Medicinal and Aromatic Plant Science and Biotechnology ©2007 Global Science Books

Anther Culture in Tea Improvement

Thayamini H. Seran

Department of Agronomy, Faculty of Agriculture, Eastern University, Chenkalady, Sri Lanka

Correspondence: **thayaseran@yahoo.com**

ABSTRACT

The important economic species in the genus *Camellia* is *C. sinensis*, which is a high priority crop in Sri Lanka and is cultivated commercially for its tender leaves. Therefore, breeding methods in tea should be directed towards leaf improvement. Improvement of tea depends on the presence of adequate genetic variability. Anther culture plays an important role in crop improvement programs to overcome barriers experienced in conventional breeding methods. Thus this technique enhances plant breeding and speeds up the release of superior cultivars within a short time in large quantities. Several workers have attempted to produce haploids that could be used for future improvement of woody plants, i.e. tea, rubber etc. In this paper, the work carried out by various workers in relation to tea improvement and potential of *in vitro* anther culture technique are discussed.

Keywords: anther culture, haploids, *in vitro*, improvement, tea
Abbreviations: BAP, benzyl aminopurine; **2,4-D**, 2-4 dichlorophenoxyacetic acid; **GA**, gibberellic acid; **IAA**, indole-3-acetic acid; **IBA**, indole-3-butytic acid; **MS**, Murashige and Skoog; **TRI**, Tea Research Institute

CONTENTS

INTRODUCTION

Tea, a perennial crop is cultivated commercially for its tender leaves, which are used as a beverage worldwide. Some species of the genus *Camellia* are grown as garden ornamentals while others, such as *Camellia sasanqua* (Thunb) are cultivated for its seed oil. The only important economic species is *Camellia sinensis*, which was introduced to Sri Lanka from India in 1839. However, the first commercial planting of tea was undertaken by James Taylor only in 1867 on 19 acres of land at Loolecondera Estate in Hewaheta, Sri Lanka (Nathaniel 1986). The present tea extent in Sri Lanka is about 210,000 ha producing 317.2 million kg as at 2005 (Anon 2005), which is the highest on record so far. Sri Lanka's economy is based mainly on agriculture, which brings the country's foreign exchange. Among agricultural exports, tea is one of the major export commodities.

NEED FOR IMPROVEMENT IN TEA

In Sri Lanka, tea plants were raised from seed until 1950, later almost all plantations were established with clonal tea (Anandappa *et al*. 1988). As tea is virtually self-sterile tea populations show considerable heterogeneity to select outstanding seedling bushes for vegetative propagation. Thus the Tea Research Institute (TRI) commenced selection of high yielding cultivars within existing genotypes. Initially, there were over 200 approved cultivars. Of these, only about 10-15 cultivars are popularly used, mostly the TRI 2020 series (Anandappa 1986). Later the TRI released several selections in the recent past for planting. These selections have been designated as the 3000 and 4000 series. These were obtained from specially bred populations using progenitor cultivars. The yield of these clonal teas in the higher and the lower elevations is around 2400 and 6700 kg/ha/year, respectively (Anon 1995). With the advent of high yielding clonal materials, biclonal and polyclonal seed gardens have also been established with the aim of obtaining better seed for estate planting (Anandappa 1986).

Received: 31 July, 2007. Accepted: 6 September, 2007.

Invited Mini-Review

Table 1 Tea production of major producing countries in 2004 and 2005.

	Production (Metric tons ,000 kg)	
Country	**2004**	**2005**
India	892.6	927.9
China	834.2	934.8
Sri Lanka	308.1	317.2
Kenya	324.6	328.6
Indonesia	164.8	165.8
Turkey	165.0	135.0
Japan	100.3	100.0
Vietnam	97.0	109.0
Bangladesh	55.6	58.6

Source: International Tea Committee Report (2005).

The biggest tea producers in the world are India and China followed by Kenya and Sri Lanka (**Table 1**). In the past three decades, there has been a fluctuation in tea production. Half of the world's tea production stems from India and China but they export about a quarter of their production; meanwhile Sri Lanka exports about 90% of its production. There is a greater demand for Sri Lankan teas internationally. However, the national average yield at 1,645 kg/ha in 2005 was much less than the average yield of competing countries such as Kenya which was found to rank highest yield at 2,325 kg/ha among the major tea producing countries. An Assam type of tea is the predominant variety grown in Kenya and Sri Lanka due to its high yield potential. In Sri Lanka, the average yield in the smallholder sector was 1,867 kg/ha in comparison to 1,358 kg/ha in the estate sector in 2005 (Anon 2005). It was noted that the smallholders, who are mainly concentrated in the low elevations, have high yielding vegetatively propagated tea, compared to the estate sector, which still has a substantial are under low yielding old seedling tea plantations (Anon 2001) and also there is depletion of soil fertility consequent to sloping land terrain especially in high and mid grown area (Anon 2005). Therefore, yield has been increased with the help of improved planting materials, balanced fertilizer application and by integrated pest management methods even though the land area is limited. Triploid clones produced larger and heavier shoots than diploids (Wachira 1994). Fertilizer application under the canopy is a desirable supplement to the space between hedges (Mitsuaki *et al*. 1999) meanwhile ammonium sulphate produced 15-20% more yield than corresponding urea treatment (Salardini 1978). Urea is normally used by the growers.

NEED FOR *IN VITRO* ANTHER CULTURE IN TEA IMPROVEMNT

From the time of seed planting, free crossing between Assam and China bushes took place resulting in a number of hybrid varieties, which show superior productivity and survival value. They were also able to withstand adverse soil and climatic conditions and therefore, received widespread acceptance (Bezbaruah 1974). Subsequently, the hybrid varieties were slowly eliminated when tea growers and selectionists unconsciously began to select broad leaf Assam types for planting (Anandappa 1986). As the demand for genetically uniform and agronomically superior planting material is increasing, the creation of new and commercially desirable cultivars becomes essential and this can be achieved only by a proper breeding program. Tea is cultivated commercially for its tender leaves, which are vegetative organs. Therefore, breeding methods in tea should be directed towards leaf improvement. Improvement of tea depends on the presence of adequate genetic variability. The greater the variability, the greater is the scope for improvement (Singh and Chakraborty 1993).

Tea is grown under various types of soil and climatic conditions with different pest and disease problems. Therefore, there is an urgent need for a greater number of high yielding cultivars possessing a combination of desirable characters (Anandappa 1986). These can be obtained by introduction, selection hybridization and genetic manipulation. "Indigenous Assam" tea seeds were introduced to Sri Lanka in 1839 from India and have been grown with success since the 1870s. However, today competition between tea exporting countries and import/export restrictions hamper the use of introduced materials. Further it would also be necessary to retest the material locally available as the effect of the environment on the performance and adaptability of tea clone has been known and demonstrated (Wickramaratne 1981). Selection of a potential cultivar is a long and laborious process. It takes about 8-10 years before the first clones are released for experimental estate planting and also it has limited potential to improve yields further because of the nature of the available seed tea material (Anandappa 1986).

The superior cultivars for commercial plantings have been selected from the seedling progenies obtained from hybridization, which combine high yield potential, leaf quality, pest and disease resistance, etc. However, self incompatibility, low seed setting ability of high yielding clones like TRI 2024, TRI 2025 and DT 1 (Anandappa *et al*. 1988), quick loss of viability of seeds and its long life cycle have been considered as obstacles in tea breeding programs. Further conventional breeding is labour-intensive, costly and time-consuming under field conditions. Therefore, application of biotechnology plays an important role in crop improvement programs to overcome barriers experienced in conventional breeding methods. Thus this technology enhances plant breeding and speeds up the release of superior clones within a short time in large quantities. Transformation technology, whose success is based on tissue culture techniques, is a useful technique for tea plant breeding to overcome pest and disease problems, etc. Generally plant tissues or protoplasts cannot be easily established for many woody plants (Matsumoto and Fukui 1998).

IN VITRO ANTHER CULTURE TECHNIQUE IN TEA IMPROVEMENT

In vitro culture technique plays a prime role in the establishment of plantations (Bajaj 1986). This technique offers an alternate tool for rapid multiplication of plants than traditional means (Murashige 1974) and also it can be used to obtain large number of plants from a rare individual or a mutant of exceptional merit or sterile hybrid (Singh 1978). Tissue culture techniques have a wide application in breeding programs, shortening the process to obtain and select new clones with interesting characteristics and also to meet increasing demand in the world. Improvement of tea using cellular and molecular biology techniques was difficult, because cell culture procedures were not well established 15 years ago (Jain and Newton 1990) but recent advances in molecular biology have seen an explosive improvement in tea breeding, molecular genetics and genetic engineering (Chen *et al*. 2006a, 2006b). Hitherto, lack of trained personnel in the different areas of biotechnology as well as the lack of sophisticated laboratory facilities has greatly retarded advancement in this area of activity in Sri Lanka. But, today tissue culture techniques are used in Sri Lanka not only as a tool in research, but also in commercial production.

Haploid plants are of great importance in genetics and plant breeding research. Because of the difficulty in obtaining pure lines in out-breeding, self incompatible plants like tea and undue delays in producing homozygous inbred lines (pure lines) through inbreeding, the scientists have been searching for naturally occurring or artificially induced haploids to accelerate plant breeding programs. Naturally occurring haploids of tea are extremely rare in nature due to their poor survival value (Singh 1978). However, haploid plants could be obtained by using anther culture and unpollinated ovule culture techniques. But culturing female tissue of angiosperms is difficult because of the inaccessibility of reproducing tissue and the complex nature of the embryo sac (von Aderkas and Dawkins 1993).

Table 2 Anther culture media for *Camellia* spp. and their *in vitro* growth responses.

Explant	Medium	Product	Reference
Camellia sinensis			
Anther	MS + Kin (2 mg/l) + 2,4-D (0.5 mg/l)	Calli	Chen 1987
Callus	N6 + Kin (2 mg/l) + 2,4-D (0.5 mg/l) + L-glutamine (800 mg/l) + L-serine 100 (mg/l)	Shoots	Chen 1987
Shoots	N6 + Zeatin (2 mg/l) + adenine (20 mg/l) + lactoprotein hydrolysate (10 mg/l)	Plantlets	Chen 1987
Callus	MS + thiamine.HCl (1.3 mg/l) + 10^{-5} M NAA + 10^{-5} M Kin	Roots	Doi 1981
Anther	½ MS + 2,4-D (2 mg/l) + BAP (1 mg/l)	Callus	Seran *et al*. 1999
Callus	½ MS + BAP (1 mg/l)	Meristemoids	Seran *et al*. 1999
Camellia japonica			
Anther	MS + *Myo*-inositol (100 mg/l) + L-glutamine (800 mg/l) + L-serine (200 mg/l)	Calli and embryos	Pedroso and Pais 1994
Anther	MS + 2,4-D (4.5 μM) + Kinetin (0.5 μM) + L-glutamine (800 mg/l) + L-serine (200 mg/l)	Calli	Pedroso and Pais 1994
Anther	MS + *myo*-inositol (100 mg/l) + BA (2.2 μM) + L-serine (200 mg/l)	Embryos	Pedroso and Pais 1994

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; BA, benzyladenine; BAP, 6-benzyl amino purine; Kin, Kinetin; MS, Murashige and Skoog (1962); NAA, naphthalene acetic acid; N6 medium (Chu 1978).

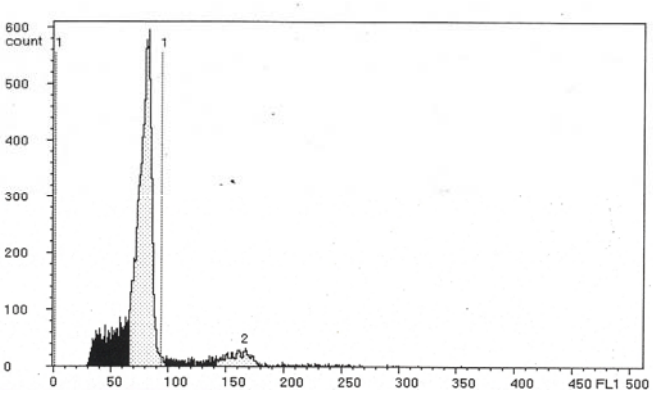


Fig. 1 The DNA histogram and peak analysis (Seran 1998). 1, n; 2, 2n.

Anther culture was introduced by Guha and Maheshwari (1964) using *Datura innoxia* (Solanaceae) as experimental material. In the past much progress has been made in anther culture of herbaceous plants. But success has been limited with woody plants largely due to a generally poor *in vitro* response (Hyun *et al*. 1986). Haploid culture of tree species has been reviewed extensively (Chen 1987; Hutchinson and Zimmerman 1987; Rohr 1987; Bonga and Aderkas 1988; Wang *et al*. 1988; Teixeira da Silva 2006). Much progress has been made in the induction of haploid plants in economically important forest and fruit trees in China (Chen 1987). Most of the cultivated forms of tea are diploids (2n=30). Besides diploids, many polyploids/aneuploids have been reported in tea (Janaki Ammal 1952; Bezbaruah 1971; Chaudhuri and Bezbaruah 1985). In tea, Katsuo first described anther culture in 1969. Okana and Fuchinone (1970) reported differentiation of anther callus into a radiated structure in which vascular bundles were found. Further work on the differentiation rate of the root derived from tea anther cultures has been described by Doi (1981). Zhenguang and Huihuo (1987) further described an elaborate technique through which plantlets were differentiated from anther callus (**Table 2**). In their experiments, callus was induced on the N6 medium devised by Chu (1978) supplemented with kinetin (2 mg/l), 2,4-dichlorophenoxyacetic acid 2,4-D (0.5 mg /l), L-glutamine (800 mg/l) and serine (100 mg/l). Differentiation was initiated on the N6 basal medium supplemented with zeatin (2 mg/l), adenine (20 mg/l) and lactalbumin hydrolysate (10 mg/l). In an experiment to regenerate haploids from cultured anther of Sri Lankan tea cultivar, Seran *et al*. (1999) reported that two levels of ploidy were present in callus where the percentage of haploid cells was more (68%) than that of diploid (6%) as shown in **Fig. 1**. The attempt has been made to produce haploids from cultured anthers of Sri Lankan tea cultivars.

FACTORS AFFECTING ANTHER CULTURE

In the above overview, there is a need to study the process of haploidization. The production of haploids through anther culture is dependent on number of factors that influence androgenesis *in vitro*, such as donor plants, pretreatment of buds, microspore stage, culture medium and other factors. This review focuses on anther culture of tea and also other species as a lesson for the production of haploids.

Donor plants

The genotype of the donor plant plays a significant role in determining the frequency of haploid plant production. When anthers are cultured *in vitro*, all rice varieties do not respond equally in producing callus and in regeneration of plants. This could be due to genetic or environmental characteristics of different varieties (Guha 1973). Rubber anthers used for inoculation must have well developed microspores and the anthers from male sterile varieties are inappropriate for inoculation (Chen 1984). In early spring and hot summer, some varieties often have a lot of unviable microspores in their anthers as a result of the influence of unfavorable environmental conditions.

The response of the pollen in culture decreases with plant age and this response is also influenced by photoperiod, temperature and the nutrient status of the plants (Sunderland 1978). Anthers taken from plants grown under short day (8 hours/day) conditions and high light intensity regime show relatively better response than under long day (16 hours/day) donor plants at the same intensity. Pollen embryogenesis can be further improved if temperature under short day conditions is maintained at 18°C and treatment of plants with pesticide (Razdan 1993). The nitrogen status of plants greatly affects the yield of microspore embryos (Sunderland 1978). Nitrogen-starved plants gave better results than those supplied with fertilizer (Dunwell 1986). In most species investigated (Tobacco: Dunwell 1976; Rice: Lupotto 1982; Oil seed rape: Thurling and Chay 1984) the first flowers or spikes produce anthers that give higher yields than those produced later in the flowering season. Of the five Sri Lankan tea cultivars tested for the callus growth in anthers, TRI 2043 exhibited relatively more callus formation from anthers cultured in medium with 2,4-D and BAP (benzylaminopurine) grown in light, followed by TRI 2023, TRI 2024, TRI 2025 and TRI 777 (Seran *et al*. 1999).

Pretreatment of floral buds

The temperature treatment is required to arrest existing metabolism in order to shift it towards the new pathway of embryogenesis instead of the usual formation of mature pollen (Razdan 1993). The buds were generally maintained in a refrigerator for short period before the anthers were excised. The optimum temperature and duration of pretreatment vary with species and it has been found that results also depend upon the type of explant and storage vessel used

(Huang and Sunderland 1982). Nitsch (1974) demonstrated that buds treated with cold temperature at 3°C or 5°C for 72 hours induced approximately 58% anthers to yield pollen embryo in some solanaceous members (*Datura*, *Nicotiana*) against 21% in anthers from buds maintained at 22°C for the same period.

Stage of microspores/pollen development

The optimum stage of pollen development at the time of harvest of the buds is of vital importance, but differs between species. Some workers believe that the most productive anthers are those which contain uninucleate microspores midway between release from the tetrad and the first pollen grain mitosis (Sunderland 1974; Niizeki 1977; Zhu *et al*. 1980). For maximum production of pollen plants, the appropriate stage of microspore development varies with plant species. Sunderland (1982) summarized that anthers of some species (post mitotic: tobacco) gives the best response if pollen is cultivated at first mitosis or later stages, whereas in most offers (premitotic: barley, wheat and rice) anthers are most productive when cultured at the uninucleate microspore stage. Anthers at a very young stage (containing binucleate mother cells or tetrads) and late stage (containing binucleate starch filled pollen) of development are generally ineffective although exceptions to the rule are known. On the other hand, *Brassica* species show better response when mature anthers or pollen are isolated from them and cultured (Sunderland 1982).

Androgenesis occurs when a microspore or pollen is induced to shift from a gametophytic pathway to a sporophytic pathway of embryo formation. This shift may occur prior to division of the microspore (premitotic) that forms the binucleate pollen grain or after microspore mitosis (postmitotic) wherein either vegetative cell or generative cell (or both) divide to undergo androgenesis (Bhojwani and Bhatnagar 1990). This is one of the most crucial aspects of the culture process since small differences in developmental age produce great differences in yield. For example, in tobacco a difference of 2 mm in corolla length may lead to a four fold difference in the yield of pollen plantlets (Dunwell 1976). According to Chen (1977), the calli recovered from differentiating rice pollen grains at early and mid uninucleate stages showed an excellent capacity to regenerate green plants, with a minimum number of albino plants. Therefore a better understanding of microspore development stage within the anther would be useful in haploid production. The exact stage of microspores is determined by a cytological examination. But for large-scale program many authors prefer to rely on a simply measured, external morphological indicator such as bud length. The microspores at mid-uninucleate stage appears to be the most responsive for callus induction from anthers excised from unopened floral buds (5 mm in length) of Sri Lankan tea cultivar, TRI 2025 (Seran *et al*. 1998).

Anther culture medium

Among the many factors, culture media affect the ability of pollen grains to produce calli and subsequently their plant development. Plant *in vitro* culture media is generally composed of several groups of components: inorganic, growth regulators and organic salts with or without a gelling agent.

Inorganic salts

Chu (1978) studied the factors that affect callus and plant production from rice anthers developed on N6 medium. He found that rice anthers were sensitive to the total nitrogen concentration. A low nitrogen concentration (490 mg/l) was beneficial for callus production whereas a higher concentration was inhibitory. Chen (1984) studied the effect of chemicals containing nitrogen on the development of microspores of rubber. He reported that the decrease of NO_3^-ion concentration in MS (Murashige and Skoog 1962) medium is favorable for the development of pollen embryos. On the contrary, the differentiation of somatic tissues of anthers requires a lower level of NH_4^+ is favorable for development of pollen embryos, because the induction of pollen embryos requires a higher concentration of total nitrogen (50.58-60.05 mM appears to be adequate). Dunwell (1986) recommended the salt mixtures for the Solanaceae and cereals be half-strength MS medium and the N6 mixture (Chu 1978), respectively. The explants turned brown before or after culture that retarded the callus induction (Sarwar 1985). Inorganic salts of MS mixture at low concentrations reduced explant browning. A beneficial effect of low salt concentration was also noted in cultured anthers of cv. TRI 2025. The results showed that callus growth was generally more in half MS media with various combinations of auxin and cytokinin than in MS media (Seran *et al*. 1998).

Growth regulators

Auxin and cytokinin together are necessary for good callus formation (Sarwar 1985). The ratio of auxin and cytokinin may have a pivotal role in plant tissue culture development (Skoog and Miller 1957). These two phytohormones interact *in vitro* to regulate cell division and differentiation. Generally *in vitro*, higher levels of the two promote callus growth (cell division) but at lower levels differentiation of adventitious shoot meristems may be initiated. A higher concentration of non-phenoxy auxin [indole-3-acetic acid (IAA), α-naphthalene acetic acid (NAA) or indole-3-butytic acid (IBA)] alone often promote root initiation rather than shoots whereas phenoxy auxin (2,4-D) promotes callus growth and embryogenesis. Cytokinin overcomes apical dominance of shoots and stimulates axillary bud growth to give shoot multiplication. Most members of the Solanaceae do not require an auxin in contrast to members of the Gramineae and Crucifereae where 2,4-D is the auxin usually supplied (Dunwell 1986). Some species (*Zea mays* and *Pennisetum americanum*) require an auxin and/or cytokinin to induce androgenesis (Nitsch *et al*. 1982).

MS medium supplemented with both cytokinin and auxin appears to be the most useful and widely used medium for the anther culture of woody plants (Chen 1987). The addition of both cytokinin and auxin to the basic medium is necessary for this development of pollen embryoids and pollen calli, but the kind and level of hormones required varies with different species of trees. Marin and Gella (1991) described that callus growth from sour cherry anthers was obtained in media composed of mineral salts and vitamins (Miller 1965) and growth regulators. A combination of 2,4-D and BA proved to be better than those of IAA, Kinetin or Zeatin. In rubber anther culture, three hormones [kinetin, NAA and gibberellic acid (GA)] are required in the differentiation medium (Chen 1984). Optimal concentration of GA promoted growth of the embryos and subsequent cotyledon formation.

Organic salts

Sucrose is an essential constituent of the culture medium where the tissue in the culture is unable to photosynthesise for itself. It is generally used at a range of 2-4% (w/v) (Razdan 1993). Originally, agar-based media were used for anther culture but then liquid media were recommended (Wernicke and Kohlenbach 1976) because agar contains compounds inhibited pollen embryo production (Kohlenbach and Wernicke 1978). A further advantage of liquid culture is that it allows the release of microspore derivatives into the medium, therefore reducing possible competition effects between developing embryos. A disadvantage in that sinking of these embryos may lead to inhibitory anaerobic conditions. Semisolid media would be used for anther culture to anchor the explants and to determine the polarity.

Culture conditions

Anther cultures are maintained in alternating periods of light (12-18 hours; 5000-10,000 lux m^2) at 28°C and in darkness (12-6 hours) regimes depending on individual systems. In responsive anthers, the wall tissues gradually turn brown and after 3-8 weeks burst open due to the pressure exerted by the growing pollen callus or pollen plant (Razdan 1993). Saha and Bhattacharya (1992) reported that anthers (from flower buds of 0.762-0.832 cm diameter collected early in the flowering season) with pollen at the uninucleate stage, were grown initially in the dark (for 2-5 weeks) and then in the light (1,500 Lux) on agar-gelled B_5 medium containing 2,4-D (0.1 mg/l) NAA (0.1 mg/l) kinetin (0.1 mg/l) glutamine (400 mg/l) and 7% sucrose at pH 5.5 and 24°C. They observed that shoot apices originated from the intervening pollen calli of tea anthers when post culture they were exposed to 35°C for 5 days prior to their transfer. The effect of elevated temperature may have helped switch off the microspore development from the male gametophytic pathway facilitating microspore regeneration.

Other factors

The tissue of the anther wall plays an important role in the induction of initial sporophytic division in pollen development (Sunderland 1978; Dunwell 1978). The time of transfer of callus to differentiation medium is critical (Chen 1984). After 20-25 days in culture, the anthers of rubber become filled with meristematic parenchymatous cells derived from the somatic tissues of anthers. He further mentioned that most of the mitotic metaphases of these callus cells are diploid (36 chromosomes). However, after 50 days of culture the somatic calli senesce while the embryos and calli that originated from microspores begin to grow vigorously and divide rapidly. At that point, about 70% of the cells have the haploid chromosome number (18 chromosomes). This time appears to be the ideal time to transfer the callusing anthers to differentiation medium to develop haploids embryos.

DEVELOPMENT OF HAPLOIDS

The microspores divide into two daughter cells that contribute to sporophyte development. The determination into either androgenic (gametophytic) or embryogenic (sporophytic) grains is possibly a function of differential gene activity (Razdan 1993). Androgenic pollen is larger and deeply staining but embryogenic pollen is smaller and weakly staining (Rashid 1983). Whatever its direct or indirect origin, somatic embryogenesis was obtained from single cells which, according to Williams and Maheswaran (1986), can be designated as predetermined embryogenic cells. It is important to note that these predetermined cells did not possess the cytological characteristics of meristematic cells (Nougarede 1967). Chen (1984) mentioned that anthers of rubber are inoculated on a medium to induce callus formation in which the microspores grow and develop into multicellular masses, haploid embryos or pollen calli under favorable conditions. There are two different processes that exit simultaneously: the formatiom of somatic calli and the initiation of microspore development into small embryos or pollen calli.

Scientists are still making attempts to produce elite cultivars because of its economic importance and high demand. Among the potential benefits to tea improvement programs rapid production of homozygous lines from heterozygous breeding lines, obtaining gametaclonal variations and early expression of recessive genes, anther culture technique plays an important role in breeding program. By doubling their chromosome numbers, homozygous diploids can be produced in a much shorter time. Preservation of germplasm, now a costly task in maintaining large tree collections, would be simplified with the establishment of gene banks by cryopreservation of *in vitro* materials (Bajaj 1986; Sakai 1986). Hence anther culture technique has the great potential in tea improvement programmes to develop a "Golden clone" and to meet its ever increasing demand in the world.

ACKNOWLEDGEMENT

The author thanks the Director and staff of Tea Research Institute, Talawakelle, Sri Lanka for collecting the materials to prepare this manuscript.

REFERENCES

Anandappa TI (1986) Planting material. In: Sivapalan P, Kulasegaram S, Kathiravetpillai A (Eds) *Hand Book on Tea*, Tea Research Institute of Sri Lanka, Talawakelle, pp 6-19

Anandappa TI, Nanayakkara R, Solomon HR (1988) Seed setting abilities of some Sri Lankan tea clones and their implications for tea breeding. In: *Proceedings of the Regional Tea (Scientific) Conference*, Colombo, Sri Lanka, pp 73-87

Anon (1995) Annual Report of the Tea Research Institute, Talawakelle, Sri Lanka, 304 pp

Anon (2001) Annual report of the Central Bank of Sri Lanka, Colombo, part I, 150 pp

Anon (2005) Annual report of the Central Bank of Sri Lanka, Colombo, part I, 150 pp

Bajaj YPS (1986) *In vitro* preservation of genetic-resources techniques and problems. In: *International Symposium on Nuclear Techniques and In Vitro Culture for Plant Improvement*, IAEA/FAO, Vienna, pp 43-57

Bezbaruah HP (1971) Cytological investigations in the family Theaceae. 1. Chromosome numbers in some *Camellia* species and allied genera. *Caryologia* **24**, 421-426

Bezbaruah HP (1974) Tea breeding - A review. *Indian Journal of Genetics and Plant Breeding* **34A**, 89-100

Bhojwani SS, Bhatnagar SP (1990) Embryology of Angiosperms, Vikas Publishing Co., New Delhi, 370 pp

Bonga JM, Aderkas PV (1988) Attempts to micropropagate mature *Latrix deciduas* Mill. In: Ahuja MR (Eds) *Somatic Cell Genetics of Woody Plants*, Kluwer Academic Publishers, pp 155-168

Chaudhuri TC, Bezbaruah HP (1985) Morphology and anatomy of aneuploid and polyploid tea (*Camellia sinensis* (L.) O. Kuntze. *Journal of Plantation Crops* **13**, 22-30

Chen C (1977) *In vitro* development of plants from microspores of rice. *In Vitro* **13**, 484-489

Chen L, Yao M-Z, Yang Y-J, Yu F-L (2006a) Collection, conservation, evaluation and utilization of tea (*Camellia* spp.) genetic resources in China. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol I), Global Science Books, Isleworth, UK, pp 578-582

Chen L, Yao M-Z, Zhao L-P, Wang X-C (2006b) Recent research progresses on molecular biology of tea plant (*Camellia sinensis*). In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol IV), Global Science Books, Isleworth, UK, pp 426-437

Chen Z (1984) Rubber (*Hevea*). In: Sharp WR, Evans DA, Ammirato PV, Yamada Y (Eds) *Hand Book of Plant Cell Vulture (Vol 2) Crop Species*, Macmillan Publishing Co., New York, pp 546-571

Chen Z (1987) Induction of Androgenesis in hardwood trees. In: Bonga JM, Durzan, DJ (Eds) *Cell and Tissue Culture in Forestry* (Vol 2), Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp 247-268

Chu CC (1978) The N6 medium and its application to anther culture of cereal crops. In: *Proceedings of Symposium on Plant Tissue Culture*, Peking, pp v43-50

Doi Y (1981) Frequency of root differentiation in anther culture of tea. *Study of Tea* **60**, 1-3

Dunwell JM (1976) A comparative study of environmental and developmental factors which influence embryo induction and growth in cultured anthers of *Nicotiana tabacum*. *Environmental and Experimental Botany* **16**, 109-118

Dunwell JM (1978) Division and differentiation in cultured pollen. In: Thorpe TA (Ed) *Frontiers of Plant Tissue Culture*, IAPIC, Calgary, pp 103-112

Dunwell JM (1986) Pollen, ovule, embryo culture as tools in plant breeding. In: Lyndsey AW, Alderson PG (Eds) *Plant Tissue Culture and its Agricultural Applications*, Butterworths, London, pp 375-389

Guha S (1973) Genotypic differences in the *in vitro* formation of embryoids from rice pollen. *Journal of Experimental Botany* **24**, 139-144

Guha S, Maheshwari SC (1964) *In vitro* production of embryos from anthers of *Datura*. *Nature* **204**, 467

Huang B, Sunderland N (1982) Temperature-stress pretreatment in barley anther culture. *Annals of Botany* **49**, 77-88

Hutchinson JF, Zimmerman RH (1987) Tissue culture of temperate fruit and nut trees. *Horticultural Review* **9**, 273-349

Hyun SK, Kim RH, Noh EW, Park JI (1986) Induction of haploid plants

Populus species. In: Lynsey W, Alderson PG (Eds) *Plant Tissue Culture and its Agricultural Applications*, Butherworths, London, pp 413-417

Jain SM, Newton RJ (1990) Prospects of Biotechnology for tea improvement. *Proceedings of the Indian National Science Academy* **5/6**, 441-448

Janaki Ammal EK (1952) Chromosome relationship in cultivated species of *Camellia*. *American Camellia* **1**, 106-114

Kohlenbach HW, Wernicke W (1978) Investigations on the inhibitory effect of agar and the function of active carbon in anther culture. *Zeitschrift für Pflanzenphysiologie* **86**, 463-472

Lupotto E (1982) Improvement in plantlet differentiation from anther culture of rice. *Genetics in Agriculture* **36**, 129-142

Martin JA, Gella R (1991) Sourcherry (*Prunus cerasus* L.). In: Bajaj YPS (Ed) *Biotechnology in Agricutlure and Forestry (Vol 16) Tree III*, Springer-Verlag, Berlin, pp 24-41

Matsumoto S, Fukui M (1998) *Agrobaterium tumefaciens*-mediated gene transfer to tea plant (*Camellia sinensis*) cells. *Japan Agricultural Research Quarterly* **32**, 287-291

Miller CD (1965) Evidence for the natural occurrence of zeatin and derivatives compound from maize which promote cell division. *Proceedings of the National Academy of Sciences USA* **54**, 1052-1058

Mitsuaki K, Koji V, Kumiko S, Yoshiyuki K (1999) The fertilizer application of lower nitrogen by using under the canopy in tea gardens. *Bulletin of the Kagoshima Tea Experiment Station, Japan* **13**, 13-55

Murashige T (1974) Plant propagation through tissue culture. *Annual Review of Plant Physiology* **25**, 135-166

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco tissue culture. *Physiologia Plantarum* **15**, 473-497

Nathaniel RK (1986) Introdution. In: Sivapalan P, Kulasegaram S, Kathiravetpillai A (Eds) *Hand Book on Tea*, Tea Research Institute of Sri Lanka, Talawakelle, pp 1-2

Niizeki M (1977) Haploid, polyploid and aneuploid plants from cultured anthers and calluses in species of *Nicotiana* and forage crops. *Journal of the Faculty of Agriculture*, *Hokkaido University, Japan* **58**, 343-466

Nitsch C (1974) Pollen culture: A new technique for mass production of haploid and homozygous plants. In: Kasha KJ (Ed) *Haploids in Higher Plants: Advances and Potential*, University of Guelph Press, Canada, pp 123-124

Nitsch C, Anderson S, Godard M, Neuffer MG, Sheridan WF (1982) Production of Haploid plants of *Zea mays* and *Pennisetum* through androgenesis. In: Earle ED, Demarley Y (Eds) *Variability in Plants Regenerated from Tissue Culture*, Praeger Publishers, New York, pp 69-91

Nougarede A (1967) Experimental cytology of shoot apical cells during vegetative growth and flowering. *International Review of Cytology* **21**, 203-351

Okano N, Fuchinone Y (1970) Production of haploid plants by anther culture of tea *in vitro*. *Japanese Journal of Breeding* **20**, 63-64

Pedroso MC, Pais MS (1994) Induction of microspore embryogenesis in *Camellia japonica* cv. Elegans. *Plant Cell, Tissue and Organ Culture* **37**, 129-136

Rashid A (1983) Angiosperm pollen-A system for cell differentiation. *Current Science* **52**, 964-967

Razdan MK (Ed) (1993) Haploid production. In: *Introduction to Plant Tissue Culture*, Intercept Ltd., Bata Barkha Nath Printers, New Delhi, pp 105-123

Rohr R (1987) Haploids (gymnosperms). In: Bonga JM, Durzan DJ (Eds) *Cell and Tissue Culture in Forestry* (Vol 2), Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp 230-246

Saha DK, Bhattacharya NM (1992) Stimulating effects of elevated temperature treatments on production of meristemoids from pollen callus of tea, *Camellia sinensis* (L.) O. Kuntze. *Indian Journal of Experimental Biology* **30 (2)**, 83-86

Sakai A (1986) Cryopreservation of germplasm of woody plants. In: Bajaj YPS (Eds) *Biotechnology in Agriculture and Forestry, (Vol I) Trees*, Springer-Verlag, Berlin, pp 113-129

Salardini AA (1978) Yield response of tea (*Camellia sinensis*) to fertilizer and irrigation in two locations of Iran. *Plant and Soil* **50**, 113-123

Sarwar M (1985) Callus formation from explanted organs of tea (*Camellia sinensis*). *Sri Lankan Journal of Tea Science* **54**, 18-22

Seran TH (1998) Studies on anther culture of tea (*Camellia sinensis* L.), MSc thesis, University of Colombo, Sri Lanka, 113 pp

Seran TH, Hirimburegama K, Hirimburegama WK, Shanmugarajah V (1998) Regeneration of plantlets from cultured anthers of tea [*Camellia sinensis* (L.) O. Kuntze]. *Tropical Agricultural Research* **10**, 271-281

Seran TH, Hirimburegama K, Hirimburegama WK, Shanmugarajah V (1999) Callus formation in anther culture of tea clones, *Camellia sinensis* (L.) O. Kuntze. *Journal of the National Science Foundation of Sri Lanka* **27**, 165-175

Singh ID (1978) Application of plant cell tissue and organ cultures in tea research and development. *Two and a Bud* **25 (2)**, 65-69

Singh ID, Chakraborty S (1993) Application of biometrical genetics in tea breeding. *Two and a Bud* **40 (2)**, 11-14

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symposium of the Society for Experimental Biology* **11**, 118-131

Sunderland N (1974) Anther culture as a means of haploid induction. In: Kasha KJ (Ed) *Proceedings of 1st International Symposium. Haploids in Higher Plants: Advances and Potential*, University of Guelph, Canada, pp 91-122

Sunderland N (1978) Strategies in the improvement of yields in anther culture. In: *Proceedings of the Symposium on Plant Tissue Culture*, Science Press, Peking, pp 65-86

Sunderland N (1982) Induction of growth in the culture of pollen. In: Yeoman MM, Truman DES (Eds) *Differentiation in Vitro*, Cambridge University Press, Cambridge, pp 1-24

Teixeira da Silva JA (Ed) (2006) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1st Edn, Vol II), Global Science Books, Isleworth, UK, 571 pp

Thurling N, Chay PM (1984) The influence of donor plant genotype and environment on production of multicellular microspores in cultured anthers of *Brassica napus*. *Annals of Botany* **54**, 681-693

von Aderkas P, Dawkins MD (1993) Haploid embryogenesis in Trees. In: Ahuja MR (Eds) *Micropropagation of Woody Plants*, Kluwer Academic Publishers, Netherlands, pp 57-65

Wachira FN (1994) Tiploidy in tea (*Camellia sinensis*). Effect on yield and yield attributes. Journal of Horticultural Science **69(1)**, 53-60

Wang D, Gui Y, Sun J (1988) Tissue culture of fruit crops in China. *Horticultural Science* **23**, 962-965

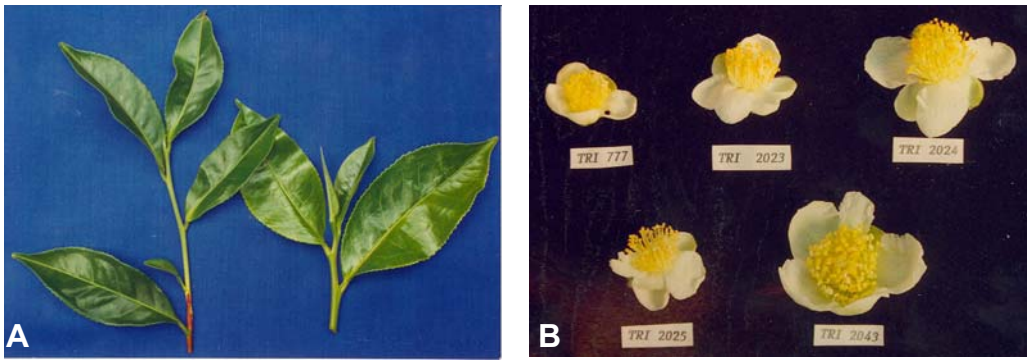
Wernicke W, Kohlenbach HW (1976) Investigations on liquid culture medium as a means of anther culture in *Nicotiana*. *Zeitschrift für Pflanzenphysiologie* **79**, 189-198

Wickramaratne WRT (1981) Genotype-environment interactions in tea (*Camellia sinensis* L.) and their implications in tea breeding and selection. *Journal of Agricultural Science (Cambridge)* **96**, 471-478

Williams EG, Maheswaran G (1986) Somatic embryogenesis: Factors influencing coordinated behaviour of cells as an embryogeneic group. *Annals of Botany* **57**, 443-462

Zhenguang, C, Huihuo L (1987) *In vitro* studies on the induction of haploid tea plants from anthers. *Proceedings of International Tea Quality Human Health Symposium*, Nov 4th - 9th 1987, China, pp 83-86

Zhu XY, Wang RL, Liang YC (1980) Induction of *Poplar* pollen plantlets. *Scientia Silvae Sinica* **16**, 190-197



(A) Dormant (left) and active (right) buds in tea shoots. (B) Flowers of different TRI clones of Sri Lankan tea.