1	TRI, Sri Lanka	Sivapalan et al. (1995)
High pubescence content	TRI/2043	TRI, Sri Lanka	Sivapalan et al. (1995)
High anthocyanin pigmentation	TRI/2025	TRI, Sri Lanka	Sivapalan et al. (1995)
High anthocyanin pigmentation	Cha Chuukanbohon Nou 6	NIVOT, Japan	Nesumi et al. (2012)
High tolerance to pH	TN-14–3	TRF, Kenya	Anon (1999)
Poor fermenter	12/2	TRF, Kenya	Anon (1999)
Mite tolerance	7/9	TRF, Kenya	Anon (1999)
Scale insect tolerant	TN 14–3	TRF, Kenya	Anon (1999)
High polyphenol content (53.7%)	Luxi white tea	TRI, China	Yu and Xu (1999)
High amino acid content (6.5%)	Anji white tea	TRI, China	Yu and Xu (1999)
Low caffeine content (0.14%)	Guangdong tea	TRI, China	Yu and Xu (1999)
High caffeine content (6.96%)	Wild tea at Yunnan	TRI, China	Yu and Xu (1999)
Water logged tolerant	TV-9	TES, Assam, India	Singh (1980)
High somatic embryogenesis	Makura-Ck2	NIVOT, Japan	Furukawa and Tanaka (2004)
Early germination	Tianfu 28	CAS, China	Wang et al. (2003)
Very early budding	Zhingcha 108	CAS, China	Yang et al. (2003)
Loop hopper resistance	EF	CAS, China	Hu et al. (2003)
Anthracnose	Abo	NIVOT, Japan	Yoshida and Takeda (2006)
Gray blight resistant	P11P11P12P12	NIVOT, Japan	Takeda (2002)
Trichomeless mutant	Progeny of Surugawase	NIVOT, Japan	Takyu et al. (2003)

UPASI United Planters Association of Southern India, *HPKV* Himachal Pradesh Krishi Viswavidyalaya, India, *TRI* Tea Research Institute, Sri Lanka, *NIVOT* National Institute of Vegetable, Ornamental Crops and Tea Science, Japan, *TRF* Tea Research Foundation, Kenya, *CAS* Chinese Academy of Sciences, China

2.4 Karyotype

Karyotype was considered to be the most important cytological marker for identification of the species. Karyotypes had been established for the most available taxa of *Camellia* including tea (Kondo 1975; Fukusima et al. 1966; Ackerman 1971; Datta and Agarwal 1992). However, karyotype grouping based on chromosome size was not successful in the *Camellia* taxa due to high stickiness of the chromosomes. Furthermore, even in the best preparation, homologous chromosome pairs could not appear identical in *Camellia* (Kondo 1975). Relatively little intraspecific karyotypic variation had been observed for the cultivated species of *Camellia* studied (Kondo 1975). Sat-chromosomes in karyotypes within mass accessions of certain *Camellia* species were morphologically and quantitatively variable. Thus, karyotypes including charac-

teristics of sat-chromosomes were not of taxonomic significance for *Camellia* taxa. Among the diploid species of *Camellia* studied, *C. japonica* L. sensu lato showed the greatest karyotypic variation; many of the studied accessions indicated similar karyotypic patterns to each other (Kondo 1975). Actually, *C. japonica* L. var. macrocarpa Masamune had satellites on four submetacentric chromosomes and the other accessions had satellites on two submetacentric chromosomes (Kondo and Parks 1980). Later, it was shown by Kondo and Parks (1979) that the C-banding method could be applied to the somatic mid-metaphase chromosomes in *Camellia* taxa. These differentially stained bands in mid-metaphase chromosomes permitted the identification of 238 individual chromosomes and made it possible to match the homologous pairs of chromosomes more precisely. Karyotypic variability and divergence among the seven accessions of *C. japonica* L. sensu lato with aceto-orcein staining were revealed by C-banding method (Kondo and Parks 1980). In this way, the cytological marker was used to sort and classify the genotypes.

The karyotype characteristics of some *Camellia* species are listed in Table 2.2. The cardinal chromosome number of the 29 species was found to be stable, $2n=30$. No polyploidy were found, excluding cultivated *C. sinensis* and *C. assamica.* It indicated that the evolution of karyotype of section Thea was mainly through the gene, similar to other tropical woody plants and different from other species of genus *Camellia* in the temperate zone. The karyotype of most species in *Thea* was M (metacentric) and SM (submetacentric) chromosomes, only few species with ST (subtelocentric) chromosomes with the order of the number was M > SM > ST.

Interestingly, *C. reticulata* had a series of polyploid varying from $2n=2x=30$, $2n=4x=60$ to $2n=6x=90$, with a basic chromosome number of $x=15$. The hypothetic allopolyploid origin and parental genomes of these polyploidy types remained unknown. Genomic in situ hybridization (GISH) was used to study the genome organization and evolution of *C. reticulata*. Total genomic DNA from closely-related diploid species (*C. pitardii* and *C. saluenensis*), with the chromosome number $2n=2x=30$, were labelled and hybridized in the presence of blocking DNA onto metaphase spreads of *C. reticulata*. The *C. pitartii* probe painted part of the tetraploid and hexaploid *C. reticulata* genomes, whereas the *C. saluenensis* probe delineated part of the hexaploid *C. reticulata* genome. The results provided compelling evidence for the allopolyploid origin of *C. reticulata* genomes and demonstrated that (1) the diploid *C. reticulata, C. pitardii* and *C. saluenensis* were the progenitors of polyploid *C. reticulata*, (2) hybridization between diploid *C. reticulata* and diploid *C. pitardii* gave birth to allotetraploid *C. reticulata* and (3) subsequent hybridization between allotetraploid *C. reticulata* and diploid *C. saluenensis* formed the allohexaploid *C. reticulata* (Gu and Xiao 2003).

2.5 Propagation

Tea and its wild species are mainly propagated by three means, i.e. seeds, vegetative cuttings and nursery graftings, although budding or grafting of mature plants are also followed but very rarely.

Seeds: Conventionally tea is propagated through seeds. Seeds are generally produced in 'seed bari' (seed orchard). A fully matured healthy seed while attached to the plants or recently dehisced are collected from the ground of the seed orchards. This is primarily due to the fact that tea seeds being recalcitrant have low viability.

After eliminating the very small seeds, the remaining seeds are transferred to a tank or trough filled with water and allowed to soak for 2–3 h. The sinker seeds are taken out of water and examined for mechanical, insect and pest damage. The usual practice is to cut open a sample of 50 to 100 seeds from the batch to examine starred, cheesy, shrunken seeds or otherwise damaged seeds by pests or diseases. Floater seeds are discarded as such seeds are found to have dried cotyledons, which normally fail to germinate. Floaters are frequently the results of punctures made by Tea Seed Bug (*Poecilocoris latus*). As soon as

Table 2.2 The karyotype of some *Camellia* species. (Liang et al. 1994; Chen et al. 2000)

Species	Karyotype
C. assamica	2n = 30 = 18m + 12sm
C. sinensis	2n = 30 = 20m + 8sm + 2st
C. grandibracteata	2n = 30 = 24m + 6sm
C. kwangnaica	2n = 30 = 22m + 8sm
C. quinquelocularis	2n = 30 = 24m + 6sm
C. tachangensis	2n = 30 = 22m + 8sm
C. gymnogynae	2n = 30 = 22m + 6sm + 2st/20m + 8sm + 2st
C. ygmnogynoides	2n + 30 = 22m + 6sm + 2st
C. jungkiangensis	2n = 30 = 20m + 8sm + 2st
C. tetracocca	2n = 30 = 22m + 8sm
C. nanchuanica	2n = 30 = 20m + 8sm + 2st/24m + 6sm
C. crassicolumma	2n = 30 = 22m + 8sm/2n = 30 = 18m + 9sm + 3st
C. atrothea	2n = 30 = 20m + 6sm + 4st
C. taliensis	2n = 30 = 22m + 8sm
C. taliensis var. bangweicha	2n = 30 = 22m + 6sm + 2st
C. irrawadiensis	2n = 30 = 18m + 12sm/22m + 8sm
C. rotundata	2n = 30 = 20m + 10sm
C. makuanaica	2n = 30 = 22m + 8sm/20m + 10sm
C. manglaensis	2n = 30 = 22m + 8sm + 4st
C. leptophylla	2n = 30 = 24m + 4sm + 2st
C. dehungensis	2n = 30 = 20m + 10sm + 2st
C. gymnigyna	2n = 30 = 20m + 8sm + 2st
C. costata	2n = 30 = 20m + 8sm + 2st
C. parvisepaloides	2n = 30 = 22m + 8sm
C. gymnagynoides	2n = 30 = 22m + 6m + 2/22m + 6 + 2/20m + 8 + 2st
C. purpurea	2n = 30 = 22m + 4sm + 4st
C. polyneura	2n = 30 = 22m + 4sm + 4st
C. sinensis	2n = 30 = 18m + 12sm + 2st
C.. sinensis var. pubilimba	2n = 30 = 20m + 10sm/18m + 10 + 2st
C. sinensis var. kucha	2n = 30 = 22m + 8sm + 2st
C. ptilophylla	2n = 30 = 22m + 8sm + 4st
C. assamica	2n = 30 = 22m + 8sm
C. yankiangcha	2n = 30 = 22m + 8sm + 4st
C. arboewscens	2n = 30 = 20m + 10sm
C. tachangensis	2n = 30 = 23m + 7sm
C. taliensis	2n = 30 = 1m + 9sm
C. crassicolumna	2n = 30 = 20m + 9sm + 1st
C. gymnigyna	2n = 30 = 21m + 8sm + 1st
C. sinensis	2n = 30 = 21m + 8sm + 1st
C. sinensis var. sinensis	2n = 30 = 20m + 9sm + 1st
C. sinensis var. assamia	2n = 30 = 22m + 7sm + 1st
C. sinensis var. pubulimba	2n = 30 = 21m + 9sm
C. quinquelocularea	2n = 30 = 22m + 8sm
*C. triloculare*a	2n = 30 = 21m + 9sm
*C. triloculare*a *var. macrophyllaea*	2n = 30 = 20m + 9sm
*C. triloculare*a *var. micromidphyllaea*	2n = 30 = 20m + 10sm
*C. triloculare*a *var. kuiea*	2n = 30 = 22m + 8sm
C. cryploneura Chang	2n = 90 = 61m + 28sm + 1st
C. oblate Chang ex Chang	2n = 30 = 24m + 5sm + 1st

Table 2.2 (continued)

Species	Karyotype
C. meiocarpa Chang	2n = 60 = 37m + 18sm + 5st
C. oleofera Abel	2n = 90 = 60m + 29sm + 1st
C. grijsii Hance	2n = 30 = 25m + 5sm
C. forrestii (Diels) Cohen-Sturt	2n = 60 = 39m + 19sm + 2st
C. tsaii	2n = 60 = 38m + 21sm + 1st
C. tsingpienensis Hu	2n = 30 = 21m + 5sm + 4st
C. yunnanensis (Pitard ex Diels) Cohen-Stuart	2n = 30 = 19m + 11sm
C. chrysantha (Hu) Tuyama	2n = 30 = 17m + 10sm + 3st
C. chrysantha var. microcarpa Mo	2n = 30 = 22m + 8sm
C. impressinervis Chang	2n = 30 = 21m + 8sm + 1st
C. impressinervis Chang	2n = 30 = 20m + 9sm + 1st

possible, the seeds are packed after grading and sorting. For transport over long distances, packing is done in wooden boxes in units of 20 kg using moist sand, subsoil, powered charcoal or ash or a mixture of two or more of these as packing material. Moisture content of the packing material varies from 10 to 30% while in the case of powered charcoal it may vary from 25 to 30%. Seeds are spread in layers along with some packing materials, and each layer is separated from the one on the top by a thin sheet of tough paper. A kilogram of graded and sorted seed may contain 300–500 seeds depending on the size of the grader used.

After 45 days, the germinated seeds are transferred to the polythene sleeves and kept under a shaded nursery for another 12–18 months.

Vegetative cuttings: Seeds were the only commercial method of propagation till the beginning of the nineteenth century. However, due to the outbreeding nature of the plant, seedlings show a wide variability for attributes such as yield, quality, etc., and this forced people to find some alternatives. The first attempt for vegetative propagation of tea was done in Indonesia by budding as well as grafting. However, due to slow speed, this method could not serve the purpose of rapid multiplication. Hence, faster propagation by single leaf cutting was developed simultaneously in India, Sri Lanka and Indonesia (Mondal 2011). This was further fine-tuned later to fit the commercial venture that exists now. Cuttings from green and semi-hard wood are usually taken from current-year growth. Cuttings are then immediately subjected to fungicide as well as commercial grade rooting-hormone treatment and inserted in the nursery for root induction for 45–60 days depending on the location, planting material and so forth. The successful rooted cuttings are then transferred to polythene sleeves filled with good virgin soils (pH around 4.5) having adequate water-holding capacity and are kept for another 8–12 months in the nursery, by which period they become ready for field transfer. Meanwhile, propagation by cuttings was attempted in several parts of the tea-growing areas around the world (Tunstall 1931a, b; Tubbs 1932; Wellensiek 1933); however, standardization of the technique of single-leaf internode cuttings, practised today, took a long time to be successful. Following this technique, TES, Assam, India released the first lot of three clones in 1949, which revolutionized the tea industry in northeastern India and more clones have since been released from time to time.

Grafting: In recent years, nursery grafting as an alternative propagation technique has gained considerable popularity. In this technique, fresh single-leaf internode cuttings of both rootstock and scion are generally taken. Scion, commonly a quality cultivar, is grafted on rootstock, and is either drought tolerant or a high-yielding cultivar. On grafting, the scion and stock influence each other and thus composite plants combine both the characters, resulting in 100% increase of yield with better quality than either of the ungrafted cultivar. Recently, a modified improved 'second-generation' grafting had been developed, where a tender *in vitro*-derived shoot was grafted on the young seedlings of tea, which

had an additional advantage over conventional grafting due to the presence of tap-root system (Prakash et al. 1999). Nevertheless, with the increasing demand for clonal tea, vegetative propagation with single-leaf internode cuttings remains the best choice in the tea industry worldwide.

2.6 Floral Biology and Pollination Mechanism

The significant differences in flower description between the China and Assam type of tea were reported by several workers (Wellensiek 1933; Bezbaruah 1975), which have been described in Sect. 1.4. Characters, such as length of the style and style arm, number and length of the outer stamens or the size of the inner petals, were able to indicate the difference in floral characters among the varieties. Importantly, anatomical study suggested that tea flower should be classified as central placental type instead of parental placental type (Bezbaruah 1975).

Tea plants showed an appreciable degree of self-sterility and invariably set a better crop of seeds with pollen from another bush nearly four times than that of selfed seeds (Wight 1938; Wu 1964). Generally, selfed seeds exhibited reduced germination. Progenies of self-pollination were inferior in vigour to those of cross pollination. Investigations on the mechanism of pollination revealed that tea pollens were heavy and sticky in nature and occurred mostly in clumps, a condition which was not favourable for carrying out by wind, rather only non-viable dried-up pollen grains can move a long distance by wind. However, insects, such as bees and wasps (*Hymenoptera*), were found to carry pollens from bush to bush. Besides, Bezbaruah (1975) observed that syrphid flies (*Diptera*) were the most common insect for natural cross-pollination. The tea flower secretes a considerable amount of honey, but they contain high phenols that cause indigestion. Perhaps, this may be the reason for not attracting the diverse insects for pollination (Barua 1989). Therefore, for higher seed setting, it may be necessary to take measures to attract insects in tea-seed orchards.

After 24–48 h of pollination, the corolla withers off and drops from the pedicel along with the anther lobes leaving the ovary exposed. The persistent calyx lobes close flatly over the ovary and the style, and the stigma gradually withers off. Although pollination takes place during flowering growth, i.e. September–January in India, the first external sign of development of fruits become evident by March and gain considerable size by May of the same year. By about August, the fruits attain full size with completely developed embryo and cotyledon. The mature embryo with two large cotyledons remains covered by a hard, deep brown testa, formed by the outer integument. The pericarp encloses 1–3 seeds inside and is made of thick, parenchymatous tissue when young but becomes sclerotic on maturity. The ripening of fruit generally takes 12 months from the time of flowering till maturity (Singh 1999).

The mechanism of self-incompatibility in tea and related species remains a challenge. A study of reproduction barrier limiting interspecific hybridization between *C. japonica* and *C. chrysantha* was undertaken in intra- and interspecies crosses. Pre-zygotic barriers were not important, pollen-type growth and penetration was good in all combination of crosses. Zygote formation and early embryo development were similar in all the crosses. While intraspecies embryos developed smoothly, interspecific crosses of embryos were aborting in various stages. Interestingly, full size but empty ovules in mature capsule resulted from embryo abortion (Hwang et al. 1992). It was reported that tea could have been considered a facultative outbreeder but with a homomorphic gametophytic self-incompatibility system. The observation of successful self-pollen tube growth in the ovary and ovule penetration clearly indicated that tea had a late self-incompatibility type of selfing control (Bezbaruah 1975).

Tea flower had also been used for value addition. Importantly, tea companies in China had begun to process fresh tea flowers for blending into specialty teas to make them flavoury. How-

ever, chemical composition of tea flowers and leaves in terms of catechins and caffeine were comparable. The flowers contained less caffeine but equivalent amount of catechins (Su et al. 2000; Lin et al. 2003). Recently, Joshi et al. (2011) did a detail characterization of various catechins and volatile compounds and found that like leaf, the unopened young flower bud contained maximum amount of flavour compounds than the fully open flower of tea.

2.7 Seed Biology

Generally, 1 to 3 seeds of 1.5–2.0 cm diameter are found in each capsule. The seeds have a hard testa outside and the embryo is covered in between two large cotyledons. Tea seeds are highly recalcitrant and lose viability within a few days after shedding from the plant (Bhattacharjee and Singh 1994). However, their viability can be maintained by surface sterilizing with mercuric chloride solution (0.01 %) for 15 min and subsequent cold storage at 4 °C. Although seeds are generally stored in moist charcoal for a few days, it is advisable to use the healthy seeds for propagation as early as possible (Singh 1999). For commercial propagation, tea seeds are produced in seed baries (orchard) planted specially for the purpose. After the release of the clonal seeds, commercial seed baries have been established for production of hybrid clonal seeds. After collection, seeds are passed through a rotary type shifter to eliminate very small seeds. Tea seeds normally vary in size from 10 to 20 mm in diameter.

2.8 Breeding Techniques

Tea breeding objectives vary from country to country, depending on the local needs (Table 2.3). However, by and large, it is aimed to improve the quality and yield. Generally, the breeding works in black tea producing countries, such as India, Kenya and Sri Lanka, are biased towards the development of high yielding and quality clones whereas the tea-producing countries near the equator, such as Japan and China, are focused on the development of cold tolerance and frost tolerance, as these countries primarily produce green tea, where the quality of made tea does not have much influence on price. Today's modern tea varieties have evolved through the sincere efforts of many years of both the tea breeders as well as experienced planters through different stages of plant introduction, selection, hybridization and physical as well as chemical breeding. The different techniques are discussed in this chapter.

2.8.1 Introduction

Introduction may involve *de novo* addition of new varieties, wild relatives or totally new species in a particular area. Often, materials are introduced from other countries or continents. In tea, the unorganized agents such as Buddhist pilgrimage or colonial soldiers made the primary introduction. However, later the secondary introduction in various tea-growing countries was done by experienced British planters or scientific communities as discussed below.

2.8.1.1 Primary Introduction

Introduction of tea into Korea was done by the troops of Emperor Wu Di during his invasion of Korea from China. In Japan, tea was introduced during the eighth century when Buddhist monk Saicho, who returned from studies of Guo Xing Si on Tian Tai Mountain of Zhe Jiang provinces, brought tea seeds to Japan and planted them at the foot of the Mountain Hi Yei in the village of Sakomoto of Omi County. Later on in 1191 A.D., Yei Sai, another Buddhist, re-introduced tea seeds from China to Japan and planted them on the hilly terrain of the Seburi Mountain, southwest of Castle Fukuoka in Chikuzen province. Yei Sai not only planted and cultivated tea plant but also regarded it as the source of a sacred remedy.

In 1690 A.D., the governor of Indonesia, J. Camphuijs brought tea seeds from China and planted the same nearby Djakarta region. Between 1828 and 1833, Mr. Jacson of East India Company

Table 2.3 Breeding objectives of tea. (Adopted from Mondal 2009 with modification)

Objectives	Importance	Regions
Improving quality	Directly linked to the profitability	Black tea producing countries such as India, East-Africa, Sri Lanka, Bangladesh and Indonesia
Increasing yield	Horizontal increase of production by extension planting is limited	Worldwide
Drought tolerance	Reduce productivity and occur all tea-growing regions of the world	Worldwide where tea grown as rain-fed crop
Reduce winter dormancy	No leaf production during winter months and occurs in North-East India, Japan and China, etc	Tea plantation near the equator
Hail/frost resistance	Causes economic loss as young leaves during rainy season are mostly affected	Hilly region of the tea-producing countries
Water log tolerance	Reduce productivity during rainy season. Generally occurs in north-eastern India	North-East India
Cold hardiness	Reduced productivity during winter due to snow. Generally occurs in China, Japan and Russia	Mainly in Japan, Russia and China
Diseases resistance, such as blister blight, stem canker, etc.	Blister blight causes severe damage as only young leaves are infected. Generally occurs in Japan, Sri Lanka, South India and Darjeeling hills of North-East India	Mainly India, Sri Lanka, Indonesia and Japan
Pest resistance, such as red spider mite, tea mosquito bug, leaf-sucking pest, etc.	Most important biotic stress as all causes severe damage to the leaves. Generally, occurs in all the tea-growing regions in the world	Worldwide
Suitability to type of manufacturing	For matching the customer's demand as well as better recovery percentage in made tea	Black-tea-producing countries such as India, East-Africa, Sri Lanka, Bangladesh and Indonesia
Low input responsive Clone	Required for organic tea farming	Organic tea

went to China and brought tea seeds again which was planted in Indonesia.

Tea was brought to Russia during 1567. Later 1735, tea was first carried overland by governmental caravans. From the Chinese border, this legendary trail lay northwestwards across 800 miles of the inhospitable Gobi Desert through Ulan Bator in Mongolia and into Russia, skirting Lake Baykal, to the town of Irkutsk.

Initially, Sri Lanka began to cultivate coffee but without success. Therefore, they decided to start tea plantation during 1841. M.B. Worms, a German who lived in Ceylon, visited China and brought tea seedlings to Pussellawa. Simultaneously, seeds were also brought from Botanical Garden of Calcutta, India during 1839 and planted in the nurseries of Royal Botanical Gardens at Peradeniya near Kandy, Sri Lanka.

2.8.1.2 Secondary Introduction

However, information regarding the secondary introduction is rather scanty due to the presence of stringent rules worldwide preventing trans-border movements of the plant propagule in later years. Additionally, the secondary introduction of tea always happened off the record by the commercial tea growers. Interestingly, the early history of the planting materials clearly indicates that being the oldest tea research Institute in the world, TES, Assam, India contributed significantly towards the secondary introduction of tea worldwide. In a conservative estimation, 60% of the world-acreage had received its initial planting materials directly or indirectly from tea genetic resources of TES, Assam, India. Initially, the commercial tea estates had been established, and later these were

used as parent source of materials for developing the new cultivars.

2.8.2 Hybridization

In natural hybridization, based on known desirable characters, such as previous performance of yield, quality or diseases tolerance capability, two parents are planted side by side in an isolated place to bear fruits. Subsequently, the seeds (F_1) are harvested, raised and planted. If average performances of these plants are found to be better than either parent, then seeds are released as hybrids or bi-clonal seeds. However, some of the outstanding performers among these progenies are marked and verified for multi-location trials and, if found suitable, are released as clones. These clones are geographically specific, and most of the tea research institutes of the world have generated the clones for their own region. Sometimes, in the above process, more than two parents are used and known as polyclonal seeds. The idea is to introduce more variability among the F_1 seeds. Since it is difficult to know about the pedigree of the cultivar (as pollen may come from any male), the chance of reproducibility of performance is low, and therefore the process is the least preferred currently.

Hand pollination or control cross, despite being an important approach, has made a limited success in tea breeding. The reasons identified could be: (1) low success rate, (2) availability (2–3 months) of tea flowers for a short period, (3) longer time taken for seed maturation (12–18 months) and (4) difference in flowering time for different clones.

2.8.3 Selection

The seeds from a particular 'seed orchard' are known by the name of that orchard or locality and are called *jats* or population, and those *jats* are the main source of planting materials of tea. There are wide variations among the offsprings for morphology, yield and quality parameters generated from a particular *jat* even though the source of the seed was the same. This is primarily due to high outbreeding nature of tea plants. Wight (1939) showed an interesting fact. About 10% of the bushes in a commercial tea garden of northeastern India produced only 2% of the total crops, i.e. green leaf and about 0.5% bushes produced as much as or more than 300% of the average crops. Thus, the planters thought that new areas planted with seeds from those 0.5% bushes will produce more uniform and better quality tea. Those selected plants were used for future plantations. Thus, the process of selection started. The first scientific attempt to select improved tea in northeastern India was made by Stiefelhagen brothers during 1860 by establishing standard sources of tea seeds. Subsequently, scientific improvements of tea by selection were followed in many countries, such as Indonesia (Wellensiek 1934), Java (Cohen Stuart 1929), Russia (Bakhtadze 1935) and northeastern India (Wight 1939). Mother bushes were selected based on morphological characters followed by anatomical (Wight 1956) and organoleptic performance of made tea (Timoshenko 1936). Indigenous Assam tea was improved by following the technique of mass selection. The yield increased considerably, because of line breeding for desirable morphological features that are genetically linked with the characteristics of Assam tea. After that, line breeding techniques were followed to improve further by mainly evolving more uniform tea plant with better quality and adaptability. In the earliest effort, two selected *jats* were hybridized to combine desirable characters into their progenies. The 'Rajghur' *jat* was developed by combining high quality of a light leaf local *jat* with the vigour of a dark leaf 'Manipuri' *jat*.

However, the seed-grown plants were not uniform as their characters were governed by genotypes of their parents, which were diverse in some phenotypic characters in relation to environmental and soil conditions. In some cases, the yield and quality were unpredictable. It was, therefore, necessary to develop clonal cultivars in tea like many other fruit crops by multiplying the selected bushes vegetatively. Today, all tea-growing countries of the world have developed clonal materials as per their requirements.

Table 2.4 Triploid tea cultivar used under commercial cultivation along with their promising characteristics. (Gunasekara and Ranatunga 2003)

Country	Polyploid cultivar	Level of ploidy	Type of polyploidy	Promising characteristic(s)	Reference
India	Sundaram	3n	Natural	High yield and quality	Sharma and Ranganathan (1986)
India	UPASI 3	3n	Natural	High yield and overall quality	Satyanarayan and Sharma (1986)
India	UPASI 20	3n	Natural	Moderate yield, highly tolerant to drought	Satyanarayan and Sharma (1986)
India	TV 29	3n	Natural	High quality	Barbora et al. (1996)
Japan	Not-know	3n	Natural	Hardier and cold resistant	Simura and Inabe (1952)
Kenya	382/1	3n	Natural	High yield	Wachira (1994)
Sri Lanka	TRI 3069	4n	Artificial	High yield and drought tolerant	Kulasegaram (1980)
Sri Lanka	HS 10A	3n	Natural	Cold resistant	Kulasegaram (1980)
Sri Lanka	GF 5/01	3n	Natural	High yielding	Anon (1973)

2.8.4 Polyploidy Breeding

Yield is the major criteria in commercial tea cultivation which depends on the size and density of the leaves in the plucking table, i.e. upper surface area of the tea bush. A positive correlation between size of the leaf and the yield in tea was well established (Satyanarayan and Sharma 1982). Therefore, the development of tea genotypes with bigger leaves through polyploidy breeding may be useful to increase the yield of tea. Further, being vegetatively propagated plants, polyploidy breeding can be used effectively. In tea, significant amount of works on polyploidy breeding had been done (Gunasekara and Ranatunga 2003), which are reviewed below.

2.8.4.1 Naturally Occurring Polyploids

Although the cultivated species of tea was diploid (Morinago et al. 1929; Barua 1989), naturally occurring intra- and interspecies polyploids of tea (Janaki Ammal 1952; Bezbaruah 1971; Jayasuriya and Govindarajulu 1975; Wachira and Kiplangat 1991) and its wild relatives were also reported (Kondo 1977). Interestingly, natural polyploids were more common in tea populations (Table 2.4) of Japan than any other countries (Banerjee 1992b; Simura and Inabe 1952). Bezbaruah, (1968) reported that in tea, the triploids, tetraploids, pentaploids and aneuploids, resulting from open-pollinated progenies, occurred naturally but in extremely low frequency.

2.8.4.2 Artificial Induction of Polyploids

Since the discovery of the effect of colchicine in 1930s on cell division for mitotic doubling of the chromosome number, it was widely used to induce the level of polyploidy in plant (Blakeslee and Avery 1937). Colchicine inhibits mitosis in cells by interfering with the structure of the mitotic spindle, thus resulting in formation of cells with a doubled chromosome number.

Similar to other plants, colchicine had been used to induce artificial polyploids in tea (Table 2.5). In Sri Lanka, Sebasthiampillai (1976) produced five tetraploid plants, namely TRI 2023, 2024, 2025, 2026 and DT 95, by treating the meristematic tissues of the terminal bud for 2–7 days with colchicine impregnated in agar. Although he found the differential response of tea genotypes with the colchicine treatment, his ploidy plants were tetraploid as he confirmed through the cytological examination of root-tip cells. However, attempts to induce polyploids using ethyl methane sulphonate (EMS) at TES, Assam, India, though tried, were not success-

Table 2.5 Details of induction of tea colchiploids. (Gunasekara and Ranatunga 2003)

Plant part used	Treatment	% success	Reference
Axillary buds of etiolated shoots	Cotton wool moistened with colchicine (0.2%) and treatment was given in the dark	13.0%	Katsuo (1966)
Terminal buds of active shoots developing from pruned bushes	Agar impregnated with colchicines (0.2–0.5%) for 5–6 days	13.5%	Sebasthiampillai (1976)
Terminal buds	Immersion in aqueous colchicines (1–2%) for 5–7 days	6–17%	Anon (1979)
Flower buds	Colchicines (0.05%) injection and drop application for 2–6 days	30.0%	Osone (1958)

ful. Nevertheless, more than 170 and 70 polyploids were subsequently generated in the same institute through conventional hybridization and colchicine treatment, respectively (Singh 1999); yet, in tea, as high as 30% ploidy had been achieved using colchicines as mutagenic agents (Table 2.5).

2.8.4.3 Morphological, Anatomical and Cytological Markers in Polyploid Teas

Screening of polyploids has not been widely exploited in tea due to lack of reliable markers. Hence, the identification of markers related to morphology, anatomy or cytology is of great importance in screening for polyploids, whether they are artificially induced or naturally occurring. Generally, the ploidy level of tea is determined by counting chromosome numbers in meristematic tissues, i.e. root-tip cells or pollen mother cells.

Chaudhuri (1979) found wide range of phenotypic and anatomical variations, such as frequency and size of stomata and sclereids among the progenies of triploid tea (Chaudhuri and Bezbaruah 1985). Similarly, to assess the effects of the level of polyploidy on the morphogenetic attributes of the F_1 seedling population, generated from a cross between diploid and tetraploid cultivars, a clear relationship was shown between ploidy levels and morphogenetic variations (Rashid et al. 1985).

Among the different morphological markers, while leaf area was found to be higher in triploid, its expansion, i.e. growth was lower in triploids in comparison with diploid tea leaves (Ng'etich and Wachira 1992). The reason for this may be that the attributes considered were more affected by environmental factors than by ploidal status.

Anatomical markers such as stomatal density were used to differentiate the triploid than diploid as it was found to be lower in triploid than diploid cultivars (Amma 1974; Chaudhuri and Bezbaruah 1985; Wachira 1994). It was found that triploid plants had a lower stomatal density than the diploid genotypes (Wachira 1994). However, this marker could not always be used as a reliable marker for identification of polyploids in tea. In fact, Chaudhuri and Bezbaruah (1985) had indeed reported that there was a lack of correlation between the level of ploidy and stomatal density. Similarly, guard-cell sizes as well as stomata size were larger in tetraploids and triploid teas than its diploid counterparts (Amma 1974). On the contrary, Wachira (1994) found that the length to breadth ratio of the guard cells was not significantly different between diploids and triploids. Later, chloroplast number in the guard cells had been identified as a reliable ploidy marker in tea (Ahmed and Singh 1993; Koskey and Wachira 2000; Ranatunaga and Gunasekare 2002; Chen and Ye 1989). Further, Koskey and Wachira (2000) found that the ratio of the guard-cell chloroplast numbers in diploids, triploids and tetraploids was found to be 2:3:4, which was the same as the ratio of their chromosome numbers (30:45:60) (Ahmed and Singh 1993). Therefore, this finding indicated that the ploidy level of tea could be accurately and rapidly identified by the chloroplast-count method, rather than by criteria based on the size and density of stomata.

Reproductive organs such as pollen grain in most of the induced tetraploid cultivars were found to be higher than that of its diploid coun-

terparts (Gunasekara 2000). But *in vitro* germination of pollen grain was poorer in tetraploid cultivars than in diploid cultivars (Thirukkumaran and Gunasekare 2001). Only 2% of the pollen grains of natural triploids were found to be viable (Bezbaruah 1971). It had been reported that pollen viability and fertility of triploid cultivars were unable to set seeds and fruits. In general, these morphological as well as anatomical markers were not consistent, which was why they were not accepted by tea breeder.

Therefore, the alternative cytological markers such as chromosome counts were found to be more reliable to differentiate the triploids from diploids. Chromosome counting in pollen mother cells, root-tip cells, and meristematic tissue cells at the shoot tip were subsequently standardized in tea (Gunasekara and Ranatunga 2003). Wachira and Muoki (1997) devised a new cytological technique to assess the activity of nucleoli and nucleolus-organizing regions of polyploids and diploids. Their study revealed that the mode of nucleolar number corresponded to multiples of the somatic cell number, and hence was a good marker for ploidy. Therefore, it is assumed that the attributes, related to anatomical features, are much more precise than the morphological characters which have been used to screen polyploidy genotypes in tea due to the fact that the later has larger environmental influence than the former.

2.8.4.4 Use of Polyploids in Tea Breeding

Generally, tea polyploids often lack desirable traits (Bezbaruah 1968; Sarmah and Bezbaruah 1984), and polyploidy breeding therefore requires planned hybridization, selection of promising polyploids and proper evaluation to confirm their performance as potential cultivars. High-yielding polyploids with low quality of made tea, had been improved through hybridization with a diploid cultivar of high quality traits (Sarmah and Bezbaruah 1984). Triploids had been produced by hybridizing tetraploid tea with diploid tea in Japan (Osone 1958), India (Chaudhuri 1979) and Bangladesh (Rashid et al. 1985). It had been shown that it was possible to combine good cup quality, with the superior vigour and hardiness of the polyploids, by crossing tetraploid progeny for commercialization. For example, open-pollinated tetraploid with inferior cup quality but with higher growth vigour (Bezbaruah 1976) was improved to higher cup quality by crossing with high-quality diploid clone as the male parent (Bezbaruah 1991).

The conventional method of producing triploids is through artificial induction of tetraploids, followed by hybridization with diploid cultivars. For example, out of 238 hybrids produced through hybridization between tetraploids and diploids at TES, Assam, India, only 79 hybrids were found to be triploids (Barbora et al. 1996). Since induction of diploid was time consuming, Osone (1958) used diploidized pollen of immature flowers to pollinate diploid plants for producing triploids. However, there is no evidence that this method had been widely practised in polyploid breeding programmes. Recently, to verify the quality of triploid cultivars of tea, Das et al. (2013) profiled caffeine and catechins of 97 F_1 segregating progenies of a common tetraploid and diploid parents. Catechins and caffeine level of the triploid progenies were compared against their diploid parents. Some of the progenies found to be better quality clones than their diploid parents. Most of the progenies of the diploid *C. sinensis* crossed with tetraploid, showed heterosis for caffeine and catechins. The genomic contributions of the diploid parents seem to be the main factor in the variation between the two populations. They demonstrated quantitative enhancement of some of the quality-related parameters in tea, providing a platform to refocus on this classical breeding approach for developing quality cultivars in tea.

2.8.4.5 Commercial Exploitation of Polyploids

Although emphasis was given to identify natural polyploids and develop artificial polyploids, reports on their performance and trait evaluation are scarce. After the discovery of natural polyploids of tea (Karasawa 1932; Bezbaruah 1971; Amma 1974; Katsuo 1966; Sebasthiampillai 1976), natural polyploids were included in cultivar selection programmes to identify desirable agronomic traits. Certain studies indicated

that natural polyploids found in Southern India possess attributes for high yields and quality (Sharma and Ranganathan 1986). On the other hand, Banerjee (1992b) had reported that though polyploids showed high vigour and tolerance to environmental stresses, they did not always contribute towards high yields, even sometimes produced low quality of tea (Bezbaruah 1968; Sarmah and Bezbaruah 1984). The prolific growth in polyploids may be attributed to increase photosynthesis owing to the increase of chloroplast number in the guard cells.

The effects of ploidy on yield and its components had been studied in tea (Amma 1974; Banerjee 1992b; Wachira 1994; Wachira and Ng'etich 1999). It was found that triploid cultivars produced larger and heavier but fewer harvestable shoots per unit area, compared to diploids due to which triploids yield less than diploids, despite higher shoot weight (Wachira 1994). In another study by Singh (1980), it was found that out of the different type of polyploids produced in India, the dry weights of five fully formed leaves in triploids and tetraploids were higher than that of diploid leaves by 14 and 109%, respectively. Other pentaploids and aneuploids, however, had relatively low leaf dry weights.

Although it was shown that polyploidy in tea enhanced the yield (Jayasuriya and Govindarajulu 1975; Kulasegaram 1980; Sharma and Ranganathan 1986), this was not always the case, as there were instances where increased polyploidy was demonstrated to depress the productivity (Banerjee 1992b; Wachira 1994). However, it was clear from those studies that though increased ploidy depressed yields, a significant difference in production could also be observed among the genotypes at the same ploidy level. In certain cases, triploids outyielded diploids, which indicated the potential for selecting or developing high-yielding polyploid cultivars. Nevertheless, rooting ability, leaf size and leaf dry weights of triploids and tetraploids were higher that of diploids but lower in pentaploids and aneuploids (Banerjee 1992b).

Two triploid cultivars were commercially successful in the tea industry of Sri Lanka. The first one, TRI 3069, which was an induced tetraploid of TRI 2025, had been accepted commercially and possesses many improved traits. The second cultivar, HS 10A, a natural triploid selected from a seedling-tea population on Hethersett Estate of Sri Lanka, was found to be better adapted to high elevations than diploid cultivars (Kulasegaram 1980). It had been reported that triploid forms of tea were hardier and more resistant to cold conditions than diploids, and a clone which was widely recommended for planting in southern India was reported to be a natural triploid (Jayasuriya and Govindarajulu 1975). Commercially acceptable polyploid tea cultivars developed in some tea-growing countries are detailed in Table 2.4 which show that only eight polyploids have found their way into cultivation, including one artificially induced polyploid.

Although extensive works have been done to identify precise markers for ploidy level in tea, it is clear from the above discussion that the results obtained are not consistent. However, among the criteria studied, the number of chloroplasts in the guard cells and the stomatal density can be used with some reliability for ploidy level analysis. These markers may be used for the screening of polyploids from a larger number of tea genotypes, although chromosome counts remain the most reliable and this procedure could minimize the time and resources needed for subsequent cytological studies.

2.8.5 Mutation Breeding

The work on mutation breeding in tea was initiated during 1967–1968 at TES, India with the objective of increasing genetic variability for possible use in evaluation of superior planting materials. However, except a preliminary report on irradiation with γ rays on cuttings, no progress had been achieved till now (Singh 1984). Studies done elsewhere, had shown that a wide range of variations can be created by irradiating various plant parts, such as seeds, leaf cuttings, auxiliary and apical buds of tea to induce mutations (Tavadgiridze 1979).

2.8.6 Pre-Breeding and Distance Hybridization

Tea breeds freely among the two cultivated species, i.e. *sinensis* and *assamica,* and up to a limited extent with few wild relatives. Earlier, Wight and Barua (1957) hybridized *C. irrawadiensis* with *C. sinensis*. Although the progenies were resembled later but failed to attack tester tong due to inferior quality. Later Bazbarua and Gogoi (1972) made a successful hybridization between *C. japonica* and *C. sinensis*. Morphologically, progenies were found to be intermediate but produced low yield as well as quality. However, a commercial high-yielding clone TV-24 was produced at TES, Assam, India from the cross between F_1 hybrids from *C. irrawadensis* and TV-2, an Assam–China hybrid. Six interspecific backcross progenies were generated by crossing hybrids of *C. saluensis* × *C. japonica* (*Camellia* × *williamsii*) back to *C. japonica*. Segregation data were obtained within these six families for five traits, three involving flavonoid constituents and two pubescence characters. A single major gene seemed to be primarily involved in each case, and two of the traits exhibited linkage behaviour. The taxonomic value of such marker trait was also discussed (Parks and Kondo 1974). Li et al. (2005) made a cross between high quality *C. sinensis* and *C. ptilophylla.* Out of the 62 progenies, 3 were identified to be commercially viable on the basis of the biochemical parameters or organoleptic test. At the same time, these three progenies were cold tolerant, a character which came from *C. ptilophylla,* thus making them suitable for cultivation in sub-tropical region of China.

Interspecific cross compatibility between tea and its allied 26 species in the genus *Camellia* was examined. The interspecific crossing abilities varied among the cross combinations, and the fruit-bearing rates were in the range of 0–42.6%. The interspecific hybrids obtained from the crossings of *C. sinensis* with *C. japonica, C. pitardii, C. assimilis, C. caudata, C. salicifolia, C. irrawadiensis* and *C. taliensis* showed very low pollen fertilities. Morphological characteristics of those hybrids, including size and shape of leaves, flowers and tree performance, were generally intermediate of their parental species. The hybrids between *C. sinensis* and *C. japonica* showed a high level of tolerance to diseases such as tea grey bright, tea anthracnose and to cold damage during winter as well. It was presumed that the F_1 hybrids which were obtained from the crossing of *C. sinensis* with *C. sasanqua, C. brevistyla* and *C. oleifera* used as a male parent might be developed through parthenogenesis of a reduced gamete. The cross compatibilities between *C. sinensis* and subgeneric sections of genus *Camellia* were found to be as follows: Thea > Camelliopsis > Paracamellia = Camellia = Theopsis > Heterogenea = Corallina (Takeda 1990).

In a natural cross between *C. taliensis* and *C. sinensis,* two low-caffeine but high-theobromine containing plants were produced. Genetic analysis indicated that caffeine-less character might be controlled by one recessive locus. Thus, this caffeineless plant might be used as a genetic resource for introducing caffeine-less trait in cultivated tea through breeding (Ogino et al. 2009).

2.9 Genetic Resources of Tea

Progress and achievements of tea breeding works in certain tea-producing countries had been well reviewed (Singh 1999; Ghosh-Hajra 2001; Deka et al. 2006). Those literatures indicated that the initial emphasis was to collect and evaluate either the indigenous or exotic germplasm for befitting the local environment. However, with the increase of the region-specific need of the industry, almost all tea-producing countries have developed their past specific clones or seed stocks which are reviewed here.

As mentioned earlier, the breeding works at TES, Assam, India which started since 1949, developed a total of 31 clones (Table 2.6), 14 bioclonal seed stocks (Table 2.7) and 134 TRA/Garden series clones (Table 2.8). In Southern India, the breeding works started at UPASI, Tamil Nadu during early 1960s, which resulted in the release of 28 clones (Table 2.9) and 5 bioclonal stocks,

Table 2.6 Descriptions of different TV clones. (Deka et al. 2006)

Name	Year of release	Preference for manufacture		Remarks
		1st preference	2nd preference	Assam–China hybrid
TV-1	1949	CTC	Orthodox	Assam type
TV-2	1949	Orthodox	CTC	Assam type
TV-3	1949	Orthodox	CTC	Assam type
TV-4	1959	Orthodox	CTC	Assam type
TV-5	1959	Orthodox	CTC	Assam type
TV-6	1959	Orthodox	CTC	Assam type
TV-7	1959	Orthodox	CTC	China hybrid
TV-8	1959	Suitable for both		Assam Type
TV-9	1959	CTC	Orthodox	Cambod type
TV-10	1963	CTC	Orthodox	Assam type
TV-11	1963	Orthodox	CTC	Assam type
TV-12	1963	Orthodox	CTC	Assam type
TV-13	1965	Orthodox	CTC	Assam type
TV-14	1967	CTC	Orthodox	Assam hybrid
TV-15	1967	Orthodox	CTC	Assam type
TV-16	1968	CTC	Orthodox	Assam hybrid
TV-17	1968	CTC	Orthodox	Assam hybrid
TV-18	1970	CTC	Orthodox	Cambod type
TV-19	1973	CTC	Orthodox	Cambod type
TV-20	1974	CTC	Orthodox	Cambod type
TV-21	1976	Orthodox	CTC	Assam type
TV-22	1976	CTC	Orthodox	Cambod type
TV-23	1976	CTC	Orthodox	Cambod type
TV-24	1979	CTC	Orthodox	Cambod and species hybrid
TV-25	1982	CTC	Orthodox	Cambod type
TV-26	1982	CTC	Orthodox	Cambod type
TV-27	1985	CTC	Orthodox	Cambod type
TV-28	1985	CTC	Orthodox	Cambod type
TV-29	1990	CTC	Orthodox	Cambod, triploid
TV-30	1993	CTC	Orthodox	Cambod type
TV-31	2006	CTC	Orthodox	Assam hybrid

Table 2.7 Descriptions of bioclonal seedling developed by TES, Assam, India. (Deka et al. 2006)

Cultivar	Parent combination	Year of release	Suitable for area
TS 378	14.5.35 X 14.6.28	1968	Hills (Darjeeling) Area
TS 379	14.5.35 X 14.12.16	1989	Hills (Darjeeling) Area
TS 397	TV-1 X 19.35.2	1976	Plains
TS 449	TV-1 X 270.2.14	1970	Plains
TS 450	TV-2 X 270.2.13	1970	Hills (Darjeeling)
TS 462	TV-1 X 124.48.8	1980	Plains
TS 463	TV-1 X TV-19	1984	Plains
TS 464	TV-1 X 19.29.2	1984	Plains
TS 491	TV-1 X S_3A_3	1989	Plains
TS 506	TV-1 X 19.22.4	1994	Plains
TS 520	TV-19 X TV-20	1992	Plains
TS 557	AV-2 X Teen Ali 17	1996	Hills (Darjeeling)
TS 569	AV-2 X Tukdah-78	1996	Hills (Darjeeling)
TS 589	TV-20 X Heeleakah 22/14	1996	Plains

Table 2.8 Descriptions of different region-specific garden series clones. (Deka et al. 2006)

Area	Clone
Darjeeling	Phoobsering 312, Phoobsering 1404, Phoobsering 1258, Kopati 1/1, Happy Valley 39, Bannockburn 157, Tukdah 145, AV2, Tukdah 253, Tukdah 246, Bannockburn 777, Rungli Rungliot 4/5, Bannockburn 688, Tukdah 78, Tukdah 383, Rungli Rungloit 17/144, CP-1, Teesta Valley 1, Badamtam 15/263, Balasun 7/1A/76, Balasun 9/3/76, Thurbo 3, Thurbo 9 and Lingia 12
South India	ATK-1 (drought tolerant clone), C-17, D-12/A2, C-1, CR-6017 (quality clone), SMP-1 (resistant to blister blight), W-35, SA-6, TTL-1, TTL-2, TTl-4 and TTL-5
Kangra Valley	Kangra and Jawala
Tripura	Huplongcherra 18, Huplongcherra 26, Meghlibundh 11, Meghlibundh 20 and Meghlibundh 25
Barak Valley	Narinpore 4, Narinpore 18, Narinpore 22, Chandighat 9, Longai 17, Longai 26, Poloi 23 and Lalamookh 7
Dooars and Terai	Hantapara 12, Huldibari 19, Leesh River 9/34, Sukna 7, Sanyasithan 8, Kamalpur 6, Moh-argung and Gulma 25

Table 2.9 Descriptions of different UPASI clones. (Sharma and Satyanarayana 1987)

Name	Description
UPASI-1	Resistant to drought and tolerant to wind, very upright and vigorous growth and forming as compact bush. Suitable for mid elevations
UPASI-2	Excellent spread with a dense plucking table fairly hardy tolerant to drought and wind. Suitable for all elevation
UPASI-3	A triploid clone with an excellent spread and dense plucking table flourishing at all elevation, bright infusion and liquor
UPASI-4	With profuse branching. Suitable for mid and high elevations
UPASI-5	Compact bush and dense plucking table suitable for mid and high elevation
UPASI-6	With good spread and high plucking density, fairly tolerant to drought, suitable for mid and higher altitude
UPASI-7	With profuse branching, good spread with a dense plucking table. Tolerant to drought and wind damage. Suitable for mid and high elevation
UPASI-8	Vigorous growth, with a good spread and dense plucking table. Can grow at all elevations particularly in warm humid zones
UPASI-9	Most popular standard clone in South India, fairly tolerant to drought and suitable to all altitude and can withstand the soil pH near neutral (6.8)
UPASI-10	Forms a broad dense plucking table, hardy clone, resistant to drought, fairly tolerant to wind. Thrives even in soils with pH near neutral (6.8)
UPASI-11	Vigorous orthotropic grower, tolerant to drought, suitable for mid altitude
UPASI-12	Semi-orthotropic, good grower, suitable for mid elevation
UPASI-13	Profuse branching with dense plucking table. Suitable for high altitude
UPASI-14	Spready bush with high plucking density. Suitable for high altitude and produced flavoury tea
UPASI-15	Resistant to drought and fairly tolerant to wind and mild frost. Suitable to mid altitude and produce quality tea thought out the year
UPASI-16	Tolerant to drought and mild frost. Suitable for all elevation. Flushes during winter
UPASI-17	Good spread and dense plucking table. Suitable for mid and high elevations. Produced very bright liquor
UPASI-18	Semi-orthotropic and vigorous grower with good branching, fairly tolerant to drought. Suitable mid and high altitude
UPASI-19	Hardy, resistant to drought and tolerant to mild frost. Flushes throughout the year. Tolerance to both drought as well as winter. Suitable for high altitude. Produced flavoury tea
UPASI-20	Semi-orthotropic grower, resistant to drought, Suitable for mid altitude. Produce bright liquor

Table 2.9 (continued)

Name	Description
UPASI-21	Excellent spread and dense plucking table. Suitable for mid and high altitude. Produce flavour, bright liquor
UPASI-22	Vigorous grower but with sparse branching. Makes good, tippy, orthodox tea with good flavour and quality
UPASI-23	Good rooter, excellent spread, tolerant to drought fairly well. Suitable for mid and high elevation
UPASI-24	Flushes thought out the year. Suitable for mid and high elevation. Produce scented flavour
UPASI-25	Recovery from pruning is quick, dense plucking point. Suitable for high altitude
UPASI-26	Recovery from pruning is quick. Resistant to drought, flushes throughout the year. Suitable for high altitude
UPASI-27	Compact plucking table with moderate spread. Tolerant to drought
TRF-1	High-yielding clone with profuse branching

Table 2.10 Clones developed by Tata Tea Ltd. (Haridas et al. 2006)

Clones	Parentage	Salient features
TTL-1	UPASI 9 X TRI-2025	High yielder and moderate quality, drought tolerant
TTL-2	Estate selection	Average yielder and excellent quality
TTL-3	Estate selection	Average yielder, moderate prone to drought
TTL-4	UPASI-10 X TRI-2025	High yielder, easy rooter, good quality
TTL-5	UPASI-10 X TRI-2025	High yielder, easy rooter, good quality, fairly tolerant to drought
TTL-6	UPASI-9 X TRI-2025	High yielder, broad leaved with larger shoots
TTL-7	UPASI-9 X TRI-2025	High yielder moderate quality, drought tolerant

i.e. BSS-1 (UPASI-10 X TRI-2025), BSS-2 (UPASI-2 X TRI-2025), BSS-3 (UPASI-9 X TRI-2025), BSS-4 (UPASI-15 X TRI-2025) and BSS-5 (CR6017 X UPASI-8). Further, to widen the genetic base, clones developed in Sri Lanka were introduced and experimented, and it was found that TRI-2024 and TRI-2025 were suitable and therefore gained popularity in the industry (Sharma and Satyanarayana 1987). Efforts to generate and conserve the tea genetic resources by private funding were also made. For example, Research and Development Department of Tata Tea Ltd, Kerala, India had developed seven promising cultivars, several bioclonal seeds stock such as TTSS-1, TTSS-2, besides they maintained a tea germplasm collection more than 100 popular tea genotypes and wild species (Haridas et al. 2006) and Table 2.10.

The plant improvement programmes in Sri Lanka started during 1930s when seeds of Betjan, Manipur and Rajghur from Assam were planted in Peradoniya Botanical Garden in Sri Lanka to establish the first tea garden. However, scientific works started only during 1937 when Dr. F. R. Tubbs brought few seeds of ST 4/10 from TES, Assam, India and seedlings were raised at Tea Research Institute of Sri Lanka and subsequently eight clones were released. Since then several clones were released which were popularly known as 20, 30 and 40 series clones. At present, Sri Lanka has more than 57% clonal tea area. Out of this, around 80% is composed by only three popular clones, i.e. TRI-2023, TRI-2025 and TRI-2026.

Bangladesh Tea Research Institute, Maulvibazar, Bangladesh had developed 13 clones and

two bioclonal seed stocks so far. These clones are known as BT-1 to BT-13. Among the two biclonal seed stocks, BST-1 and BST-2, the former is more popular, which is a cross between BT-1 and the popular Indian tea cultivar TV-1 (Deka et al. 2006).

Initially, the Tea Research Institute of Vietnam, Vinh-Phu had started the breeding of tea by collecting the planting materials from India. Many elite clones and *jats* such as PH-1, PH-3 and IA had been evolved through selection and hybridization (Tien 1993). Later, two clones namely LDP1 and LDP 2 had been released with a yield potential of 17,500 kg green leaf/ha and 16,900 kg green leaf/ha, respectively. Two quality clones 276 and 215 (cross between PH1 and Shan tea) had also been developed (Toan and Tao 2005).

In Indonesia, the breeding of tea which was initiated during 1980s was confined to selection only. Later ten clones, namely GMB-1 to GMB-10 had been released which were developed by hybridization at Research Institute for Tea and Cinchona, Gambung, Indonesia. While GMB-1 to GMB-5 had the potential to produce 3,500 kg/ha/year of made tea, GMB-6 to GMB-10 had a productivity potential of 5,000 kg/ha/year of made tea. Interestingly, few clones were also found to be tolerant to blister blight (Arfin and Semangun 1999).

Tea improvements in Kenya started with the introduction of seeds from Assam, India which were used to establish the first tea plantation. Since these progenies had not been particularly selected for high yield and quality, the resultant seedling populations of mixed genotypes were genetically inferior, though diverse. With this population, organized tea improvement started with the formation of *Tea Research Institute of East Africa* in 1961, and later the Tea Research Foundation of Kenya in 1980 with a mandate for research on all aspects of tea. Thus, in the first phase of the tea improvement, mass selection among introduced seedling jats based on morphological characteristics was done. As a result, several cultivars such as TRFK 6/8, TRFK 7/3, TRFK 7/9, TRFK 7/14, TRFK 11/4, TRFK 12/12, TRFK 12/19, TRFK 31/8, TRFK 31/11, TRFK 31/27, TRFK 31/28, TRFK 31/29, TRFK 54/40, TRFK 55/55, TRFK 55/56, TRFK 56/89, TRFK 100/5 and TRFK 108/82 were released for the industry. However, being heterogeneous genotypes, they formed good breeding materials for the second phase of mass selection. It was basically done by hybridization of selected parental stocks, superior in certain attributes that they were selected for. Several cultivars such as TRFK 303/35, TRFK 303/152, TRFK 303/156, TRFK 303/179, TRFK 303/186, TRFK 303/199, TRFK 303/216, TRFK 303/231, TRFK 303/259, TRFK 303/248, TRFK 303/352, TRFK 303/366, TRFK 303/388, TRFK 303/577, TRFK 303/745, TRFK 303/791, TRFK 303/978, TRFK 303/999, TRFK 303/1199, TRFK 347/314, TRFK 347/326, TRFK 347/336 and TRFK 347/573 were developed. The third phase involving selections from bi-clonal full-sib progeny resulted in the release of clones TRFK 337/3, TRFK 337/138 and TRFK 338/13. Later, the limitations of phenotypic selection encouraged, spurred the search and development of superior but genetically uniform tea clones. Presently, 45 clones had been developed out of which 24 were selected from seedling populations including the most popular clone 6/8. Thus in total, 27 clones (60%) shared the genetic pedigree of clone 6/8. Among the cultivars, few were credited with some special characters such as S 15/10, a high-yielding clone recently developed and registered for 10,000 kg made tea/ha/annum. Similarly, SFS 150 and 303/577 were accounted for drought tolerant and TN 14–3 for tolerant to high soil pH, SFS 150 and TN 14–3 for cold tolerant, 12/2 for poor fermenter, 311/287 for tetraploids and 7/9, 57/15, SC 31/27, S 15/10 for mite tolerant (Seurei 1996).

About 2,665 accessions of tea including a few species of *Camellia* were maintained at the China National Germplasm Tea Repository which had contributed to develop more than 200 improved cultivars (Wang et al. 2011). Out of which, 97 national registered cultivars, among them 17 were *jats*, 80 were clones, 30 were landraces and 67 were improved clones. They were bred by 23 different institutions including the national and local tea research institutes, Agricultural Universities, local agricultural departments, tea ex-

perimental stations, etc. There were also about 130 registered cultivars, among them 16 were *jats*, 114 were clones, 29 were landraces and 101 were improved clones, respectively. However among them, only 54 were popular clones suitable for green tea, 32 cultivars for black tea and 33 cultivars were for oolong tea. Few cultivars, such as Zhuyeqi and Fuding Dabaicha, processed stronger prune-shock. The plucking surface of Zhuyeqi and Fuding Dabaicha contained more dense leaves, branches and shoots than that of popular clone 'Xianggbolu', hence the former two were recommended for mechanical plucking (Yongming 1999; Chen and Zhou 2005).

Apart from the Chinese main land, commendable works on tea breeding had also been done at Taiwan Tea Experimental Station, Taoyuan, Taiwan. Among the 66 cultivars, Chisin Oolong, Chinsin Dapan, TTES No. 12, TTES No. 13 and Shy jih Chuen were very popular. Nevertheless, Chinsin Oolong was the most important tea cultivars in Taiwan tea industry occupying half of total tea acreage. Besides, Chinsin Dapan had a wide manufacturing adaptability, suitable for making green tea, paochung tea, white-tip oolong tea and black tea. TTES No 12 (Kinshen) was known for its light milky flavour and hence very popular. On the other hand, TTES No. 13 had satisfactory tolerance levels of environmental stress such as drought and die-back diseases as compared to Chinese Oolong tea (Toan and Tao 2005).

In Korea, tea breeding began at Tea Experimental Station, Boseong which was established in 1992 and developed seven tea cultivars till recently. These cultivars were being propagated as cuttings for distribution to tea farmers. Some of the popular cultivars were Bohyang, Myungseon, Chamnok, Seonhyang, Mihyang, Jinhyang and Oseon. Since then, massive breeding works such as introduction of green tea cultivars, hybridization using cv. Yabukita as parent had been undertaken (Jeong et al. 2005).

Tea breeding was started way back in 1920 at Tea Experimental Station, Shizuoka, Japan. Additionally, several private tea breeders started the varietal improvement of tea which resulted in the development of many clones. Among them, Hikosaburo Sugiyama (1857–1941), a noted tea breeder popularly known as 'Burbank of tea' after US plant scientist, Luther Burbank, developed the popular clones of Japan 'Yabukita', 'Koyanishi' and 'Rokuro'. At present, 'Yabukita' alone planted 76% of all Japanese tea plantations. However, clonal selection programme was more intensified during 1950 and as a result many good clonal cultivars were released in 1970. These newly developed cultivars had contributed much to the modern Japanese tea industry. There were 70 registered varieties and few of them were, Ooiwase, Yabukita, Surugawase, Sayamakaori, Yamakai, Kurasawa, Kanayamidori, Okuhikari and Sawamizuka (Takeo 1992).

2.10 Bottlenecks of Tea Breeding

Although conventional tea breeding is well established and contributed much for tea improvement over the past several decades, it is time consuming and labour intensive. The bottlenecks of conventional breeding are: (1) perennial nature, (2) long gestation periods, (3) high inbreeding depression, (4) self-incompatibility, (5) unavailability of distinct mutant of different biotic and abiotic stress, (6) lack of distinct selection criteria, (7) low success rate of hand pollination, (8) short flowering time (2–3 months), (9) long duration for seed maturation (12–18 months), (10) clonal difference of flowering time and fruit bearing capability of some clones. Similarly, although vegetative propagation is an effective method of tea propagation, it is limited by several factors, such as, (1) slower rates of propagation, (2) unavailability of suitable planting material due to winter dormancy, drought in some tea growing areas and so on, (3) poor survival rate at nursery due to poor root formation of some clones and (4) season-dependent rooting ability of the cuttings, etc. Therefore, to overcome the problems related to tea breeding, scientists across the world started finding some alternatives through biotechnological approaches which are discussed subsequently.

2.11 Conclusion

Conventional breeding in tea is well established and contributed significantly. Several region-specific clones and bi-parental hybrids have been developed across the tea growing regions of the world. However, several important aspects of tea breeding are in their initial stages which need to be strengthened. Some of them are: (1) association mapping which has tremendous potential to identify the Quantitative trait loci (QTL) particularly in tea; (2) development of large-scale molecular markers and their utilization in marker-assisted breeding; (3) although pseudo-test cross has been utilized for developing the initial linkage map, it is necessary to develop the bi-parental mapping population for various applications where progress is very much limited in comparison to other similar woody perennials such as coffee and eucalyptus.

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