Chapter 2
Camellia

Tapan Kumar Mondal

2.1 Introduction

The genus *Camellia* includes shrubs and trees belonging to the family Theaceae and is native to eastern Asia. The genus was named by Linnaeus in the honor of Jesuit missionary G. J. Kamel, who first recommended to grow the Japanese rose in Europe. In his systema Naturae of 1735, Linnaeus gave the name *Camellia tsubaki* to the plant found in Japan as the tsubaki and it only acquired its present systematic name, *Camellia japonica* in his *Species Plantarum*.

The center of origin of the genus *Camellia* is in South and southwestern China, centering the provinces of Yunnan, Guangxi and Guangdong straddling the Tropic of Cancer, the area bounded by longitude 85°W and 150°E and latitudes 37°N and 10°S, but its distribution within this area is not uniform. Apparently 90% of *Camellia* species originated in South and Southeast China though some species, viz. *C. japonica* and *C. lancelolata* originated in Japan and Philippines as well as Indonesia, respectively.

In the East, different species of *Camellia* were spread from country to country by Buddhist monks. Their introduction into Europe began some 300 years ago, when the first attempt was to cultivate ornamental *Camellia* plant brought by sea from the East. Those *Camellia* flourished all over the Europe from England to France, Belgium, and Italy and from Portugal to Spain in their acid soil and temperate humid climate. *Camellia* was introduced to the USA at the beginning of the eighteenth century. From England, ornamental *Camellia* was introduced to Australia during the nineteenth century.

The economic importance of the genus *Camellia* is largely due to *C. sinensis*, whose young leaves are used to prepare tea. This single species is the economic backbone of several South Asian countries including India, China, Japan, Korea, Sri Lanka, Indonesia, erstwhile USSR, and African countries such as Malawi, Kenya, etc. Amidst the wild species, economic value of *C. japonica* ranks the highest due to its beautiful ornamental flowers so much so that more than 3,000 cultivars are available now worldwide. Other wild species with ornamental values are *C. reticulata*, *C. sasanqua*, and *C. saluensis*. A few wild species such as *C. oleifera*, *C. semiserrata*, and *C. chekiangolomy* are used to produce oil from their seeds, which is used in Chinese cookery and has pharmaceutical value. Finally, *Camellia* leaves contain a number of substances used in the pharmaceutical industry, including xanthine, teophiline, teabromine, adenine, tearine, and oleic acid.

2.2 Botany

2.2.1 Morphology

Camellias are broad-leaved, evergreen shrubs, which may grow up to a height of 25 ft, but more often to 6–12 ft. It has a spread of 6–10 ft. The dark-green leathery leaves are 4 in. long. The flowers, which

T.K. Mondal (✉)
Biotechnology Laboratory, Uttar Banga Krishi Viswavidhalaya, PO: Pundibari, Dist Cooch Behar 736 165, West Bengal, India
and
National Research Center of DNA Fingerprinting, National Bureau of Plant Genetic Resources, Pusa, New Delhi 110 012, India
e-mail: mondaltk@yahoo.com

C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources*,
DOI 10.1007/978-3-642-21201-7_2,

range in color from white to pink and red, are 3–5 in. in diameter. The flowers generally bloom between September and April either as single, semi-double, or double and are pollinated mainly by bees. Botanically, leaves are coriaceous, pinnately veined, often serrated, petiolated, rarely sessile, and amplexicaul. Flowers are hermaphroditic, solitary or in clusters at the branch terminals or leaf axils, pedicellate or sessile, bracts usually 2–8, sepals usually 5–6, sometimes the differentiation between bracts and sepals is indistinct, becomes perulate to 21 perules, deciduous or persistent, corollas white, red, or yellow, petals 5–12, basically connate, stamens numerous in 2–6 series, outer filament whorl often connate into filament tube adnate to petal bases, anthers dorsifixed or occasionally basifixed, 2-locular, longitudinally cleft, ovaries superior, 3–5 locular, sometimes unilocular, 3–5 valvate usually dehiscent from the top, columella persistent or lacking; seed globose or polygonal, seed coast corneus (Chang and Bartholomew 1984).

2.2.2 Conventional Propagation

Camellia plants are propagated either through seeds or cuttings. Usually mature seeds are collected from mother plant, pre-soaked for 24 h in water and the hard covering around the micropyle should be filed down to leave a thin covering and then sown in polythene sleeves in the nursery where it takes 12–18 months to attain more than 15 cm height before transferring to the field. Nevertheless, seed-grown plants show a high degree of variability. Alternatively, it can be multiplied vegetatively wherein cuttings with an axillary bud are planted in moss peat under shade followed by the transfer of the rooted plants to the field. Two other methods known as layering and grafting are also used for propagation. Layering can be done with the plants of more than 2 year old where leaves and shoots of the branch hanging down are removed. Then using a sharp knife, a small incision is introduced in a single place and the branch down is trained to the ground in such a way to secure the tip in the soil with the clip, which is then covered with wet, moist peat soil. Roots formed after 2 weeks from the incision allow the shoot tip to be separated from the parent plant to be planted individually.

In the nineteenth century, when modern methods of root cutting were not developed, it was common to propagate Camellias by grafting. Normally, crown grafts were made at the base of young *C. japonica* stocks, though different kinds of side cleft graft and approach graft could be used (Laborey 1986). Juvenile grafting onto hypocotyls of seedling has proved to be both very effective and simple to practice (Vieitez and Vieitez 1983). In Japan, particularly in the Camellia Centre of Kyuohu Island, it is common for highly prized bonsai Camellias to be created by grafting onto segments of root 50–60 cm long and 2–3 cm in diameter taken from wild *C. japonica*; flowering 3–4 years later (Vieitez et al. 1991).

2.2.3 Genetic Diversity

The genus *Camellia* had reportedly 40 species in 1920. The number of species was increased to 87 by Sealy 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 (Prince and Parks 2000) that indicates genetical instability and high outbreeding nature of the genus. In a conservative estimation, there are more than 3,000 cultivated varieties of ornamental *Camellia* worldwide of which more than 2,500 have been registered in the American Camellia Society.

The *Camellia* is the largest genus of the family Theaceae. The genus is valued for tea due to the content of caffeine and apurine alkaloids, which act as stimulus for central nervous system of human being. Nagata and Sakai (1984) reported the distribution of caffeine in 23 species of the genus *Camellia*. The caffeine content on a dry weight basis in some of them was as follows: *C. sinensis* var. *sinensis* (2.78%), *C. sinensis* var. *assamica* (2.44%), *C. taliensis* (2.54%), and *C. kissi* (0.02%). *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. Chang and Bartholomew (1984) divided the whole *Camellia* genus into 4 subgenera and 20 sections totally, which are depicted below with the example of some prominent species in each section.

I Subgenus	*Protocamellia*
Section I	*Archecamellia*
	Camelia granthamiana
	Camellia albogigas
	Camellia pleurocarpa
Section II	*Stereocarpus*
	Camellia krempfii
	Camellia dormoyana
	Camellia yunnanensis
	Camellia liberistyla
	Camellia liberistyloides
Section III	*Piquetia*
	Camellia piquetiana
II Subgenus	*Camellia*
Section IV	*Olifera*
	Camellia gauchowensis
	Camellia sasanqua
	Camellia vietnamensis
	Camellia oleifera
Section V	*Furfuracea*
	Camellia integerrima
	Camellia polypetala
	Camellia latipetiolata
	Camellia crapnelliana
	Camellia furfuracea
	Camellia oblate
	Camellia gaudichaudii
	Camellia parafurfuracea
Section VI	*Paracamellia*
	Camellia grijsii
	Camellia confuse
	Camellia kissii
	Camellia lutescens
	Camellia fluviatilis
	Camellia brevistyla
	Camellia obtusifolia
	Camellia maliflora
	Camellia miyagii
	Camellia shensiensis
	Camellia brevissima
	Camellia puniceiflora
	Camellia tenii
	Camellia microphylla
	Camellia phaeoclada
	Camellia weiningensis
Section VII	*Pseudocamellia*
	Camellia szechuanensis
	Camellia chungkingensis
	Camellia trichocarpa
	Camellia ilicifolia
	Camellia henryana
Section VIII	*Tuberculata*
	Camellia tuberculata
	Camellia anlungensis
	Camellia obovatifolia
	Camellia rhytidocarpa
	Camellia litchi
	Camellia parvimuricata
Section IX	*Luteoflora*
	Camellia luteoflora
Section X	*Camellia*
	Camellia omeiensis
	Camellia polydonta
	Camellia lapidea
	Camellia mairei
	Camellia villosa
	Camellia kweichowensis
	Camellia albovillosa
	Camellia albescens
	Camellia tunganica
	Camellia trichosperma
	Camellia phellocapsa
	Camellia semiserrata
	Camellia multiperulata
	Camellia lungshenensis
	Camellia reticulate
	Camellia pitardii
	Camellia hiemalis
	Camellia uraku
	Camellia edithae
	Camellia xylocarpa
	Camellia hongkongensis
	Camellia cryptoneura
	Camellia oviformis
	Camellia compressa
	Camellia setiperulata
	Camellia saluenensis
	Camellia boreali-yunnanica
	Camellia lucidissima
	Camellia magnocarpa
	Camellia japonica
	Camellia subintegra
	Camellia longicaudata
III Subgenus	*Thea*
Section XI	*Corallina*
	Camellia coralline
	Camellia tonkinensis
	Camellia wardii
	Camellia pilosperma
	Camellia fleuryi
	Camellia nitidissimia
	Camellia paucipunctata
	Camellia lienshanensis
	Camellia pentamera
	Camellia scariosisepala
	Camellia acutiserrata
Section XII	*Brachyandra*
	Camellia muricatula
	Camellia szemaoensis
	Camellia pachyandra
	Camellia xanthochroma
	Camellia amplexifolia
	Camellia brachyandra
	Camellia nervosa
	Camellia nematodea
	Camellia gilbertii
	Camellia crassipetala
	Camellia yangkiangensis
	Camellia parviflora
Section XIII	*Longipedicellata*

(*continued*)

(*continued*)

	Camellia amplexicaulis
	Camellia petelotii
	Camellia longipedicellata
	Camellia indochinensis
Section XIV	*Flavae*
	Camellia flava
	Camellia aurea
Section XIV	*Chrysantha*
	Camellia chrysantha
	Camellia flavida
	Camellia impressinervis
	Camellia chrysanthoides
	Camellia tunghinensis
	Camellia pingguoensis
	Camellia pubipetala
Section XV	*Calpandria*
	Camellia lanceolata
	Camellia connata
Section XVI	*Thea*
	Camellia crassicolumna
	Camellia pentastyla
	Camellia taliensis
	Camellia irrawadiensis
	Camellia crispula
Section XVII	*Longissima*
	Camellia longissima
Section XVIII	*Glaberrima*
	Camellia gymnogyna
	Camellia costata
	Camellia yungkiangensis
	Camellia leptophylla
	Camellia pubicosta
	Camellia angustifolia
	Camellia sinensis
	Camellia assamica
	Camellia pubilimba
	Camellia waldenae
	Camellia fangchensis
	Camellia ptilophylla
	Camellia parvisepala
	Camellia glaberrima
	Camellia kwangtungensis
IV Subgenus	*Metacamellia*
Section XIX	*Theopsis or Eriandra*
	Camellia macrosepala
	Camellia cuspidata
	Camellia grandiflora
	Camellia chekiangensis
	Camellia longicuspis
	Camellia crassipes
	Camellia longicalyx
	Camellia forrestii
	Camellia acutisepala
	Camellia buxifolia
	Camellia minutiflora
	Camellia parvicuspidata
	Camellia acutissima
	Camellia subacutissima
	Camellia callidonta
	Camellia handelii
	Camellia triantha
	Camellia costei
	Camellia tsaii
	Camellia synaptica
	Camellia transnokoensis
	Camellia rosthorniana
	Camellia lutchuensis
	Camellia euryoides
	Camellia trichoclada
	Camellia parvilimba
	Camellia brevipes
	Camellia elongate
	Camellia longicarpa
	Camellia parvilapidea
	Camellia stuartiana
	Camellia transarisanensis
	Camellia fraternal
	Camellia dubia
	Camellia percuspidata
	Camellia membranacea
	Camellia rosaeflora
	Camellia campannisepala
	Camellia lancilimba
	Camellia tsingpienensis
	Camellia pubisepala
	Camellia parviovata
	Camellia viridicalyx
	Camellia lancicalyx
	Camellia parvicaudata
	Camellia subglabra
	Camellia nokoensis
	Camellia tsofuii
	Camellia trichandra
	Camellia villicarpa
	Camellia cratera
	Camellia punctata
	Camellia lawii
	Camellia trigonocarpa
	Camellia cordifolia
	Camellia wenshanensis
	Camellia melliana
	Camellia candida
	Camellia caudate
	Camellia assimiloides
	Camellia assimilis
	Camellia edentate
	Camellia salicifolia

(*continued*)

2.2.4 Karyotype and Genome Size

Based upon the analysis by flow cytometry and staining by propidium iodide, the genome size of *Camellia japonica* ($2n = 30$; basic chromosome number, $x = 15$) was found to be 4G bases though the triploids have 1.5 times higher DNA than diploids (Tanaka et al. 2005). Generally, the chromosomes are small in size and tend to clump together due to stickiness. The length of *Camellia* chromosome ranges from 1.28 to 3.44 μm (Bezbaruah 1971). The *r* value (ratio of long

arm to short arm) for all the 15 chromosomes range from 1.00 to 1.91. The consistency in diploid chromosome number suggests a monophyletic origin of all *Camellia* species.

Cytological markers of the genus *Camellia* were elaborately studied in the early 1970s with many interesting features. Chromosome number has been established for the most available taxa of *Camellia* including tea (Beretta et al. 1987), which was reviewed by Kondo (1975).

Karyotypic data of *Camellia* had also been accumulated in past for several species (Fukushima et al. 1966; Ackerman 1971; Kondo 1975). Unfortunately, karyotype grouping by chromosome size was difficult 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 are morpholo gically and quantitatively variable. Thus, karyotypes including characteristics of sat-chromosomes are 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 accessions studied indicated similar karyotypic patterns to each other (Kondo 1975). For instance, *C. japonica* L. var. *spontanea* (Makino), *C. japonica* L. var. *macrocarpa* Masamune, *C. japonica* L. subsp. *rusticana* (Honda) Kitamura and four cultivars including "Aka-Wabisuke," "Fukurin-Wabisuke," "Kuro-Wabisuke" and "Wabisuke" carried same, most common standard acetoorcein-stained karyotype if the presence of satellites is not considered; 16 metacentric, 8 submetacentric, and 6 subtelocentric chromosomes. 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 can be applied to the somatic mid-metaphase chromosomes in *Camellia* taxa. These differentially stained bands in somatic mid-metaphase chromosomes permit the identification of 238 individual chromosomes and make it possible to match the homologous pairs of chromosomes more precisely and possibly even measure chromosome divergence among different clones within the same species with same or similar karyotypes. Karyotypic variability and divergence among the seven accessions of *C. japonica* L. *sensu lato* with same acetoorcein-stained karyotype were revealed by C-banding method (Kondo and Parks 1980). By this way, the cytological marker was used to sort and classify the vast number of cultivars. However, due to the development of more sensitive biochemical techniques, attention was shifted toward the search of biochemical markers.

2.3 In Vitro Culture in *Camellia* Species

2.3.1 Micropropagation

Since the propagation of some *Camellia* species by conentional methods is difficult and slow, other means have been sought. Several reviews on micropropagation of *Camellia* including tea and related species have been published (Kato 1989a; Vieitez et al. 1991; Dood 1994; Das 2001; Mondal et al. 1998). It is evident from the literature that while Bennett (1977) was pioneer for initiation of tissue culture of ornamental Camellias yet, Vieitez et al. (1991) did a systematic study of micropropagation with *C. japonica,* which elaborately highlighted various factors that affect multiplication rate in in vitro and subsequent hardening processes. Depending on the species-specific requirements among the wild *Camellias*, various factors that influence the micropropagation are briefly reviewed below.

2.3.1.1 *C. japonica*

In the late 1970s, the use of in vitro culture methods was suggested as a means to solve the constraints in propagation mainly due to shy rooting in vegetative cuttings (Bennett and Scheibert 1982). Since then, several protocols have been described for the micropropagation of *C. japonica*. The first elaborate report to regenerate plants from shoot tips and axillary buds was made by Creze and Beauchesne (1980), who took meristems with one or two leaf primordial of 0.5 mm long from 1-year-old rooted cuttings or from 3- to 4-year-old seedling and cultured them on a MS medium supplemented with adenine (20 mg/l); IAA (0.1 mg/l); 1 mg/l each of kinetin, BAP, and GA_3; and polyvinylpyrrolidine (10 g/l). Although cultures were established and elongated to produce shoots more rapidly than shoot tips, no rooting and transfer to the soil were not described.

In *C. japonica*, buds of juvenile origin gave consistently better results in terms of both growth and vigor on MS as compared to other macronutrient formulae of Lepoivre (Quoirin and Lepoivre 1977), Knop (Tabachnik and Kester 1977), Schenk and Hildebrandt (1972), and modified Heller (1953). However, regeneration from adult material of *C. japonica* cv. Alba Plena was poor on MS (Vieitez et al. 1989a). In a series of shoot multiplication experiments, Vieitez et al. (1989a) found that WPM was the best among the six macronutrient formula tested (modified Heller 1953), MS, half-strength MS, WPM, Gresshoff and Doy (1972) and Anderson (1984). In contrast to Carlisi and Torres (1986), who found that MS and half-strength MS were the best for culturing of *C. japonica*, the observations recorded by Vieitez et al. (1989a) were poor in these media. The different response observed by Carlisi and Torres (1986) was probably genotype-dependent.

For *C. japonica*, the most widely used cytokinin was BAP (Table 2.1). However, Creze and Beauchesne (1980) reported the importance of 2-iP (1 mg/l) as an essential component for shoot proliferation, kinetin was also found to have no effect on shoot multiplication when used either alone or in combination with BA (Samartin et al. 1984). The GA_3 (5–10 mg/l) was also used for proliferation of shoots in cv. Purple Dawn (Carlisi and Torres 1986; Torres and Carlisi 1986). Among the auxins, IAA and IBA are used for shoot proliferation, but there are no reports on the use of NAA and 2,4-D for culture of *C. japonica* (Creze and Beauchesne 1980; Vieitez et al. 1989b). Apart from plant growth regulators (PGR), another factor, which was found to be important, is the physical condition of the media. In general, liquid medium was more suitable than solid medium for shoot proliferation in *C. japonica* (Carlisi and Torres 1986; Vieitez et al. 1989a).

2.3.1.2 *C. oleifera*

Very little work has been done on this important oil-yielding species. Tian-Ling (1982) used MS medium supplemented with BAP (4 mg/l) and NAA (2 mg/l) for induction of adventitious buds leading to plantlet regeneration. In another study, lateral buds of adult trees were also used by Yan et al. (1984) for induction of axillary bud proliferation.

2.3.1.3 *C. reticulata*

Heller's (1953) macroelements with the addition of $(NH_4)_2SO_4$ (0.13 mg/l) in combination with MS vitamins were found to be the best for induction of axillary buds. WPM was also found to be superior to modified Heller (1953) and the recipes of Anderson 1984 (San-Jose and Vieitez 1990). A combination of BAP and zeatin has also been successfully used for promoting the growth and proliferation of axillary shoots (San-Jose and Vieitez 1990; San-Jose et al. 1991). Multiplication rates in terms of both number of axillary buds and the length of shoots could further be improved by horizontal placement of the explants (San-Jose and Vieitez 1990).

2.3.1.4 *C. sasanqua*

While Torres and Carlisi (1986) preferred MS medium, Samartin (1991) found B_5 (Gamborg et al. 1968) macronutrients supplemented with micronutrients of MS to be suitable for the growth and proliferation of axillary shoots. A combination of BAP and NAA was found to be the most suitable for shoot multiplication in both of these studies.

2.3.1.5 *Camellia* Hybrids

Despite the availability of limited information, the medium of Tukey (1934) was found to be the best for in vitro seedling growth of three different interspecific hybrids including *C. japonica* × *C. cuspidata*, *C. japonica* × *C. reticulata*, and *C. japonica* × *C. saluenensis* (Lammerts 1958). Creze and Beauchesne (1980) made the first attempt to regenerate plants from shoot tips and axillary buds on *C. saluenensis* × *C. chrysantha,* details on which were mentioned in their report.

2.3.2 Rooting and Hardening

Like other woody plants, rooting is a major limitation in micropropagation of *Camellia*. Rooting of in vitro raised shoots was achieved either upon continuous

Table 2.1 Summary of micropropagation studies in *Camellia*

Species/cultivar	Explant	Medium				Remarks	Response time	Reference
		Initiation	Multiplication	Regeneration/ organogenesis	Rooting			
C. saluenensis × *C. japonica*	In vitro shoot	–	–	–	1/2 MS	–	–	Beretta et al. (1987)
C. japonica, Purple Dawn	Shoot tips and nodal segments	1/2 MS + BA (1)	1/2 MS + BAP (1) + GA_3(5)	–	–	–	–	Carlisi and Torres (1986)
C. saluenensis × *C. chrysantha*	Shoot tips of seedlings	MS + Kn (1) + 2ip (1) + GA_3(1) + IAA (1) + PVP (10 g/l)	MS + Kn (1) + 2ip (1) + GA_3 (1) + IAA (1) + PVP (10 g/l)	–	–	–	–	Creze and Beauchesne (1980)
C. japonica	Shoot tips and nodal segments of 3–4-year-old seedlings	MS + Kn (1) + 2ip (1) + GA_3(1) + BA (1) + IAA (1) + Adenine (20) + PVP (10 g/l)	MS + Kn (1) + 2ip (1) + GA_3 (1) + BA (1) + IAA (1) + Adenine (20) + PVP (10 g/l)	–	–	–	–	Creze and Beauchesne (1980)
C. japonica	Shoot tips and nodal segment	MS + modified vit + BA (1) + IAA (0.1)	Same basal medium (MS) + BAP (1)	–	1/2 MS with same supplement	–	10 weeks, 4 weeks	Samartin et al. (1984)
C. japonica	Shoot tips 2–3-month-old seedling	MS + BA (1)	–	–	1/2MS with modified vit + IBA (1 g/l)	18 days dark treatment before placing rooting media enhances rooting	16 days	Samartin et al. (1986)
C. sasanqua, Onigoromo Thunb.	Shoot tips and nodal segments	–	–	–	Gamborg's (B_5) medium	–	Gamborg (B_5) + modified vita + BAP (0.5) + NAA (0.1)	Samartin (1991)
C. reticulata, "Captain Rawes"	Terminal shoot tips and nodes	Heller's (1953) macro + $(NH_4)_2SO_4$ $_{(132.14)}$ + MS vit+ BAP (2) + Zeatin (2) + IBA (0.01) + 2ip (2)	WPM + BAP (2) + Zeatin (2) + 2ip (2) + IBA (0.01)	–	1/2 WPM + dipping in IBA solution (1 g/l) for 30 min	Time of explant collection influenced shoot multiplication	16 weeks, 4 weeks	San-Jose and Vieitez (1990)
C. reticulata, Captain Rawes	In vitro leaf	Heller's (1953) macro + $(NH_4)_2SO_{4(132.14)}$ + vit + BAP (2) + Zeatin (2) + IBA (0.01) + 2ip (2)	WPM + BAP (2)+ Zeatin (2)+ 2ip (2)+ IBA (0.01)	WPM + BAP (2) + IBA (1)	1/2 WPM + sucrose (6%) + agar (0.6%)	Shoots from adventitious origin rooted very poorly in comparison with those axillary origin, under same culture condition	2–3weeks, 10–12 weeks respectively	San-Jose and Vieitez (1992)

(*continued*)

Table 2.1 (continued)

Species/cultivar	Explant	Medium				Remarks	Response time	Reference
		Initiation	Multiplication	Regeneration/ organogenesis	Rooting			
C. reticulata cv. Captain Rawes	Shoot tips and nodes of adult trees	Heller's (1953) macro + 1 mm $(NH_4)_2SO_4$ + MS vit + BAP (2) + Zeatin (2) + IBA (0.01) + 2ip (2)	WPM + BA (2) + Zeatin (2) + 2ip (2) + IBA (0.01). Horizontal position were better than vertical position for shoot multiplication	–	1/2 macro WPM + full micro + vit + sucrose (6%)	–	8 weeks, 16 weeks respectively	San-Jose et al. (1991)
C. oleifera	Immature cotyledons and embryos	–	–	MS + BA (4) + NAA (2)	–	Liquid medium filter bridge support was better for rooting	–	Tian-Ling (1982)
C. sasanqua, Day Dream	Shoot tips, stem segment	MS + BAP (1) + NAA (0.1) for juvenile plant MS + BAP (1) for adult material	1/2 MS + modified MS vit + NAA (0.1) + BAP (2) + GA_3 (5–10) + sucrose (3%)	–	1/2 MS + modified vit	–	8 weeks for shoot proliferation and 8 weeks for plantlet regeneration	Torres and Carlisi (1986)
C. ×williamsii, Debbie	Internode	WPM macro + MS micro + MS vit + BAP (0.5) + IBA (0.01)	–	MS + IBA (0.1) + Phytagel (0.25%) + TDZ (2.75)	–	Phytotoxic levels of antibiotic kanamycin and cefotaxime have been detected	Callusing, plantlet regeneration	Tosca et al. (1996)
C. japonica, Alba Plena	Shoot tips and nodal segment	Vieitez et al. (1989a, b)	Vieitez et al. (1989a, b)	–	WPM macro after dipping in IBA 1 g/l for 15 min followed by 12 days darkness	Supporting media (agar or paper bridge) did not significantly affect rooting	4 weeks	Vieitez et al. (1989a)
C. japonica cv. Alba Plena	Shoot tip (2–4 cm), nodal segment, and whole shoots of field grown plant	Heller's (1953) macronutrient increased by factor 1.25 + 1 mM $(NH_4)_2SO_4$ + MS micronutrient+ BAP (1) + IBA (0.01) + *m*-inositol (100) + Jacquiots vit (Gautheret 1959)	WPM + BAP (2) + Zeatin (2) + 2ip (2) + IBA (0.01)	–	WPMO after giving IBA (1 g/l) treatment for 15 min	The rate of shoot proliferation depends upon the explant used	2–3 weeks for rooting	Vieitez et al. (1989b)
C. oleifera	Lateral buds of adult trees	–	–	–	–	–	–	Yan et al. (1984)

Figures in parenthesis denote concentration (mg/l) *TDZ* Thidiazuron; *vit* Vitamin

exposure to a low concentration of auxin or initially to a less exposure to a high auxin concentration followed by their transfer to an auxin-free medium. In vitro rooting of *Camellia* species has been reported by a number of workers (Table 2.1). Reduction of MS salt concentrations to half-strength favored both induction and elongation of rooting in *Camellia* species (Samartin et al. 1984, 1986; Kato 1985). However, Vieitez et al. (1989b) did not find any significant difference in rooting of in vitro raised shoots of *C. japonica* cv. Alba Plena using half-strength MS medium.

In *Camellia,* IBA (0.5–8 mg/l) has been shown to give better results than NAA for in vitro root initiation. Roots induced by NAA were shorter, thicker, and with accompanying calli, which were undesirable features for the subsequent transplanting. On the other hand, with IBA treatments, rooting occurred much later but were long and fibrous (Samartin et al. 1986).

Liquid medium with filter paper bridge was beneficial for rooting in *C. olerifera* (Tian-Ling 1982; Kato 1985; Nakamura 1987b). Torres and Carlisi (1986) reported that a pulse treatment of shoots with 500 mg/l IBA for 30 min before placing on a root induction medium gave best results in *C. sasanqua*. Beretta et al. (1987) obtained increased rooting in *C. saluenous* × *C. japonica* hybrids with 1–2 g/l IBA treatment for 15 min. In comparison to other woody species, the *Camellia* spp. require higher IBA concentration and longer immersion time (San-Jose et al. 1988) and such high treatments were not as deleterious for Camellias as in other woody species like *Prunus avium* (Riffaud and Cornu 1981). Dark treatment after dipping the shoots in auxin was reported to favor rooting in *C. japonica* (Samartin et al. 1986) and *C. reticulata* (San-Jose and Vieitez 1990). However, Samartin (1991), who also worked with *C. japonica,* did not find any significant difference between the effects of light and dark treatments on rooting.

Rooting mixture alone influences the survival rate at nursery. While 75% survival of *C. japonica* was obtained in petrite:soil (1:1) by Samartin et al. (1984), a higher survival rate of 70–90% of the same species was achieved in soil:quartz (1:1) mixture by Samartin et al. (1986) and Vieitez et al. (1989b). In *C. reticulate,* rooted shoots were transferred to pot containing 1:1 mixture of peat and quartz and placed in a plastic tunnel with mist/fog system in lamps to a give a 16-h photoperiod, which resulted 80% survival (San-Jose et al. 1991).

However, there is no report on either of any nursery performance or any field performance of micropropagated Camellias so far.

2.3.3 Somatic Embryogenesis

Somatic embryogenesis is considered to be the most efficient regeneration system of *Camellia* (Jain and Newton 1990). However, the efficacy of such a system for plant production depends on the multiplication and conversion rate of somatic embryo. The recent biotechnological advances, including gene cloning and gene transfer, offer great promise for rapid improvement of genotypes for desirable traits and integrate well with the technique of somatic embryogenesis. Although somatic embryogenesis has been fully exploited in herbaceous species, there remain difficulties with woody species like *Camellia*. However, it has a tremendous potential in clonal propagation and most importantly in genetic transformation (Mondal et al. 1999). In *Camellia*, it has been successfully used for artificial seed production (Ballester et al. 1997), cryopreservation for long-term storage of germplasm (Janeiro et al. 1996), and some interspecific crosses of *Camellia* (Nadamitsu et al. 1986), where immature somatic embryos were rescued and cultured before abortion. It can also be used for the production of disease-free and androgenic or haploid plants (Pedroso and Pais 1994). The various factors, which govern the somatic embryogenesis of *Camellia* species, are concisely summarized below.

2.3.3.1 Explant Choice

Although somatic embryogenesis has been reported from various explants of ornamental Camellias, most of the workers have, however, used mature cotyledon slices or zygotic embryos (Table 2.2). In *C. japonica* and *C. reticulata*, immature cotyledons and zygotic embryos, roots, stems, and leaves have been used for the induction of somatic embryogenesis (Plata and Vieitez 1990; Vieitez and Barciela 1990; Plata 1993; Pedroso and Pais 1993; Zhuang and Liang 1985a). Dark period of at least 14 weeks appeared to be necessary for somatic embryogenesis from in vitro leaf for *C. reticulata* (San-Jose and Vieitez 1993). Vieitez

Table 2.2 Summary of somatic embryogenesis in *Camellia*

Species and cultivar	Explant	Medium				Reference
		Induction	Maturation	Germination	Multiplication	
C. japonica	Mature cotyledon	MS + BAP (1)	–	–	–	Barciela and Vieitz (1993)
C. japonica	Immature Cotyledon	MS with modified vit + BAP (1–2) + IBA (0–2)	–	–	MS + modified vit + BAP (1)	Barciela and Vieitz (1993)
C. japonica	Mature cotyledon	Knop + BA (0.5–5)	–	–	–	Bennett and Scheibert (1982)
C. japonica	Mature cotyledon	MS + BA (0–5) + IBA (0–2)	–	MS + GA_3 (1)	–	Kato (1986a)
C. sasanqua	Mature cotyledon	MS + BAP (0–10) + IBA (0–2)	–	–	–	Kato (1986b)
C. japonica	Primary somatic embryo	MS + GA_3 (1) + colchicine (0.1%)	–	MS + GA_3(1)	–	Kato (1989b)
C. vietnamensis × *C. chrysantha*	Mature cotyledon	MS + BA (3) + NAA (1)	–	MS + GA_3(1) + Coconut milk (10%)	–	Nadamitsu et al. (1986)
C. sinensis with 13 cultivars, *C. japonica* 3 cultivars, *C. sasanqua*, *C. brevistela*, *C. nokoensis*, *C. japonica* (cv. Kosyougatu) × *C. granthamiana*	Mature sliced cotyledon	MS + BA (1–5)	–	–	–	Nakamura (1988a)
C. japonica cv. Elegans	In vitro leaf	1/2 MS + DTT (2.5) but ferrous sulfate was replaced by ferric citrate (2.5)	Same as induction medium	Full strength, induction media + D-glucose (25 g/l) + BA (1) + IBA or IAA (0.1)	–	Pedroso and Pais (1993)
C. reticulata	Mature and immature cotyledons	MS + IBA (0.5–1) MS + BA (1) + IBA (0.5)	–	MS+GA_3 (3–5) + IAA (1–2)	–	Plata and Vieitez (1990)
C. reticulata cv. Mouchang	Immature zygotic embryo	–	–	–	MS + Modified vit + BAP (0.5) + IBA (0.1)	Plata et al. (1991)
C. japonica "Alba Plena" and *C. retieulata* Mouchang	In vitro leaf	MS + BAP (8) + IBA (0.5)	MS + BAP (8) + IBA (0.5)	MS + $GA_{3(3)}$ + IAA (1)	Secondary somatic embryogenesis was multiplied in MS + 4.4 μM BAP + IBA (0.1)	San-Jose and Vieitez (1993)

C. japonica	Immature and mature zygotic embryo	MS with modified vit + BAP (1–2) + IBA (0–2)	MS with modified vit + BAP (1–2) + IBA (0–2)	MS with modified vit + BAP (1) + (0.1)IBA + GA_3(5) + IAA (2)	MS + GA_3 (1/2) generally induced secondary embryogenisis	Vieitez and Barciela (1990)
C. japonica cv. Alba Plena	In vitro roots	MS with thiamin (1) + nicotinic acid (0.1) + pyrodoxine-Hcl (0.1)	MS with thiamin (1) + nicotinic acid (0.1) +pyrodoxine-Hcl (0.1)	MS+GA_3 (5) + IAA (1)	–	Vieitez et al. (1991)
C. japonica "Alba Plena"	In vitro roots of juvenile origin	MS + Zeatin (1) + BA (0–2) + IBA (0–2)	MS + Zeatin (1) + BA (0–2) + IBA (0–2)	MS+GA_3 (5) + GA_3 (1–2)	MS + IBA (0.1) + BAP (1)	Vieitez et al. (1991)
C. japonica × *C. chrysantha*	Immature zygotic embryos	MS + Kn (0.1–0.5) + NAA (0.5–1) + YE (1)	–	Anderson (1984) basal medium + 2ip (0.2–0.5) + GA_3 (5) + PVP (5 g/l)	–	Yamaguchi et al. (1987)
C. oleifera	Mature cotyledons	–	–	–	–	Yan et al. (1984)
C. chrysantha	Mature cotyledons	MS + BA (1) + NAA (0.2–0.5)	–	–	–	Zhuang and Liang (1985b)
C. sasanqua	Mature cotyledons	MS + BA (1) + NAA (0.2–0.5)	–	MS + BA (2) + IAA (0.5) + ABA (0.2) + glutamine 500 or MS+GA_3 (1)	–	Zhuang et al. (1988)
C. reticulata	Mature cotyledon	MS + BA (1) + NAA (0.2)	–	B_5 or liquid MS + BA (0.1–0.2) + IAA (0.1–0.5)	–	Zhuang and Liang (1985a)

Figures in parenthesis denote concentration (mg/l) *DTT* Dithiothreitol, *PVP* Polyvinylpyrrolidone, *Vit* Vitamin, *YE* Yeast extract

et al. (1991) reported somatic embryogenesis from the in vitro roots of *C. japonica* clones cv. "Alba Plena."

2.3.3.2 Physiological Stage

Successful induction of somatic embryos from cotyledon explants depends upon the physiological maturity of the cotyledons. In *C. japonica,* Vieitez and Barciela (1990) collected seeds in July, September, and October to determine the right stage for induction of somatic embryogenesis. They achieved 94% embryogenesis in seeds collected in September in contrast to 20% of those collected in October under the climatic condition prevailing at Spain. The seeds collected in September were fully grown but were still immature suggesting a transitory dormancy stage.

2.3.3.3 Genotypic Variation

Genotype plays a major role in the induction of embryogenesis. Nakamura (1988a) screened four *Camellia* species including Japanese tea cultivars among which the embryogenic response varied between 0 and 50%. Of all the cultivars screened, the best response was reported in "Yabukita" and "Kurasawa" tea cultivars. Among the other *Camellia* species, high differentiation rates of somatic embryos were obtained with *C. japonica* (48–58%), *C. sasanqua* (9–81%), and *C. brevistela* (93%).

2.3.3.4 Basal Medium and Growth Regulators

The type, concentration, and time of application of different growth regulators in culture media have been extensively worked out. In general, a high cytokinin-to-low auxin or low cytokinin alone was found to be necessary for induction of somatic embryos in *Camellia*, but cytokinins have been often reduced or omitted in subsequent subculturing. In general, direct somatic embryos in *Camellia* can be obtained on a wide range of culture conditions: full to 1/3 strength modified MS, 15–30 g/l sucrose, D-glucose or combinations of both, 0–10 mg/l auxin and 0–10 mg/l cytokinin, in liquid, semi-solid, or solid medium. Light was an important parameter for somatic embryo formation, especially from stem and leaf explants (direct and indirect). Somatic embryos either did not form in the dark or their number was significantly lower than that for cultures under photoperiod. Successful conversion of cotyledon-derived embryos into plants ranged from 35 to 79%, depending on the culture medium used (Pedroso-Ubach 1994). Identical results were obtained for leaves, cultured on modified MS medium supplemented with 20 g/l sucrose or 25 g/l D-glucose, 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), and 0.1 mg/l kinetin (Pedroso-Ubach 1991). Leaves (31%) cultured in MS liquid medium with 1.0 mg/l BAP and 0.5 mg/l 2,4-D produced clusters of 3–23 somatic embryos/leaf. Less frequently (2–3%), globular embryos detached from the remaining differentiated leaf tissues and developed singularly in the liquid culture. Only the embryos arising in clusters developed into plantlets.

Among the PGRs, cytokinin such as BAP (0–10 mg/l) has been widely used for *Camellia* (Table 2.2), though Vieitez et al. (1991) claimed that zeatin (1 mg/l) in combination with BAP and IBA was essential for the induction of somatic embryos in *C. japonica* cv. Alba Plena. In auxins, IBA (0–2 mg/l) was widely used in the induction medium for *Camellia,* though NAA has also been used in different concentrations in different species such as 0–2 mg/l in *C. reticulata* (Zhuang and Liang 1985a), 0.2–0.5 mg/l in *C. sasanqua* (Yamaguchi et al. 1987), 0.5–1.0 mg/l in *C. japonica* × *C. chrysantha*, and 1 mg/l in *C. vietnamensis* × *C. chrysantha* (Nadamitsu et al. 1986).

The other species on which embryogenesis was carried out are *C. brevistela* (Nakamura 1988), *C. chrysantha* (Zhuang and Liang 1985b), *C. oleifera* (Yan et al. 1984), and *C. sasanqua* (Nakamura 1988; Zhuang et al. 1988), and with new hybrids whose development is hindered by poor fertility or embryo viability (Nadamitsu et al. 1986; Yamaguchi et al. 1987). In all these reports, somatic embryogenesis was achieved using MS medium containing a cytokinin (usually BAP) with or without auxin (usually NAA). However, none of these studies involved comprehensive experiments to determine optimum conditions for embryogenesis.

2.3.3.5 Secondary Embryogenesis

There are two kinds of growth patterns for secondary embryogenesis in *Camellia* (1) somatic embryo-to-somatic embryo, commonly known as repetitive

embryogenesis and (2) callus-to-somatic embryo in which multiplication of somatic embryos depends upon subculturing of callus (Vieitez 1994), although the former pathway is more frequent in *Camellia* (Plata and Vieitez 1990; Vieitez and Barciela 1990). The works on secondary embryogenesis in different species of *Camellia* are discussed here.

C. japonica

Secondary embryogenesis has been reported primarily from embryos derived from a wide range of initial explants, i.e., from cotyledons or from excised embryos (Kato 1986a; Vieitez and Barciela 1990), roots (Vieitez et al. 1991), or from in vitro leaves (San-Jose and Vieitez 1993). Primary embryo upon transfer to MS medium with or without growth regulators gave rise to secondary embryogenesis within 3–4 weeks. In general, growth regulators used for *C. japonica* were higher concentration of BAP along with lower concentration of IBA.

C. reticulata

High frequency (65%) secondary embryogenesis was reported in *C. reticulata* on cotyledonary and hypocotyl region of isolated primary embryos by Plata and Vieitez (1990). This response was evinced on a medium containing BAP (0.5 mg/l) and IAA (0.5 mg/l). According to these workers, although a combination of BAP (2 mg/l) and IBA (1 mg/l) provided more embryos per explant, this gave the lowest responsive explant for secondary embryos.

Plata et al. (1991) studied the anatomical sequence of events, which led to the differentiation of secondary embryogenesis in *C. reticulata* cv. Mouchang. They found that embryogenesis occurred mainly on the hypocotyl region of primary embryos. Histological monitoring revealed that secondary embryos apparently had a multicellular origin from embryogenic areas originating in both epidermal and subepidermal layers of hypocotyl region. This morphogenic competence was related to the presence of relatively undifferentiated cells in superficial layers of the hypocotyl of the primary embryo.

2.3.3.6 Developmental Biology of the Somatic Embryo

The origin of somatic embryo originates from either single cell or group of cells, which depends upon the plant. The histological and anatomical aspects of somatic embryogenesis in *Camellia* have been mentioned by Kato (1986), Plata and Vieitez (1990), and Vieitez et al. (1991) without giving details on the cellular events and anatomical changes that occurred during embryogeneis. Barciela and Vieitz (1993) made a detailed study on the origin and anatomical development of somatic embryos differentiated on *C. japonica* cotyledon sections. This study used computer-aided image analysis for cytological quantification, and the measurements of cell starch and protein contents as the stained cell areas by staining with periodic acid Schiff (PAS) stain and by mercuric bromophenol blue, respectively. Barciela and Vieitz (1993) observed that small protuberances or nodules began to appear on the abaxial epidermis of the cotyledons from 7-day-old in vitro tissue. The nodules continued to develop and by 30th day they were 4–6 mm in diameter and became moderately prominent, and bore embryos in several different stages of development. After 2 months of culture, embryos were 6–8 mm long and could be isolated either for germination or secondary embryogeneis. Only the abaxial surface of the cotyledon explants was morphologically competent and had multicellular origin. To determine whether the embryogenic nodules could be maintained indefinitely in culture, they were isolated from the initial cotyledons, removed from in vitro produced somatic embryos, and then cultured for 6 months with monthly transfer to a fresh medium. The parenchymatic tissue of the nodule failed to proliferate or grow and turned progressly necrotic.

The above histological analysis suggests that the nodules associated with the occurrence of somatic embryogenesis can be considered as small localized callus tissue, which is necessary for the redetermination of embryogenic cells. Histological observations of embryogenesis in *C. reticulate* (Plata and Vieitez 1990) suggested that somatic embryos develop directly from cotyledon without any apparent callus phase. However, the differentiation of the embryos was nevertheless related to the developing swollen parts

(Kato 1986), swollen whitish areas, or compact bulging tissue of cotyledon explant (Plata and Vieitez 1990). Such swellings might be equivalent to the nodules observed in *C. japonica* (Vieitez and Barciela 1990; Barciela and Vieitz 1993). Though morphologically visible, the nodules that develop on *C. japonica* cotyledons cannot be ascribed as true callus.

2.3.3.7 Morphology of Somatic Embryo

The morphology of the *Camellia* somatic embryos is influenced by the concentration of cytokinin in the medium. In case of *C. japonica* (Vieitez and Barciela 1990; Vieitez et al. 1991), most embryos could be classified into the following two clearly distinct types (1) seed like embryos, which were yellowish-white with large cotyledons alike to mature zygotic *Camellia* embryos; and (2) bud like embryos, which were green with cotyledons resembling true leaves. They generally developed in media with relatively high BAP concentration. Ammirato (1985) stated that reasonably high levels of cytokinins partially or totally inhibit the development of somatic embryo cotyledons and the shoot apex grows out to form the first mature leaves so that the somatic embryo looks more like a shoot. The observed bud-like embryos may be an example of such cytokinin-induced premature shoot emergence. Anomalies such as polycotyledonary cotyledon, hypertrophy, or fascination were also observed to various extents among both seed-like and bud-like embryos, but both kinds were genuinely bipolar having both shoot and root meristems.

2.3.4 Cold Storage and Cryopreservation

The potential of using in vitro systems for germplasm collection and conservation as well as for multiplication has been broadly discussed in several reviews and feature articles (Kartha 1985; Engelmann 1997). The application of in vitro techniques to germplasm storage is of particular interest for the conservation of plants such as *Camellia* species that are normally propagated vegetatively and/or have recalcitrant seeds.

The storage of *Camellia* seeds in genebanks is problematic because *Camellia* seeds are classified as recalcitrant (Pence 1995). They are sensitive to low temperatures and desiccation and are unable to retain their viability through long-term storage (Kato 1989). Even when they are maintained under moist conditions at 3–5°C, their viability is relatively short-lived (Salinero and Silva-Pando 1986). The most common method for preserving the genetic resources of species with recalcitrant seeds or those vegetatively propagated, is as plants in field genebanks. Limited work has been carried out on cold storage and cryopreservation of *Camellia* with both material obtained ex vitro (seeds) and material cultured in vitro (somatic embryos, embryonic axes, and shoot apices) and are presented in Table 2.3.

Table 2.3 Summary of cold storage and cryopreservation studies in wild *Camellia*

Species	Explant	Storage method	Response	Reference
C. japonica	Somatic embryo clusters	Short- to medium-term storage at 2–4°C	Reduced embryogenic competence after 6 months, improved germination capacity after 2 months	Janeiro et al. (1995)
	Encapsulated somatic embryos	Short- to medium-term storage at 2–4°C	Reduced embryogenic competence after 2 months, 30–40% plant recovery after 2 months	
	Somatic embryos and encapsulated somatic embryos	Cryopreservation	No survival of frozen material	Janeiro et al. (1995)
	Embryonic axes from mature seeds	Cryopreservation after 2–3 h desiccation	100% survival and 40% plant recovery, 18% somatic embryogenesis rate	Janeiro (1996)
C. reticulata	Somatic embryo clusters	Cold storage at 2–4°C	76% germination after 2 months	Chaudhury et al. (1991)

2.3.4.1 Short-Term Storage

The attempts of preserving the wild *Camellia* explants by short-term storage has been made with three different explants such as somatic embryos, shoot tips, and their encapsulated forms, which are elaborated here. The effects of short- to medium-term cold storage on the maintenance of embryogenic capacity and germination of somatic embryos of *Camellia* were investigated by Janeiro et al. (1995). Four embryogenic lines were used: three belonging to *C. japonica* (1, 2 and SY-89) and one to *C. reticulata* cv. Mouchang. Lines 1 and 2 of *C. japonica*, which were used to study survival and the preservation of embryogenic capacity of somatic embryos induced directly on the roots of in vitro grown plantlets (Vieitez et al. 1991). The effect of cold storage on the germination of *Camellia* somatic embryos into whole plants was studied in greater detail (Janeiro et al. 1995). Cold treatment for 8 weeks significantly improves the secondary embryogenesis, but it depends on genotypes. The shoot and root length of the germinated plantlets were also significantly increased by 2 months cold treatment, but it depends on genotypes. However, the incidence of secondary embryogenesis during germination also decreased after cold treatment.

Somatic Embryos

The potential uses for artificial seeds are numerous including storage, handling, and delivery of elite germplasm. The possibility of using cold storage to preserve synthetic *Camellia* seeds was investigated by Janeiro et al. (1996). In that study, the effects of cold storage of *C. japonica* somatic embryos on the maintenance of embryogenic competence and germination of encapsulated embryos were determined. Somatic embryos were encased in sodium alginate (3%) beads made in MS basal medium with 3% sucrose. The beads were then stored for 1–2 months in darkness at 2–4°C. After 1 month, the encapsulated embryos exhibited a significant reduction in both survival rate and competence for secondary embryogenesis, however, additional 1 month cold storage had further little reduction effect. The survival and secondary embryogenesis rates, 68% and 69% respectively, when placed in the maintenance medium following 60 days storage at 4°C were still acceptable. However, the productivity (number of secondary embryos per responsive encapsulated embryo) was dramatically reduced from 62.6 for unstored encapsulated embryos (control) to 5.4 secondary embryos indicating the negative influence of cold.

The reduced competence for secondary embryogenesis of cold-stored encapsulated embryos of *Camellia* appears to reflect increased maturity, since their capacity for germination is better preserved than their embryogenic competence. In this respect, short- or medium-term cold storage of synthetic *Camellia* seeds destined for germination appears to be feasible as long as a 30–50% fall in plant recovery rate could be tolerable. In contrast, cold storage alone cannot be used to maintain embryogenic competence, since the productivity of cold-stored encapsulated embryos is seriously reduced.

Shoot Tips

Ballester et al. (1997) reported almost 100% survival frequencies in seven of the eight clonal shoot cultures of *C. japonica* tested, when stored at 2–4°C for up to 12 months. Shoot tips of *C. japonica* encapsulated in alginate beads and stored at 2–4°C survived for a shorter period of time than uncapsulated ones. Encapsulated material had survival rates of 75, 50, and 10% on 30, 60 and 75 days, respectively.

2.3.4.2 Long-Term Storage and Cryopreservation

Since the embryogenic competence of *Camellia* somatic embryos is clearly not preserved during short-term cold storage under the conditions used in the studies described in previous sections, the feasibility of using cryopreservation techniques was investigated (Janeiro 1996; Janeiro et al. 1996).

In these studies, somatic embryos of *C. japonica* (2–5 mm size) were subjected to several protective pretreatments to prevent the formation of ice crystals inside the cells. Following pretreatment, half of the somatic embryos in each experiment (controls) were placed directly in maintenance medium (MS medium supplemented with 4.40 μM BA and 0.49 μM IBA) and the other half was placed in sterile 2 ml polypropylene cryovials and immersed in liquid nitrogen for

24 h. Somatic embryos were thawed (by immersing the cryovials in water for 1–2 min at 35–38°C) and transferred to the maintenance medium. Both treated and untreated embryos were cultured in a growth chamber under the standard conditions specified above. After 10 weeks, they found that no frozen somatic embryos survived regardless of the desiccation period; however, survival rate of unfrozen embryos was 100% after 15 min of desiccation treatment, which was further reduced to 53% after 2 h of desiccation indicating an acceptable tolerance of these somatic embryos to dehydration (Janeiro 1996).

Janeiro et al. (1996) also investigated the feasibility of cryopreservation of *C. japonica* embryonic axes. The explants were isolated from mature seeds. After sterilization of seeds, the embryonic axes were excised from the cotyledons with 1–2 mm of petiole to protect the plumule, and were either used as such or dehydrated for 1.5 or 3 h in sterile laminar air flow. Half the material was placed directly in MS maintenance medium (controls), and the other half was placed in cryovials and frozen in liquid nitrogen for 24 h before transfer to the same MS medium. They found that the capacity of *Camellia* embryogenic axes to produce somatic embryos, especially on the hypocotyl region, is maintained and even enhanced after the stress produced by cryoexposure.

2.3.5 Haploid Culture

Microspore culture presents a number of potential advantages mainly in relation to in vitro selection strategies and to genetic studies for developing doubled-haploid mapping population, etc. In *C. japonica*, embryogenesis was induced from microspore. Among the various media composition and PGR formulation, MS along with 2,4-D (4.5 μM) and kinetin (0.5 μM) were reported to be the best. The development of microspore derived proembryos was obtained in MS medium supplemented with 2.2 μM BAP and reached the highest level when the microspores were cultured in this medium. However, the development of microspore-derived embryos ceased at maturation stage (Pedroso and Pais 1994) and no further work on this area has been reported so far.

2.3.6 Embryo Rescue

Camellia breeders from all over the world have desired to develop yellow flowered *Camellia*. The discovery of *C. chrysantha* has generated great excitement among the *Camellia* growers and breeders as a potential source for a new range of *Camellia* floral colors. Although numerous interspecific hybridizations have been attempted, crossing of *C. chrysantha* with some other species is very difficult. In this regard, several cultivars of *C. japonica* that contributes to about 70% of the current horticultural needs and *C. chrysantha* with its potential for new color were thought to be especially important. However, probably due to the phylogenetic distance between the two species, the interspecific hybridization is extremely difficult (Yoshikawa and Yoshikawa 1990). Hwang et al. (1992), therefore, did a systematic investigation to understand the nature of reproductive barrier between *C. japonica* and *C. chrysantha* with intra- and interspecific crosses using two different lines of each species. They found that zygote formation and early embryo development were similar in intra- and interspecific crosses. Full size but empty ovules in mature capsules resulted from embryo abortion. Liang et al. (1986) reported that interspecific hybrid embryos of *C. pitardii* var. *yunnanensis* × *C. chrysantha* developed normally, reached torpedo stage, and differentiated normally. However, a complete successful protocol of embryo rescue will be immensely helpful to develop the long-waited yellow-colored *Camellia* using *C. chrysantha* as a source.

2.4 Employment of Markers

2.4.1 Morphological Markers

The progress of *Camellia* breeding has been slowed down due to the lack of reliable selection criteria (Kulasegaram 1980). Though a number of morphological and biochemical markers have been reviewed in the past mainly involving *Camellia sinensis* (Ghosh-Hazra 2001), yet they have only marginally improved the efficacy of selection for desired agronomic traits. Morphological marker such as pollen morphology of

eight species of *Camellia* was examined using light microscope and scanning electron microscope. Results showed that the pollen size in genus *Camellia* was moderate or big mostly showing prolate from the equatorial view and trioblate-circular from the polar view. The aperture was 3-colporate with fine and long colpi and multishaped. The muri and lumina varied in size and shape among the species. The pollen morphology of the genus was relatively identical indicating that the genus *Camellia* was a natural group (Chen et al. 1997). The phylogenic classifications under section *Thea* in genus *Camellia* were briefly reviewed based on flower morphology, tree habit, etc. The geographical distribution and the evolutionary tendency of the species and varieties were also described by Chen et al. (2001).

However, most of the morphological markers are influenced greatly by the environmental factors and hence show a continuous variation with a high degree of plasticity. Therefore, these markers cannot be used to discrete groups for taxonomic identification of tea (Wickremaratne 1981).

2.4.2 Biochemical Markers

Biochemical markers were widely used for characterization of different plant germplasm (Das et al. 2002). Presence of calcium oxalate crystals and their quantity in parenchymatous tissue of leaf petioles, nomenclatured as phloem index, have been suggested to be a suitable criterion for classifying tea hybrids (Wight 1958).

Paper chromatography was also used to investigate the taxonomy of different species of *Camellia* under the section *Thea*. It has been found that species within the *Thea* section of the genus *Camellia* are closely similar in chemical composition, the general pattern of which bears no obvious relationship to the chemical composition of non-*Thea* Camellias (Roberts et al. 1958).

Though *Camellia saluensis* is found to cross readily with *C. japonica,* identification of their F_1 hybrid known as *C.* ×*williamsii* was a challenge for breeders at the young stage. Parks and Case (1968) on the basis of flavor and other coloring compounds using paper chromatography were successful to identify the true hybrid seedlings.

2.4.3 Isozyme Markers

Genetic analysis of isozyme variation was used for cultivar identification in a wide range of plants (Hirai and Kozaki 1986). Similarly, in *Camellia*, isozymes have also been analyzed for studying the genetic tendencies, cultivar identification, and implication in hybrid breeding, which are discussed below.

Wendel and Parks (1982) analyzed 17 isozymes of different cultivars of *C. japonica*. They found that 15 isozymes produced two to nine polymorphic loci while two produced one to three monomorphic bands. Based on the segregation of 12 loci by eight enzymes, they postulated codominant inheritance of single-gene traits. They also suggested that two pairs of genes are linked, that is, aspartate amino-transferase with phosphoglucomutase and 6-phosphogluconate dehydrogenase with phosphoglucomutase. In a further study, the same authors (Wendel and Parks 1983) reported isozyme variations at 15 loci from 12 enzymes with 205 genotypes of *C. japonica*. All loci were polymorphic and a total of 64 alleles were detected. Peroxidase and 6-phophogluconate dehydrogenase (6-PGDN) isozyme were also used to differentiate between varieties of *C. sinensis* and *C. japonica* (Ikeda et al. 1991). They concluded that alcohol dehydrogenase isozymes in *C. japonica* are encoded by two genes *adh*-1 and *adh*-2. Both loci are expressed in seeds and their products are randomly associated with intra- and intergenic dimers. Electrophoresis of leaf extracts produced only the products of *adh*-2. Formal genetic analysis indicated that the two *adh* loci are tightly linked. Most segregations fit the expected Mendelian ratios but in some individuals distorted segregation was also observed (Wendel and Parks 1984). Starch gel electrophoresis was used to score allelic variation at 20 loci in seeds of *C. japonica* collected from 60 populations distributed throughout Japan. In comparison with other plant species, the genetic diversity within the population is very high, that is, 66.2% of loci were polymorphic per population, which gave an average number of 2.16 alleles per locus. They also reported genotypic proportions at most of the loci in majority of all the populations and found a good fit of the Hardy–Weinberg expectations (Wendel and Parks 1985).

2.4.4 Molecular Marker

Due to widespread cultivation of clonal tea by elite planting material, the genetic diversity is diminishing gradually. Therefore, germplasm characterization at molecular level of *Camellia* will help (1) varietal improvement of *Camellia* for agronomically important characters; (2) to preserve the intellectual property right of *Camellia* breeders; (3) identification of individual *Camellia* hybrid cultivar by making a molecular passport; (4) prevention of duplicate entry of different genotypes in *Camellia* gene pool; (5) efficient selection of the varieties for hybridization program, graft compatibility in composite plant production, and so on; and (6) taxonomic classification of *Camellia* genotypes on the basis of molecular markers which is still fragile. The various molecular markers are discussed below.

2.4.4.1 RAPD Markers

Since the discovery of random amplified polymorphic DNA (RAPD) marker (Williams et al. 1990), it is being used for a number of areas in plant taxonomy. In *Camellia*, a considerable amount of work has been carried out.

The genetic diversity and molecular phylogeny of 24 ornamental *Camellia* species and varieties were investigated by RAPD analysis. Fifteen decamer oligonucleotide primers were selected from the 61 screened, which generated a total of 95.3% polymorphism of the amplified bands. The molecular phylogenetic dendrogram of 24 species was constructed using UPGMA that generated two groups, corresponding to 3- and 5-locular ovary in morphology. The genetic relationship and the molecular phylogeny among section *Thea* were discussed by Chen and Yamaguchi (2002).

Maternal inheritance of chloroplast DNA (cpDNA) in some cross progenies between *C. vernalis* and *C. japonica* was investigated using the polymorphism of *atp*H–*atp*I region by RAPDs. The cpDNAs of all *C. vernalis* cultivars showed the same type as those of *C. sasanqua*, and all the progenies from *C. vernalis*, either open-pollinated or crossed, had the same cpDNA type as their maternal plants (Tateishi et al. 2007).

Internal transcribed spacer (ITS) of nrDNA has been widely employed for reconstructing phylogenetic relationships in plants, especially at the species level. In order to assess the efficacy of nrITS in elucidating the interspecific relationships of *Camellia*, Vijayan and Tsou (2008) conducted an experiment with seven closely or distantly related species. Extensive study of *Camellia,* based on *Pfu*-amplified ITS sequences, showed well-resolved interspecies relationships. Thus, the potential of nrITS in deducing the phylogenetic relationships in *Camellia* was demonstrated.

RAPD markers were used for identification of *C. japonica* and related species as well as their hybrids. A wide range of markers such as random 10-oligomer to chloroplast-specific sequences were used and checked with the previously published monogram on *Camellia* for phylogenetic relationship. Finally, the taxonomic classification as mentioned in the Chang's manual for different *Camellia* species was confirmed (Prince and Parks 1997, 2000; Thakor 1997; Tiao and Parks 1997, 2003; Yoshikawa and Parks 2001; George and Adam 2006; Orel et al. 2007).

2.4.4.2 ISSR Markers

Intersimple sequence repeat (ISSR) has been used for genetic characterization of various plant species (Tsumura et al. 1996). Because of the greater length of ISSR primers, they show greater repeatability and stability of map position in the genome while comparing genotypes of closely related individuals (Zietkiewicz et al. 1994).

C. euphlebia, a rare and endangered species of China, is distributed in a small region in the Guangxi province. Wei et al. (2005) studied the level and pattern of the genetic diversity of 84 individuals from natural populations by using 100 ISSRs. Their results indicated a relatively low level of genetic diversity in *C. euphlebia* at the species level and at population level and a relative degree of differentiation among populations. Gene flow among populations was also found to be low. Inbreeding and limited gene flow might be the key factors resulting in the observed genetic structure of *C. euphlebia*. Strategies are proposed for the genetic conservation and management of this species.

C. nitidissima Chi (Theaceae), with its golden-yellow flowers, is a popular ornamental species. Due to deforestation and collection of seedlings, its natural populations have receded greatly in recent decades. Genetic diversity and genetic differentiation of 12 natural populations and one ex situ conserved population of *C. nitidissima* in China were analyzed using ISSR markers. Their study indicated a low level of genetic diversity at both species as well as population levels and a relatively high degree of differentiation among populations in naturally occurring populations. In contrast, the ex situ population contained higher genetic variability compared to each natural population. Thus, they suggest that all the wild *C. nitidissima* populations should be protected in situ (Wei et al. 2008).

2.4.4.3 Microsatellite Markers

Simple sequence repeats (SSRs), known also as microsatellites, are tandemly repeated DNA sequence motifs (usually 2–5 bp long) that are highly polymorphic in plant genomes (Wu and Tanksley 1993). Due to their hypervariability, relative ease of scoring by PCR, codominant nature, and high reproducibility, they are now considered to be one of the most reliable genetic markers.

Ueno et al. (1999) were pioneer to develop the SSRs from *C. japonica*, a closely related species of tea. Out of the total 339 RAPD amplifications, 21 were found to contain microsatellite repeats. Finally, four primer pairs were developed, which yielded single-locus polymorphic amplification products. Using these primer pairs, 53 *C. japonica* ecotypes were genotyped and population genetic parameters were calculated. The following year, Ueno et al. (2000) investigated the spatial genetic structure of *C. japonica* using four of these microsatellite primers. Spatial distribution of individuals was also assessed to obtain an insight into spatial relationships between individuals and alleles. Morisita's index of dispersion plotted 518 individuals of *C. japonica* in a single clump and Moran's *I* spatial autocorrelation coefficient revealed weak genetic structure, indicating a low level of allele clustering among the individuals.

Recently, an initial study of sequence tagged microsatellite site (STMS) variation was undertaken by Matteo et al. (2010) with 132 accessions of *Camellia* spp., which included 24 accessions representing 22 different species or varieties as well as 63 cultivars of *C. japonica*, 33 cultivars of *C. sasanqua*, 7 cultivars of *C.* × *vernalis*, 3 cultivars of *C.* × *hiemalis*, and 2 cultivars of *C. hybrida*. The four primer sets used (MSCJAF37, MSCJAH46, MSCJAF25, and MSCJAH38) successfully amplified polymorphic alleles in all the species analyzed, showing cross-transferability. Overall, 96 alleles were scored. MSCJAH38 primers produced the highest number of bands (30), while MSCJAH46 primers yielded the lowest number (15). The genetic distance between pairs of accessions was estimated on the basis of the Nei coefficient and a principal coordinate analysis was performed. The plot revealed a main differentiation between the *C. japonica* cultivars and the winter Camellias. The distribution of the genetic variation, attributed by AMOVA, particularly highlighted genetic overlap among *C. sasanqua* cultivars and the cultivars belonging to *C.* × *vernalis*, *C.* × *hiemalis*, and *C. hybrida*. The study demonstrated that STMS markers offer a suitable method for detection of genetic variability and molecular study of camellia genotypes.

The two major molecular phylogenetic investigations of the genus *Camellia* (one by Vijayan et al. 2009 with nrITS sequences and the other with nDNA *RPB2* sequences by Xiao and Parks 2003) have provided considerable insight into the interspecies relationships of *Camellia*, which could not be provided by many previous attempts with use of cpDNA sequences (Orel et al. 2003; Yang et al. 2006). These two molecular phylogenetic investigations share many important findings. Both studies revealed the need to revise the existing classifications, both supported the monophyly of sections *Thea* and *Furfuracea* and that the species *C. hongkongensis* should be shifted from section *Camellia* to section *Furfuracea*, and both revealed that sections *Eriandra* and *Theopsis* were closely related and not separable and that species of sections *Tuberculata* and *Chrysantha*, as well as *C. szechuanensis* from section *Pseudocamellia*, were closely related. Finally, results of both studies equally supported the section *Camellia* as polyphyletic. The species from the section *Camellia* formed groupings based on geographical origin and distribution, and species in this section distributed in the southeastern and eastern China, Korea, and Japan are well separated from those in southern and southwestern China.

Nevertheless, both studies disagreed on many points. The most notable disagreement was the monophyly of the section *Paracamellia* defined by Ming (2000) and Sealy (1958), which was supported by Xiao and Parks (2003), but our study showed a bifurcation of the section *Paracamellia* and supported Chang's (1981) creation of the section *Oleifera* from the section *Paracamellia*. A recent study of leaf anatomical characters also supported the separation of *Oleifera* from the section *Paracamellia* (Lin et al. 2008). Other important differences are, first, species of the section *Eriandra* and *Theopsis* formed a monophyletic clade in our tree, but mixed together with species from the section *Camellia* and divided into two well-separated clades in the study by Xiao and Parks (2003). Second, the positions of some species in small sections and isolates differed; for example, *C. amplexicaulis* of section *Longipedicellata* was isolated and was a sister to the clade of *Eriandra* and *Theopsis* in our trees, but was associated with clades of species in sections *Camellia*, *Oleifera*, and *Paracamellia* in the Xiao and Parks (2003) dendrogram trees. Also, *C. yunnanensis* of section *Stereocarpus* was embedded in the clade consisting of sections *Chrysantha* and *Tuberculata* in our trees, but was allied to section *Furfuracea* in the Xiao and Parks (2003) dendrogram trees. These types of conflicts in results are not uncommon in molecular phylogeny and can arise from both analytical and biological factors (Rokas et al. 2003a). Analytical factors that generally affect phylogenetic reconstruction are choice of optimality criterion (Huelsenbeck 1995), data availability (Cummings et al. 1995), taxon sampling (Graybeal 1998), and specific assumptions in the modeling of sequence evolution (Yang et al. 1994). The major biological factor that affects phylogenetic reconstruction is the evolutionary dynamics that may cause the history of the genes under analysis to obscure the history of the taxa (Rokas et al. 2003b).

2.5 Genomic Resources

Genomics and its global expression profile (proteomics) offer an additional advantage for rapid identification of genes and pathway to control important plant traits. Multigenic characters, such as abiotic stress, particularly drought and frost, etc. on being the major production constraints in *Camellia* cultivation could be studied in-depth by employing genomics.

Caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) are two purine alkaloids that are present in high concentrations in some species of *Camellia*. However, most members of the genus *Camellia* contain no purine alkaloids. Tracer experiments using [8-^{14}C] adenine and [8-^{14}C] theobromine showed that the purine alkaloid pathway is not fully functional in leaves of purine alkaloid-free species. In five species of purine alkaloid-free *Camellia* plants, sufficient evidence was obtained to show the occurrence of genes that are homologous to caffeine synthase. Recombinant enzymes derived from purine alkaloid-free species showed only theobromine synthase activity. Unlike the caffeine synthase gene, these genes were expressed more strongly in mature tissue than in young tissue (Mariko et al. 2009).

Among the wild species, *C. oleifera* yields 55% oil from its kernel, which is considered to be one of the best plant oils, as it contains ~90% unsaturated fatty acids (~80% oleic acid, ~10% linoleic acid and linolenic acid). As the enzyme controlling the first-step desaturation during the biosynthesis of plant unsaturated fatty acids, stearoyl-ACP desaturase (SAD) directly regulates the proportion of saturated fatty acids and unsaturated fatty acids by dehydrogenating saturated fatty acids bonded to ACP (acyl carrier protein) to form oleic acids. Therefore, Zhang et al. (2008) cloned the full-length cDNA of *C. oleifera* SAD (CoSAD) gene, which will help in future for genetic improvement on other oil plants.

Partial cDNA sequences of three anthocyanin biosynthetic genes (*F3H*, *flavanone 3-hydroxylase*; *DFR*, *dihydroflavonol 4-reductase*; *ANS*, *anthocyanidin synthase*) were isolated from the petals of *C. japonica*. Their deduced partial amino acid sequences shared high homologies with those of woody plant species (CjF3Ha, 98.0%; CjF3Hb, 91.2%; CjDFR, 99.0% with *Camellia sinensis*; CjANS, 90.3% with *Rhododendron* × *pulchrum*). Some important amino acid residues for enzymatic activities were also conserved in the isolated clones, suggesting that the genes were the homologs of *C. japonica* (Tateishin et al. 2010).

C. reticulata is found as diploid, tetraploid, as well as hexaploid and hence expected to have a complex genome. Physical maps of the 18S–26S rDNA ribosomal RNA genes (rDNA) were generated by fluorescent in situ hybridization (FISH) for *Camellia*

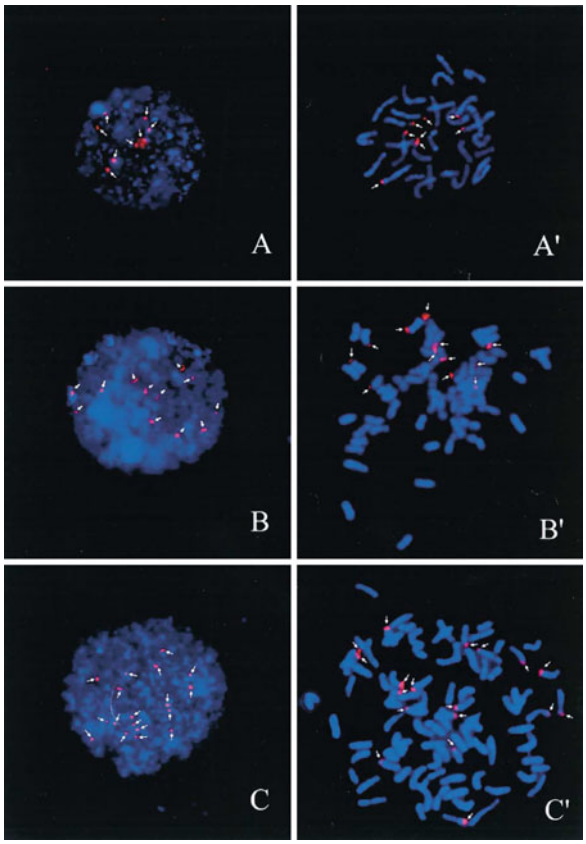

Fig. 2.1 Fluorescent in situ hybridization of *Camellia* interphase nuclei and metaphase chromosomes with the 18S–26S rDNA probe (*red color*) and blue fluorescence shows DNA counterstained with DAPI. The *white arrows* point the site of signals. (A, A′) The hybridization signals of diploid *C. reticulata*. (B, B′) The hybridization signals of tetraploid *C. reticulata*. (C, C′) The hybridization signals of hexaploid *C. reticulata* (Source: Gu and Xiao 2003)

reticulata polyploid complex, including three types of ploidy of *C. reticulata* and its related species, *C. japonica*, *C. yunnanensis*, *C. pitardii*, and *C. saluenensis*. An advanced method was used for preparing chromosome spreads (Fig. 2.1). Eight, twelve and eighteen rDNA sites were observed on the genomes of diploid, tetraploid, and hexaploid *C. reticulata,* respectively. Eight, four, five, and four rDNA loci were located on the chromosomes of *C. pitardii*, *C. japonica*, *C. saluenensis*, and *C. yunnanensis,* respectively. The number and position of rDNA sites in these species were compared for analysis. The results support some of the earlier phylogenetic speculation about this complex genome and suggest the occurrence of some structural rearrangements in chromosome (Gu and Xiao 2003).

2.6 Future Thrust

So far, genetic improvement of *Camellia* has mainly been achieved by conventional breeding. However, in the past three decades, serious attempts have been made to intervene in some of the conventional breeding approaches employing biotechnology. It is noteworthy to mention that since the work of Bennett (1977), the pioneer of *Camellia* micropropagation, the technique has been worked out well for various applications, albeit not commercially exploited. This is perhaps due to the fact that vegetative propagation techniques are well established and cost effective. On the other hand, many aspects of somatic embryogenesis have been studied in detail for ornamental *Camellia* including the regeneration pathway. Despite the fact that transgenic technology has tremendous scope for *Camellia*, surprisingly no transgenic plants have been developed so far. However, it is evident now among the different techniques of gene transfer, *Agrobacterium tumefacians*-mediated transformation has been attempted by different groups in tea, which therefore will be suitable for *Camellia* also. So far, DNA markers are concerned, several DNA markers have been used to make fingerprints, which need to be documented systematically and should be made available for public use to preserve the intellectual property rights of *Camellia* breeders. Although in several ways molecular biology of *Camellia* can be directed, yet priority should be given to the followings:

- Undertake a massive germplasm characterization effort across the world through a common "*Camellia* germplasm characterization consortium," which already exists for several similar crops
- DNA markers need to be identified to do early selection at nursery stage for various biotic (such as flower blight) and abiotic stresses (such as cold hardiness), which will revolutionize *Camellia* breeding where works suffer due to the lack of selection criteria and long gestation periods
- To develop the molecular markers for hybrid identification
- To generate and characterize the expressed sequence tags of *Camellia*

Lastly, the researches on transgenic *Camellia* need to be carried out to address some of the problems of the *Camellia* nursery growers by exploiting the highly regenerative system, which is fortunately available for *Camellia*.

References

Ackerman WL (1971) Genetic and cytological studies with *Camellia* and related genera. Technical Bulletin No 1427, USDA, US Government Printing Office, Washington DC, USA, 115 p

Ammirato PV (1985) Patterns of development in culture. In: Henke RR, Hughes KW, Constantin MP, Hollaender A (eds) Tissue culture in forestry and agriculture. Plenum, New York, USA, pp 9–29

Anderson WC (1984) A revised tissue culture medium for shoot multiplication of rhododendron. J Amer Soc Hort. Sci 109:343–347

Ballester A, Janeiro LV, Vieitez AM (1997) Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica* and *Camellia reticulate* Lindley. Sci Hortic 71:67–78

Barciela J, Vieitz AM (1993) Anatomical sequence and morphometric analysis during somatic embryogenesis on cultured cotyledon explants of *Camellia japonica* L. Ann Bot 71:395–404

Bennett WY (1977) Tissue culture for Camellias? Am. Camellia Yearb: 188–190

Bennett WY, Scheibert P (1982) In vitro generation of callus and plantlets from cotyledons of *Camellia japonica*. Camellia J 37:12–15

Beretta D, Vanoli M, Eccher T (1987) The influence of glucose, vitamins and IBA on rooting of *Camellia* shoots in vitro. In: Abstracts of symposium on vegetative propagation of woody species, Italy, p 105

Bezbaruah HP (1971) Cytological investigation in the family Theaceae – I. Chromosome numbers in some Camellia species and allied genera. Caryologia 24:421–426

Carlisi JC, Torres KC (1986) In vitro shoot proliferation of *Camellia* 'Purple Dawn'. HortScience 21:314

Chang HT (1981) A taxonomy of the genus *Camellia*. Acta Scientarum Naturalium Universitatis, Sunyatseni, Monographic series, vol 1, pp 1–180

Chang H, Bartholomew B (1984) *Camellias*, Basford, London pp 67–72

Chaudhury R, Radhamani J, Chandel KPS (1991) Preliminary observation in the cryopreservation of desiccated embryonic axes of tea (*Camellia sinensis*) L.O.Kuntze seeds for genetic conservation. Cryoletters 12:31–36

Chen L, Yamaguchi L (2002) Genetic diversity and phylogeny of tea plant (*Camellia sinensis*) and its related species and varieties in the section Thea genus *Camellia* determined by randomly amplified polymorphic DNA analysis. J Hortic Sci Biol 77:729–732

Chen L, Tong Q, Gao Q, Jilin S, Fulian Y (1997) Obsebation on pollen morphology of 8 species and 1 variety in the genus *Camellia*. J Tea Sci 17:183–188

Chen L, Yu F, Lou L, Tong Q (2001) Morphological classification and phylogenic evolution of section Thea in the genus *Camellia*. In: Proceedings of international conference on O-Cha culture and science, 5–8 Oct 2001, Shizuoka, Japan, p 37

Creze J, Beauchesne MG (1980) *Camellia* cultivation in vitro. Int Camellia J 12:31–34

Cummings MP, Otto SP, Wakeley J (1995) Sampling properties of DNA sequence data in phylogenetic analysis. Mol Biol Evol 12:814–822

Das SC (2001) Tea. In: Parthasarathy VA, Bose TK, Deka PC, Das P, Mitra SK, Mohandas S (eds) Biotechnology of horticultural crops, vol 1. Naya Prokash, Calcutta, India, pp 526–546

Das A, Gosal SS, Sidhu JS, Dhaliwal HS (2002) Biochemical characterization of induced variants of potato (*Solanum tuberosum* L.). Indian J. Genet. 62: 146–148

Dood AW (1994) Tissue culture of tea (*Camellia sinensis* (L.) O. Kuntze) – A review. Inter J Trop Argic 12:212–247

Engelmann F (1997) In vitro conservation research activities at the International Plant Genetic Resources Institute (IPGRI). Plant Tiss Cult Biotechnol 3:46–52

Fukushima E, Iwasa S, Endo N, Yoshinari T (1966) Cytogenetics studies in *Camellia*. I. Chromosome survey in some Camellia species. Jpn J Hortic 35:413–421

Gamborg O, Miller R, Ojima K (1968) Nutrient requirement suspensions cultures of soybean root cells. Exp Cell Res 50: 151–158

Gautheret (1959) La culture des tissus végétaux : techniques et réalisations. Masson Edit

George O, Adam M (2006) Investigation into the evolutionary origins of Theaceae and genus *Camellia*. Int Camellia J 38: 78–89

Ghosh-Hazra N (2001) Advances in selection and breeding of tea – a review. J Plantation Crops 29:1–17

Graybeal A (1998) Is it better to add taxa or characters to a difficult phylogenetic problem? Syst Biol 47:9–17

Gresshoff PM, Doy C H (1972) Development and differentiation of haploid *Lycopersicon esculentum*. Planta 107:161–170

Gu Z, Xiao H (2003) Physical mapping of the 18S-26S rDNA by fluorescent in situ hybridization (FISH) in *Camellia reticulata* polyploid complex (Theaceae). Plant Sci 164: 279–285

Heller R (1953) Recherches sur la nutrition minerale des tissus vegetaux cultives in vitro Annales des Sciences Naturelles (Bot) Biol Veg 14:1–223

Hirai M, Kozaki I (1986) Isozymes of citrus leaves. In: Kitaura K, Akihama T, Kukimura H, Nakajima H, Horie M, Kozaki I (eds) Development of new technology for identification and classification of tree crops and ornamentals. Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Government of Japan, Tokyo, pp 73–76

Huelsenbeck JP (1995) Performance of phylogenetic methods in simulation. Syst Biol 44:17–48

Hwang Y-J, Okubo H, Fujieda K (1992) Pollen tube growth, fertilization and embryo development of *Camellia japonica L.* × *C. chrysantha* (Hu) Tuyama. J Jpn Soc Hortic Sci 60: 955–961

Ikeda N, Kawada M, Takeda Y (1991) Isozymic analysis of *Camellia sinensis* and its interspecific hybrids In: Proc. Inter. Symp.of Tea Science, Shizuoka, Japan, Aug. 26–28 (98)

Jain SM, Newton R J (1990) Prospects of biotechnology for tea improvement. Proc Indian Natl Sci Acad 6: 441–448

Janeiro LV (1996) Almacenamiento en frio de especies lenosas propagandas in vitro. PhD Thesis, University of Santiago de Compostela, Santiago de Compostela, Espana

Janeiro LV, Ballester A, Vieitez AM (1995) Effect of cold storage on somatic embryogenesis systems of *Camellia*. J Hortic Sci 70:665–672

Janeiro LV, Ballestter A, Vieitez AM (1996) Cryopreservation of somatic embryos and embryonic axes of *Camellia japonica* L. Plant Cell Rep 15:699–703

Kartha KK (1985) Meristem culture and germplasm preservation. In: Kartha KK (ed) Cryopreservation of plant cells and organs. CRC, Boca Raton, FL, USA, pp 115–134

Kato M (1985) Regeneration of plantlets from tea stem callus. Jpn J Breed 35:317–322

Kato M (1986a) Micropropagation through cotyledon culture in *Camellia japonica* L. and *Camellia sinensis* L. Jpn J Breed 36:31–38

Kato M (1986b) Micropropagation through cotyledon culture in *Camellia sasanqua*. Jpn J Breed 36:82–83

Kato M (1989a) Polyploids of *Camellia* through culture of somatic embryos. Hortic Sci 24:1023–1025

Kato M (1989b) *Camellia sinensis* L. (Tea): in vitro regeneration. In: Bajaj YSP (ed) Biotechnology in agriculture and forestry, vol 7: Medicinal and aromatic plants II. Springer, Berlin, Germany, pp 82–98

Kondo K (1975) Cytological studies in cultivated species of *Camellia*. PhD Thesis, University of North Carolina, Chapel Hill, NC, USA, 260 p

Kondo K, Parks CR (1979) Giemsa C-banding and karyotype of *Camellia* (banded karyotypes can tell more detail on inter and intra-specific relationships in *Camellia*). Am Camellia Yearb 34:42–47

Kondo K, Parks CR (1980) Giemsa C-banding and karyotype of *Camellia*. In: Proceedings of international Camellia congress, Kyoto, Japan, pp 55–57

Kulasegaram S (1980) Technical developments in tea production. Tea Q 49:157–183

Laborey J (1986) Les camellias. Flamarion, La maison rustique, Paris, France, pp 1–238

Lammerts WE (1958) Embryo culture in *Camellia* seed germination. In: Tourje EC (ed) Camellia Culture (171–174) Southern California Camellia Society, Pasadena, California

Liang H, Zhang Z, Zhang X (1986) Investigation of the sexual process in interspecific crosses between *Camellia pitardii* var. *yunnanesis* and *C. chrysantha*. Acta Bot Yunn 8:147–152

Lin XY, Peng QF, Tang X, Hu ZH (2008) Leaf anatomy of *Camellia* sect. *Oleifera* and sect. *Paracamellia* (Theaceae) with reference to their taxonomic significance. J Syst Evol 46:183–193

Mariko I, Naoko K, Kouichi M, Natsu T, Misako K (2009) Occurrence of theobromine synthase genes in purine alkaloid-free species of *Camellia* plants. Planta 229:559–568

Matteo C, Marinoni T, Daniela VS (2010) Microsatellite-based genetic relationships in the genus *Camellia*: potential for improving cultivars. Genome 53:384–399

Ming TL (2000) Monograph of the Genus *Camellia*. Kunming Institute of Botany, Chinese Academy of Sciences, Yunnan Science and Technology Press, Kunming, China

Mondal TK, Bhattacharya A, Sood A, Ahuja PS (1998) Micropropagation of tea using thidiazuran. Plant Growth Reg. 26:57–61

Nadamitsu S, Andoh Y, Kondo K, Segawa M (1986) Interspecific hybrids between *Camellia vietnamensis* and *C. chrysantha* by cotyledon culture. Jpn J Breed 36:309–313

Nagata T, Sakai S (1984) Differences in caffeine, flavanols and amino acids contents in leaves of cultivated species of *Camellia*. Jpn J Breed 34:459–467

Nakamura Y (1987b) In vitro rapid plantlet culture from axillary buds of tea plant (*C. sinensis* (L.) O. Kuntze). Bull. Shizuoka Tea Expt Station 13:23–27

Nakamura Y (1988) Efficient differentiation of adventitious embryos from cotyledon culture of *Camellia sinensis* and other *Camellia* species. Tea Res J 67:1–12

Orel G, Marchant AD, Wei CF, Curry AS (2007) Molecular investigation and assessment of C. azalea (syn. C.changii Ye 1985) as potential breeding material. Int Camellia J 39: 64–75

Orel G, Marchent A, Richards G (2003) Evolutionary relationships of yellow-flowered *Camellia* species from Southeast Asia. Int Camellia J 35:88–96

Parks CR, Case KF (1968) Chromatographic evidence for the genetic contamination of *Camellia saluenesis* in cultivation. American Camellias Year book, pp 124–134

Pedroso MC, Pais MS (1993) Direct embryo formation in leaves of *C. japonica* L. Plant Cell Rep 12:639–643

Pedroso MC, Pais MS (1994) Induction of microspore embryogenesis in *Camellia japonica* cv. Elegans. Plant Cell Tiss Org Cult 37:129–136

Pedroso-Ubach MC (1991) Contribuicao para a preservacao e o melhoramento de *Camellia japonica* L. Master's Thesis (English abstract). Faculdade de Ciencias da Universidade de Lisboa, Lisboa, Portugal, pp 23–50

Pedroso-Ubach MC (1994) Somatic embryogeneisis in *Camellia japonica* L. a search for markers. PhD Thesis, Faculdade de Ciencias da Universidade de Lisboa, Lisbon, Portugal

Pence VC (1995) Cryopreservation of recalcitrant seeds. In: Bajaj YSP (ed) Biotechnology in agriculture and forestry, vol 32, Crypreservation of plant germplasm I. Springer, Berlin, Germany, pp 29–50

Plata E (1993) Morphogenesis in vitro de *Camellia reticulata*: Proceson de embryogenesis somatica regeneration de plants. Doctoral Thesis, University of Santiago de Compostela, Santiago de Compostela, Espana

Plata E, Vieitez AM (1990) In vitro regeneration of *Camellia reticulata* by somatic embryogenesis. J Hortic Sci 65:707–714

Plata E, Ballester A, Vieitez AM (1991) An anatomical study of secondary embryogenesis in *Camellia reticulata*. In vitro Cell Dev Biol Plant 27:183–189

Prince L, Parks CR (1997) Evolutionary relationships in the tea subfamily Theoideae based on DNA sequence data. Int Camellia J 29:135–144

Prince L, Parks CR (2000) Estimation on relationships of Theoideae (Theaceae) inferred from DNA Data. Int Camellia J 32:79–93

Quoirin M, Lepoivre P (1977) Improved media for in vitro culture of *Prunus* sp. Acta Horti 78:437–442

Riffaud JL Cornu D (1981) Utilization de la culture in vitro pour la multiplication de merisiers adultes (*Prunus avium* L.) selectionnes en foret. Agronomie 1:633–640

Roberts EAH, Wight W, Wood DJ (1958) Paper chromatography as an aid to the taxonomy of thea Camellias. New Phytol 57:211–225

Rokas A, King N, Finnerty J, Carroll SB (2003a) Conflicting phylogenetic signals at the base of the metazoan tree. Evol Dev 5:346–359

Rokas A, Williams BL, King N, Carroll SB (2003b) Genome scale approaches to resolving incongruence in molecular phylogenies. Nature 425:798–804

Salinero MC, Silva-Pando FJ (1986) La multiplication delas camellias. In: Diputacion provincial de Pontevedra (ed) La Camellia. C Peon. Pontevedra, Spain, pp 175–184

Samartin A (1991) Potential for large scale in vitro propagation of *Camellia sasanqua* Thunb. J Hortic Sci 67:211–217

Samartin A, Vieitez AM, Vieitez E (1984) In vitro propagation of *Camellia japonica* seedlings. HortScience 19:225–226

Samartin A, Vieitez AM, Vieitez E (1986) Rooting of tissue cultured camellias. J Hortic Sci 61:113–120

San-Jose MC, Ballester A, Vieitez AM (1988) Factors affecting in vitro propagation of *Quercus robus* L. Tree Physiol 4:281–290

San-Jose MC, Vieitez AM (1990) In vitro regeneration of *Camellia reticulata* cultivar "Captain Rawes" from adult material. Sci Hortic 43:155–162

San-Jose MC, Vieitez AM (1992) Adventitious shoot regeneration from in vitro leaves of adult *Camellia reticulate*. J Hortic Sci 67:677–683

San-Jose MC, Vieitez AM (1993) Regeneration of *Camellia* plantlets from leaf explant cultures by embryogenesis and caulogenesis. Sci Hortic 54:303–315

San-Jose MC, Vidal N, Vieitez AM (1991) Improved efficiency of in vitro propagation of *Camellia reticulata* cv. captain leaves. J Hortic Sci 66:755–762

Schenk RU, Hildebrandt A (1972) Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can J Bot 50:199–204

Sealy JR (1958) A revision of the genus *Camellia*. Royal Horticultural Society, London, UK

Tanaka T, Mizutani T, Shibata M, Tanikawa N, Parks CR (2005) Cytogenetic studies on the origin of *Camellia* × *vernalis*. V. Estimation of the seed parent of *C.* × *vernalis* that evolved about 400 years ago by cpDNA analysis. J Japan Soc Hor. Sci 74:464–468

Tabachnick L, Kester DE (1977) Shoot culture for almond and almond peach hybrid clones in vitro Hort Sci 12:545–547

Tateishi N, Ozaki Y, Okubo H (2007) Occurrence of ploidy variation in Camellia vernalis. J Fac Agric Kyushu Univ 52: 11–15

Tateishin N, Ozaki Y, Okubo H (2010) Molecular Cloning of the Genes Involved in Anthocyanin biosynthesis in *Camellia japonica*. J Fac Agric Kyushu Univ 55:21–28

Thakor BH (1997) A re-examination of the phylogenetic relationships within genus *Camellia*. Int Camellia J 29:131–135

Tian-Ling LU (1982) Regeneration of plantlets in cultures of immature cotyledons and young embryos of *Camellia oleifera* Abel. Acta Biol Exp Sin 15:393–403

Tiao JX, Parks CR (1997) Identification of closely related *Camellia* hybrid and mutant using molecular markers. Int Camellia J 29:111–116

Tiao JX, Parks CR (2001) Research for a new classification system for the genus *Camellia*. Int Camellia J 33:109–112

Tiao JX, Parks CR (2003) Molecular analysis of the genus *Camellia*. Int Camellia J 35:57–65

Torres KC, Carlisi JA (1986) Shoot and root organogenesis of Camellia sasanqua Plant. Cell Rep 5:381–384

Tosca A, Pondofi R, Vasconi S (1996) Organogenesis in *Camellia x williamsii:* cytokinin requirement and susceptibility to antibiotics. Plant Cell Rep 15:541–544

Tsumura Y, Ohba K, Strauss SH (1996) Diversity and inheritance of inter-simple sequence repeat polymorphism in douglus fir (*Pseudotsuga menziessi*) and sugi (*Cryptomeria japonica*). Theor Appl Genet 92:40–45

Tukey HB (1934) Artificial culture methods for isolated embryos of deciduous fruits. Amer Soc Hort Sci Proc 32:303–322

Ueno S, Yoshimaru H, Tomaru N, Yamamoto S (1999) Development and characterization of microsatellite markers in *Camellia japonica* L. Mol Ecol 8:335–336

Ueno S, Tomaru N, Yoshimaru H, Manabe T, Yamamoto S (2000) Genetic structure of *Camellia japonica* L. in an old-growth evergreen forest, Tsushima. Jpn Mol Ecol 9: 647–656

Vieitez AM (1994) Somatic embryogenesis in *Camellia* spp. In: Jain S, Gupta P, Newton R (eds) Somatic embryogenesis in woody plants. Kluwer Academic, Dordrecht, Netherlands, pp 235–276

Vieitez AM, Barciela J (1990) Somatic embryogenesis and plant regeneration from embryonic tissues of *Camellia japonica* L. Plant Cell Tiss Org Cult 21:267–274

Vieitez ML, Vieitez AM (1983) Propagation of camellias by the hypocotyls grafting. American Camellias Year book, pp 1–4

Vieitez AM, Barciela J, Ballester A (1989a) Propagation of *Camellia japonica* cv. Alba Plena by tissue culture. J Hortic Sci 64:177–182

Vieitez AM, San-Jose MC, Ballester A (1989b) Progress towards clonal propagation of *Camellia japonica* cv. Alba Plena by tissue culture techniques. J Hortic Sci 64:605–610

Vieitez AM, San-Jose C, Vieitez J, Ballester A (1991) Somatic embryogenesis from roots of *Camellia japonica* plantlets cultured in vitro. J Am Soc Hortic Sci 116:753–757

Vijayan K, Tsou CH (2008) Technical report on the molecular phylogeny of *Camellia* with nr ITS: the need for high quality DNA and PCR amplification with *Pfu*-DNA polymerase. Bot Stud 49:177–188

Vijayan K, Zhang WJ, Tsou CH (2009) Molecular taxonomy of Camellia (Theaceae) inferred from nrITS sequences. Am J Bot 96:1348–1360

Wei X, Wei JQ, Cao HL, Li H, Ye WH (2005) Genetic diversity and differentiation of *Camellia euphlebia* (Theaceae) in Guangxi, China. Ann Bot Fenn 42:365–370

Wei X, Hong-Lin C, Yun-Sheng J, Wan-Hui YE, Xue-Jun GE, Feng LI (2008) Population genetic structure of *Camellia nitidissima* (Theaceae) and conservation implications. Bot Stud 49:147–153

Wendel JF, Parks CR (1982) Genetic control of isozyme variation in *Camellia japonica* L. J Hered 73:197–204

Wendel JF, Parks CR (1983) Cultivar identification in *Camellia japonica* L. using allozyme polymorphisms. J Am Soc Hortic Sci 108:290–295

Wendel JF, Parks CR (1984) Distorted segregation and linkage of alcohol dehydrogenase genes in *Camellia japonica* L. (Theaceae). Biochem Genet 22:739–748

Wendel JF, Parks CR (1985) Genetic diversity and population structure in *Camellia japonica* L. (Theaceae). Am J Bot 72: 52–65

Wickremaratne MR (1981) Variation in some leaf in tea (*Camellia sinensis* L.) and their use in the identification of clones. Tea Q 50:183–189

Wight W (1958) The agrotype concept in tea taxonomy. Nature 181:893–895

Williams JGK, Kubelik AR, Livak KJ, Rafaliski JA, Tingey SV (1990) DNA polymorphism amplified by arbitory primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535

Wu KS, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellite in rice. Mol Genet 241: 225–235

Xiao TJ, Parks CR (2003) Molecular analysis of the genus *Camellia*. Int Camellia J 35:57–65

Yamaguchi S, Kunitake T, Hisatomi S (1987) Interspecific hybrid between *Camellia japonica* cv. choclidori and *C. chrysantha* produced by embryo culture. Jpn J Breed 37: 203–206

Yan MQ, Ping C (1983) Studies on development of embryoids from the culture cotyledons of *Thea sinensis* L. Sci. Silv. Sin. 19:25–29

Yan MQ, Ping C, Wei M, Wang YH (1984) Tissue culture and transplanting of *Camellia oleifera*. Sci Silvae Sin 20: 341–350

Yang Z, Goldman N, Friday A (1994) Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. Mol Biol Evol 11:316–324

Yang JB, Li HT, Yang SX, Li DZ, Yang YY (2006) The application of four DNA sequences to studying molecular phylogeny of *Camellia* (Theaceae). Acta Bot Yunn 28: 108–114

Yoshikawa N, Parks CR (2001) Systematic studies of *Camellia japonica* and closely related species. Int Camellia J 33: 117–121

Yoshikawa K, Yoshikawa N (1990) Inter-specific hybridization of *Camellia*. Bull Seibu Maizuru Bot Inst 5:56–75

Zhang DQ, Tan XF, Xie LS, Chen HP, Qiu J, Hu FM (2008) The cDNA cloning and characteristic of stearoyl-acp desaturase gene of *Camellia oleifera*. Acta Hortic 769:55–61

Zhuang C, Liang H (1985a) In vitro embryoid formation of *Camellia reticulata* L. Acta Biol Exp Sin 18:275–281

Zhuang C, Liang H (1985b) Somatic embryogenesis and plantlet formation in cotyledon culture of *Camellia chrysantha*. Acta Bot Yunn 7:446–450

Zhuang C, Duan J, Zhou J (1988) Somatic embryogenesis and plantlets regeneration of *Camellia sasanqua*. Acta Bot Yunn 10:241–244

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple-sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics 20: 176–183

http://www.springer.com/978-3-642-21200-0

Wild Crop Relatives: Genomic and Breeding Resources
Plantation and Ornamental Crops
Kole, C. (Ed.)
2011, XXVII, 303 p., Hardcover
ISBN: 978-3-642-21200-0