Review Article

doi:10.19071/jpc.2017.v45.i2.3302
Journal of Plantation Crops, 2017, 45(2):77-90

Scope and significance of biotechnological improvement of tea

Mainaak Mukhopdhayay, Akan Das[1] and Tapan Kumar Mondal[2]*

University of Kalyani, Kalyani-741235, Nadia, West Bengal, India
[1]University of Science and Technology, Ri-Bhoi-793 101, Meghalaya, India
[2]ICAR-National Bureau of Plant Genetic Resources, New Delhi-110 012, India

(Manuscript Received: 13-06-17, Revised: 13-07-17, Accepted: 28-07-17)

Abstract

Tea is an important plantation crop of India, which generates huge employment opportunities in rural and hilly backward places. Being a woody perennial crop with an economic life span of more than 60 years, it also has a role in sustaining the ecosystem. Because of its long gestation period, as observed from conventional breeding, alternative methods such as molecular breeding is highly relevant, which is rather limited in tea breeding programmes. Therefore, adoption of biotechnological approaches is a better option to shorten the breeding cycle of tea. Recent developments from the biotechnological research works on tea and related species are summarized in the review.

Keywords: AFLP, biotechnology, breeding, *Camellia sinensis*, micropropagation, molecular markers

Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze], belonging to Theaceae family, is an important cash crop worldwide which is perennial in nature. It is an evergreen shrub cultivated in humid and sub-humid tropical, sub-tropical, and temperate regions of the world. Due to its perennial life cycle, tea plants recurrently come across wide range of stresses that reduces growth and yield. On the other side, tea consumption by the world population is increasing day by day due to its potential medicinal benefits. Hence, improving tea production requires suitable research and development programmes for developing new clones with high yield, superior quality and stress tolerance. Historically, tea breeding commenced long back and conventional methods took the focal point. Conventional tea breeding is a long procedure but contributed a great deal towards the improvement of tea over the past several decades. Tea is propagated either through seeds or vegetative cuttings. However, grafting is also used for propagation and subsequently achieved substantial recognition.

Tea is the most widely consumed beverage, prepared from the green leaves of the tea plants, having slightly bitter, astringent flavour (Mondal *et al.,* 2004). China is the hometown of tea plantations, processing and consumption for the past 2000 years. Some tea plants more than 1500 years old are still thriving in their native forests of Yunnan province in the South Western China (Hara *et al.*, 1995).

According to the Chinese medical book, the 'Ben Chao' tea and Chinese history can be dated back to the year 2737 B.C., when the then Emperor Shen Nong discovered tea (Hara *et al.*, 1995). The people in South-West China used tea for paying tribute to the Chinese emperors way back to 1066 B.C.

The probable centre of origin of tea is South-East China near the source of the river Irrawaddy (Taylor and McDowell, 1993). From this place it spread to the southern part of China and further to some South-East Asian countries. Subsequently, tea had stretched into many tropical and subtropical countries by 221 B.C. along with the migration of

*Corresponding Author: mondaltk@rediffmail.com

war-affected people from China to other countries like Vietnam, Myanmar, Laos, Thailand *etc*.

Charles Bruce, a Scottish traveler collected tea seeds from the Singpo tribe of Assam in early 19th century and planted tea bushes in a hilly region of Assam called Sadiya. Unfortunately, this region was unearthed by the flood of Brahmaputra that wrecked havoc in the year 1952. Earlier, in 1834, under the initiative of Lord Bentinck, the British started cultivating tea in India by importing plants, expertise and labour from China. The first shipment of Assam tea was forwarded to England in the year 1838 and private entrepreneurs started their foray into the tea business in the year 1939.

Tea is produced in almost all of the continents with a few exceptions. India, China, Sri Lanka, Vietnam, Bangladesh, Indonesia, Iran, Taiwan, Japan and Malaysia in the Asian continent, Argentina and Brazil in the South America, Rwanda, Kenya, Cameroon and Zimbabwe in Africa, Turkey and Georgia in Europe are the significant tea producers in the world.

Today tea is considered as one of the healthiest natural beverages and a stimulant fit to be consumed by people of all age group (Mukhopadhyay, 2012). As a consequence, tea consumption in India has gone up throughout the social strata. Furthermore, being an agricultural commodity, tea production is amenable to various stresses and thus the disparity between production and demand cannot be avoided. Therefore, in order to cope up with the incremental demand of tea, efforts have focused upon enhancing crop production with an emphasis on yield, quality and stress tolerance. Consequently, these parameters of made tea remained the major criteria for tea breeding.

Tea production in India

In 2003, world tea production was 3.21 million tonnes, while in 2008, world production reached over 4.73 million tonnes. The leading producers of tea are China, India, Kenya, Sri Lanka, and Turkey (Mukhopadhyay, 2012). Though India was the major producer of tea in the world once, of late, China emerged as the major tea producer. During 1951-60, India contributed around 40 per cent of the world tea production, which declined to 26 per cent during 2004 (Nagoor, 2009). However, an increasing trend was observed for some years before the slump in the year 2008-09.

Table 1. Total production of Indian tea from 2002-03 to 2014-15

Year	Total production (million kg)
2002-03	845.97
2003-04	878.65
2004-05	906.84
2005-06	948.94
2006-07	973.07
2007-08	987.02
2008-09	972.77
2009-10	991.18
2010-11	966.04
2011-12	1095.46
2012-13	1135.07
2013-14	1224.48
2014-15	1197.18

* source: www.teaboard.gov.in

Presently, India is the second largest tea producer in the world and also the world's largest consumer of black tea. The domestic market of India consumes more than 1200 million kg of tea (Table 1) and increasing continuously as tea is a low-priced drink. On the other hand, India's position is fourth in terms of tea exports, which reached around 200 million kg during 2014-15 that earned 619.96 million US$ (teaboard.gov.in).

Hence, it is apparent that, there is paramount necessity for developing superior planting materials and innovative technologies for enhancing tea production in order to meet the increasing demand for domestic consumption as well as for export. Conventional methods adopted in tea breeding have their own limitations for developing suitable elite clones with desired quality and are highly time consuming. However, biotechnology can contribute immensely towards the uninterrupted supply of elite tea planting materials for sustainment of tea industry. This review article will give an outline on the conventional breeding methods that contributed a lot for the betterment of tea industry and will cover the some of the recent developments in tea breeding research.

Conventional propagation methods of tea

Seeds, vegetative cuttings and nursery grafting make up the primary mode of propagation of tea, apart from budding or grafting of mature plants, which is done rarely. Conventional tea breeding is well-known, but slow due to its perennial life cycle and long (4-5 years) gestation period. Seeds were the only commercial method of propagation until the beginning of the 19th century. Seedlings show an extensive inconsistency for attributes like yield and quality, and these bottlenecks forced people to experiment on vegetative cuttings. Seed-borne plants are heterogeneous due to their highly allogamous nature; consequently, it is difficult to maintain their superior character (Mondal, 2014). Additionally, tea breeding did not show much progress due to lack of reliable selection criteria. On the other hand, vegetative propagation also did not come up in large scale due to time-consuming development, poor survival rate of some clones and need for large number of planting material. Faster propagation by single leaf cutting has been developed simultaneously in India, Sri Lanka and Indonesia (Mondal, 2011). The first lot of three clones, developed by vegetative cutting, was released in Assam in 1949, which revolutionized the tea industry of North-East India and more clones have since been released from time to time. Fresh single-leaf internode cuttings of both rootstock and scion were generally taken for grafting. It has been demonstrated that hardening period could be shortened by micro-grafting of *in vitro* raised tea shoots on seedling raised root stock of the same age, due to faster root growth of root stock and proliferation of the scion (Mondal *et al.*, 2005). With the mounting demand for elite tea clones, vegetative propagation with single-leaf internode cuttings remains the best choice in the tea industry worldwide.

Although few morpho-chemical markers are available for the identification of superior cultivars, these markers are greatly influenced by environmental factors and show a continuous variation with a high degree of plasticity (Mondal, 2014). However, due to several impediments in conventional breeding methods, focus has been set on tissue culture techniques and micropropagation (Mondal, 2002a). Furthermore, these techniques deserve the potential for varietal improvement through the ways other than usual breeding procedure.

Tea tissue culture

It is evident from the available literature that while Forrest (1969) was pioneer for initiating the work on tissue culture of tea while Kato (1985) did a systematic study on micropropagation of tea. However, the emphasis on survival rate including hardening to increase survival rates of micropropagated tea was given only on early 1990s (Mondal *et al.*, 2004).

Generally, shoot tips and nodal segments with dormant axillary buds of either juvenile or adult origin of current year growth are commonly used as explants for tea micropropagation. Nevertheless, zygotic embryos, immature and mature cotyledons for the induction of adventitious buds, epidermal layers of stem segments, stem segments without epidermal layer and intact stem segments for the regeneration of shoots has been employed (Mondal *et al.*, 2004). The most common basal medium is either full or half-strength MS salts (Murashige and Skoog, 1962). The effect of thidiazuron on micropropagation of tea was compared with that of 6-benzyladenine using nodal segments from *in vitro* raised seedlings on woody plant medium (WPM) and MS was superior to WPM in all combinations (Mondal *et al.*, 1998). However, like other woody perennials, major problems encountered in tea micropropagation are exudation of phenolic substances from explants and microbial contamination in tissue culture medium. Borchetia *et al.* (2009) used RAPD and microsatellite markers to find out the genetic fidelity of micropropagated plants from the explants of field grown mother bush and *in vitro* developed seedlings. The micropropagated plantlets showed both cytological and genetic stability. SSR primers also confirmed complete stability between the regenerants. Thus, it was concluded that micropropagated plants derived from axillary as well as adventitious mode of propagation can be genetically true to type. This is also a very cost effective technique and would be helpful in quick propagation at a commercial scale.

Genetic engineering can be considered as an extra tool for overcoming some of the bottlenecks of tea breeding and that requires *in vitro* regeneration system. Somatic embryogenesis was considered as

the most efficient regeneration system of tea (Jain and Newton, 1990). However, plant production depends on the efficiency of multiplication and conversion rate of somatic embryos. The advantage of somatic embryogenesis (Mondal *et al.*, 2002a) is the development of adventitious embryos from explants without an intervening callus phase, which helps in maintaining genetic fidelity (Bano *et al.*, 1991). It has a tremendous potential in clonal propagation (Mondal *et al.*, 2001a) and most importantly through genetic transformation of tea (Mondal *et al.*, 1999). It can also be used for disease free plant production and androgenic or haploid plant production of tea (Chen and Liao, 1982). However, somatic embryos are incompatible for low temperature storage in tea, whereas nodal explants are suitable for such storage and sprouts early (Mondal *et al.*, 2004). Thus, much achievement could not be attained by somatic hybridization.

Apart from that, protoplast culture is also significant in tea. However, the success of protoplast culture depends upon the source material, which is obtained *via* cell suspension culture. This technique has tremendous potential in tea crop improvement, but nothing noteworthy has been achieved. Many of the wild relatives of tea have agronomically important biotic and abiotic stress resistant characters, which can be incorporated into the cultivated variety of tea through creation of hybrid or cybrid. Thus, protoplast culture has tremendous potential for varietal improvement of tea. Another important tissue culture technique adopted for tea improvement was anther culture. Production of homozygous diploids is of great importance in tea improvement because it is heterozygous and heterogeneous. Initial attempts in anther culture were made by Katsuo (1969) and later on Okano and Fuchinone (1970) produced roots from anther-derived callus. However, Chen and Liao (1982) made the first successful attempt from anther culture of tea. In spite of multiple attempts, noteworthy success could not be obtained. Haploid tea plants could not be regenerated and development of micro-calli remained only citable achievement.

Genetic transformation

Transgenic technology, to an extent, has been replaced conventional breeding successfully as it tenders several advantages. Through transgenic technology (Mondal, 2008), gene transfer to plants was very effective, with added advantage of saving considerable time compared to conventional breeding process. In woody plants, *Agrobacterium tumefaciences* mediated transformation has been most widely accepted. Several workers (Matsumoto and Fukui, 1998, 1999; Biao *et al.*, 1998; Mondal *et al.*, 1999, 2001b, 2001c; Luo and Liang, 2000) attempted genetic transformation using different explants such as *in vitro* leaves, somatic embryos *etc*., but the first healthy transgenic plants were produced by Mondal *et al.* (2001c), using somatic embryos as explants. Matsumoto and Fukui (1998, 1999) reported stable transformations in callus after molecular characterization through PCR and southern hybridization. Mondal *et al.* (2001c) confirmed the stable integration of transgene by molecular characterization. *Agrobacterium* mediated transformation has been exploited to reduce caffeine by suppressing glutathione synthetase (Mohanpuria *et al.*, 2008) or to produce low caffeine containing tea by silencing caffeine synthase gene (Mohanpuria *et al.*, 2011). However, production of transgenic tea plant remains difficult until recently due to low transformation efficiency as well as difficult regeneration system, which can be attributed to the presence of high intensity of polyphenols in explants (Biao *et al.*, 1998). In contrast, Zehra *et al.* (1996), infected 35-day-old *in vitro* leaves with *Agrobacterium rhizogenes*, causal agent of the 'hairy root' disease, and explants were co-cultivated with bacterial cell and confirmed the stable integration. Konwar *et al.* (1998) also transformed *in vitro* tea shoots but the technique has not been exploited commercially. Several attempts have been made to standardize the process but transgenic tea plants remained elusive.

Molecular markers

Conventionally, morphological, cytological, biochemical markers were used to detect the genetic diversity. Nevertheless, all of them suffer from reduced reproducibility and other bottlenecks whereas molecular markers are reasonably consistent. On the other hand, extreme care is required when using clones of different origins, otherwise extensive cultivation of clonal tea can wipe out genetic diversity. Although, a few gene banks for tea germplasm conservation have been established, rationalization has not been done.

However, for future sustainability rationalization of collections is urgent (Mondal *et al.*, 2003; Mishra *et al.*, 2009). Molecular markers or DNA markers, identify genomic region that are responsible for desirable traits. Based on detection, they are of different types. As they are located in the non-coding regions, they are selectively neutral and not affected by environmental factors or developmental stage (Bandyopadhyay, 2011). Identification of highly reliable molecular tool is extremely important to reveal the unexplored genetic variation in tea. Currently tea plantation is based on genotype superiority, but widespread cultivation of clonal tea may be fatal to genetic diversity. Hence, germplasm characterization will assist varietal improvement of tea. The different molecular markers, applied to tea, include rapid amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), simple sequence repeat (SSR), cleaved amplified polymorphic sequence (CAPS), restriction fragment length polymorphism (RFLP) *etc*. Since, application of molecular markers in tea has ramified a lot, and various groups have already carried out enormous quantity of work, consideration of all the contributions is beyond the scope of this article.

RAPD is considered to be first generation dominant marker due to its swiftness, easy protocol and non-requirement of radioactive materials (Mondal *et al.*, 2004). In tea, substantial quantity of work has been carried out. At the inception, Wachira *et al*. (1995) characterized different cultivars of Kenyan tea for which 23 primers were used that generated 157 polymorphic bands. The maximum polymorphisms of 20 bands were detected. The amplified fragments and similarity matrix, among the clones, ranged from 0.3 to 3 kb and 43 to 96 per cent, respectively. The constructed dendrogram clearly discriminated different varieties of China, Cambod and Assam tea. Tanaka *et al.* (1995) used 10-mer and 12-mer primers to distinguish dissimilarity among Indian, Japanese, Vietnamese, Korean and Chinese tea. Primer OPF-2 was found to be highly polymorphic. They concluded that, Korean tea had undergone modest genetic diversification following introduction from China. On the contrary, Japanese tea had closer relationship with Chinese and Indian tea. RAPD markers (Mondal, 2000, Mondal *et al.*, 2000a, Mondal and Chand 2002) characterized 25 Indian tea cultivars. In a modification to RAPD technique, Tanaka and Taniguchi (2002) added nucleotides to the 3' end to make the RAPD bands clearer in tea. Singh *et al.* (2004) developed intra-clonal genetic variability in vegetatively propagated tea by RAPD. The genetic diversity, kin-ness relationship and molecular identification of 15 well-known and extensively planted traditional elite Chinese tea genetic resources were investigated using RAPD markers. By using the RAPD markers all the investigated tea genetic resources could be easily identified. Thus, RAPD markers were found to be suitable to evaluate the genetic diversity and relationship, and to identify tea genetic resources (Chen *et al.*, 2005). The enzyme polyphenol oxidase (PPO), present in chloroplasts, participates in tea fermentation. PPO has remarkable specificity for the ortho-dihydroxy functional group of the tea catechins. Ramkumar *et al.* (2011) investigated fifteen accessions for total PPO activity based on the PPO activity range that were again separated into three groups like; high, moderate and low PPO activity. RAPD analysis, using 20 decamer primers, revealed genetic diversity. Genetic similarity (GS) among the accessions ranged from 0.64 to 0.918. This genetic diversity study has been the initial assessment of partitioning the intra specific level of genetic variation correlated to the total PPO enzyme activity. Genetic diversity in 13 tea genotypes of Pakistan was performed using RAPD markers which yielded high level of diversity. Unweighted pair group method using arithmetic averages (UPGMA) based cluster analyses grouped the genotypes into five main groups. Broad and narrow leaved genotypes were accommodated in separate sub-clusters. Similarly, samples from narrow leaved genotypes were grouped in different main clusters reflecting the geographical origins of tea samples (Afridi *et al.*, 2011). Similarly, in Bangladesh molecular characterization of 18 tea clones was made using 20 decamer random primers. In these 18 tea clones, 755 bands were produced with an average of 37.75 bands per primer and among them 97.41 per cent bands were polymorphic. The molecular size of the amplified DNA fragments ranged from 250 to 5000 bp. Ten unique bands were amplified from the genome of the 18 tea clones. The values of pairwise genetic distance ranged from 24.0 to 59.0 indicating the presence of a wide range of genetic diversity

(Boonerjee *et al.*, 2013). Apart from that due to limited degree of polymorphism, attention was given for alternative advance markers.

Restriction fragment length polymorphism (RFLP) has the advantage of showing co-dominant alleles. Besides their high genomic abundance and random distribution, RFLPs have better reproducibility. RFLP has been used to investigate genetic diversity in cultivated plants and wild relatives (Tanksley *et al.*, 1989). In tea, this technique was used to assess the genetic variation of the Japanese green tea using phenylalanine ammonia-lyase (PAL) as probe (Matsumoto *et al.*, 1994). In order to prevent adulteration, Japanese tea 'Yabukita' was studied by employing STS-RFLP using the sequence information of PAL, chalcone synthase and dihydroflavonol 4-reductase genes (Kaundun and Matsumoto, 2003). The genetic diversity among 30 tea cultivars in Sichuan of China was investigated by RFLP analysis of cp DNA. Upon amplification, 135 bands were detected, among which 72.59 per cent were polymorphic. The outcome suggested relatively higher level of genetic polymorphism in tea cultivar could be detected at the nuclear genome level, whereas cpDNA PCR-RFLP markers could estimate relatively lower level genetic polymorphism (Chen *et al.*, 2012). There are some disadvantages, RFLP analysis such as is more complicated, expensive and time-consuming. Another major drawback for RFLP is probe availability.

AFLP is highly reproducible and reliable method that allows assessment of large genome and preferred for its wide genome coverage. It is suitable in genome mapping, DNA fingerprinting and marker assisted breeding. It is also useful in detection of polymorphism between closely related genotypes (Mishra *et al.*, 2009). Another advantage of AFLP is its capability to produce multilocus fingerprints in a single analysis, significantly reducing the cost of analysis and increasing the possibility of detecting polymorphisms (Vos *et al.*, 1995). In tea, Paul *et al.* (1997) were the first to employ AFLP markers to detect diversity and genetic differentiation of 32 tea clones comprising Indian and Kenyan origin. Five enzyme-primer combinations revealed total 73 unambiguous polymorphic bands. The size of polymorphic bands ranged from 106 to 218 bp. Genetic diversity within population showed that the chinary types were more variable than Assam or Cambod type. The similarity matrix co-efficient varied from 35 to 96 per cent. The dendogram constructed on the basis of shared fragment into three known types, *i.e.,* Assam, China and Cambod, which were generally consistent with the existing knowledge on the biosystematics of tea. According to the PCA, Assam clones from India and Kenya clustered closely indicating a common ancestry. In India, Rajashekaran (1997) first reported AFLP analysis of 42 tea clones. It included twenty-three UPASI, 17 South Indian and two Kenyan tea clones and 90 per cent of the UPASI clones were found to be inbred and incompatible for commercial cultivation. Resistant and susceptible clones to blister blight were grouped, and thus this study paved the way to develop blister blight resistance markers. Genetic characterization of 29 Darjeeling tea cultivars was performed using AFLP markers and 677 bands were observed, among which 469 were polymorphic and the dendogram obtained using UPGMA method, divided the clones into Assam, China and Cambod types. The study clearly indicated extensive cross breeding between species within the genus *Camellia* (Mishra and Sen-Mandi, 2001). Similarly, 49 tea cultivars from south India produced a total number of 1555 DNA fragments. Systematic analysis revealed that all these tea cultivars could be clearly distinguished into three groups as discussed earlier. The Chinary type showed a maximum diversity index of 0.612 and the minimum of 0.285 was observed within the Assam type. Genetic distance was maximum (0.946), between Assam and Cambod and minimum (0.852) between Assam and China. Finally the study divulged that the south Indian tea germplasm has narrow genetic diversity and hence a sustained effort is required to preserve tea germplasm resources and development of superior variety by means of wide genetic crosses (Balasaravanan *et al.*, 2003). Analogous results obtained from AFLP analysis of Darjeeling tea. Because among the 29 tested clones similarity index of China type population ranged from 0.712 to 0.815, 0.711 to 0.800 in Cambod type population and 0.716 to 0.778 in Assam type population (Mishra *et al.*, 2009). A cDNA-AFLP approach can be utilized to identify transcripts expressed in response to drought in tea. Drought effect on both tolerant and susceptible cultivars and genetic

differences can be visualized as polymorphisms in the transcriptome. The cluster analysis has revealed clustering-type I, which separated the tolerant and susceptible cultivar, whereas type II has separated as early and late responsive transcripts. Several transcript derived fragments has been identified on the basis of differential expression in tolerant genotypes of which more than eighty sequences could be obtained of which some showed homology in the public databases. The genes have been found to be related to carbohydrate metabolism, stress response, protein modification and translation. The genes strongly expressed in response to drought in tolerant genotype can assist in identifying and determining the genetic basis of mechanisms involved in conferring drought tolerance in tea (Gupta *et al.* 2013). AFLP is highly acknowledged for its high genomic abundance, significant reproducibility, generation of numerous bands, wide range of applications, and non-requirement of sequence data for primer construction. Disadvantages include the need for high molecular weight purified DNA, dominance of alleles. On top, due to the high number and different intensity of bands strict selection criteria is sought.

Inter-simple sequence repeat (ISSR) analysis is a PCR-based technique with primers composed of microsatellite sequences (Mondal, 2002c) with one to three selective bases anchored at the 3' or 5' ends of the primer and it can be applied for genomic fingerprinting at the inter-specific level (Zietkiewicz *et al.*, 1994). ISSR-PCR method can be used potentially for genetic fingerprinting and taxonomic classification of tea genotypes. Because twenty-five diverse tea cultivars were analyzed using the ISSR and based upon the polymorphism, 12 selected primers amplified 130 bands of which 84 per cent were polymorphic. The dendogram revealed three distinct clusters which concur with the known taxonomical classification of tea (Mondal, 2002b). Habitat fragmentation is a crucial problem for ancient tea populations. Because, in Yunnan, China *C. sinensis* var. *assamica* populations were cultivated for more than 10 centuries. Genetic diversity and differentiation were examined by using ISSR markers. The average genetic diversity within populations was approximately 0.2809. The polymorphic loci of the populations ranged from 56.5 to 90.91 per cent. The result is merely a reflection of highly out crossing in the tea species and hence, in order to conserve these ancient tea populations *in situ* and *ex situ* strategies would be highly required (Ji *et al.*, 2011). ISSR markers can be applied for the discrimination of tea germplasm at the inter-specific level. Using ISSR markers 134 tea varieties preserved in the China National Germplasm Tea Repositories were analyzed and eighteen primers upon amplification generated 99.4 per cent polymorphic bands. Nei's gene diversity and Shannon's Information index indicated a wide gene pool (Liu *et al.*, 2012). The disadvantages of ISSR marker includes requirement of clean DNA template, similar concentrations among accessions for standardization of reactions. Furthermore, bands are scored as dominant markers and genetic diversity estimates are based on diallelic characters (Bakkappa *et al.*, 2011). Even with these few limitations, ISSR markers provide an attractive alternative to other markers (Wolfe and Liston, 1998).

Simple sequence repeats (SSRs) are powerful molecular markers applied in genetic diversity studies due to their abundance and wide distribution throughout the genome. They are co-dominant in nature, and are highly reproducible. SSRs are also known as microsatellites as these are short repeat motifs present in both coding and non-coding regions of DNA. SSRs show a high level of length polymorphism due to mutations of one or more repeats. The use of SSRs as molecular marker is being favored due to their multi-allelic nature, reproducibility, high abundance and extensive genome coverage (Kantety *et al.*, 2002)**.** Freeman *et al.* (2004), Zhao *et al.* (2007) and Hung *et al.* (2008) utilized SSR while Kaundan and Matsumoto (2003) used Cleaved Amplified Polymorphic Sequence (CAPS). The first attempt to study genetic relationships among Sri Lankan tea accessions using SSR markers was done by Ariyaratne *et al.* (2009). In 27 accessions of Sri Lankan tea germplasm, SSR markers were used to assess genetic relationships. Nine genomic and ten EMST-SSR primers were used in this study that produced a total of 122 alleles, all of which were 100 per cent polymorphic. The outcome indicated that the genetic base of most of the accessions is from Assam or open pollinated lines of Assam. In a different approach, SSRs developed from Expressed Sequence Tags (ESTs), known as EST-SSRs, are extensively utilized and

provide important source of gene based markers. The EST sequence information in the publicly available databases is increasing in a faster rate and the emerging approach offers a better alternative process of development of SSR markers from the ESTs than the conservative methods (Sahu *et al.*, 2012). A total of 12,851 EST sequences of *C. sinensis*, downloaded from National Center for Biotechnology Information (NCBI) were mined for the development of microsatellites. Finally, 6148 (4779 singletons and 1369 contigs) non-redundant EST sequences were found. Out of total 3822.68 kb sequences 26.61 per cent EST sequences containing 2371 SSRs were detected with a density of one SSR per 1.61 kb leading to development of 245 primer pairs. These mined EST-SSR markers will help in the study of variability, mapping and evolutionary relationship in *C. sinensis*. Additionally, these SSRs can also be applied across species (Sahu *et al.*, 2012). Genetic fingerprinting and relationship of 21 Tanzanian tea clones were analyzed using SSR markers that showed 90 to 100 per cent polymorphism with an average of 98.33 per cent. The genetic diversity averaged at 0.7043 with a mean heterozygosity of 0.6042 while the genetic similarity ranged from 0.11 and 1.00 signifying high genetic diversity. This study shows that the selected tea cultivars bear significant genetic diversity and genetically diverse genotypes can be used as parents in the breeding programs (Ndunguru *et al.*, 2014). In a recent study, twenty-one genomic and genic microsatellites markers were used to evaluate genetic diversity and DNA fingerprinting of 15 popular tea accessions. Each accession had a unique marker profile, indicating that microsatellite markers were useful in differentiation studies among the tea collections. The study indicated that the populations from western Himalayan possessed a moderate to high level of genetic diversity, which could provide valid guidelines for genetic improvement of tea (Bhardwaj *et al.*, 2014).

Current efforts have demonstrated that analysis of expressed sequence tags (ESTs) is an appropriate strategy for identifying genes involved in specific biological functions in model plants and even in non-model plants in which genomic data are not available. The suppression subtractive hybridization (SSH) technique enables specific cloning of ESTs representing genes that are differentially expressed in different mRNA populations and isolates genes without prior knowledge of their sequence or identity. Moreover, the availability of databases of known genes and proteins and gene ontology (GO) annotation provides an opportunity to predict the functions of newly isolated putative gene sequences (Das *et al.*, 2012). In another study, seven cDNA libraries from various organs has been constructed and used to generate ESTs to increase the amount of genomic information of tea. A total of 17,458 ESTs has been generated and assembled into 5,262 unigenes. About 50 per cent of the unigenes were assigned annotations by GO. Some are homologous to genes involved in important biological processes, such as nitrogen assimilation, aluminum response, and biosynthesis of caffeine and catechins. About 67 unigenes has been expressed differentially among the seven organs. SSR motif searches among the unigenes identified 1,835 unigenes (34.9%) harboring SSR motifs of more than six repeat units. A subset of 100 EST-SSR primer sets has been tested for amplification and polymorphism in 16 tea accessions. Seventy-one primers sets successfully amplified EST-SSRs and 70 EST-SSR loci are polymorphic. Furthermore, these 70 EST-SSR markers are transferable to 14 other *Camellia* species. These markers will improve the study of important traits and the molecular genetics of tea plants (Taniguchi *et al.*, 2012).

Omics

In tea, several genes have been cloned and characterized (Mukhopadhyay *et al.*, 2015). Takeuchi *et al.* (1994) started functional genomics study with the isolation of the chalcone synthase gene from a Japanese tea cultivar, thus revealing its organ-specific and sugar-responsive expression. Tea leaf contains very high amount of polyphenols (35%), the majority of which are catechins. Several biosynthesis related genes, like PAL, chalcone synthase, flavanone 3-hydroxylase and flavonoid 3p have been cloned. Caffeine synthesis pathway related genes such as caffeine synthase, 7-N-methyltransferase have been isolated. Isolation of these genes resulted in caffeine-free tea plant production through RNAi technology (Kato *et al.,* 2000; Mohanpuria *et al.,* 2011). Theanine, a major amino acid of tea, has a great influence on the taste of tea. Therefore, manipulating theanine biosynthesis would be useful for improving the

quality of green tea. Glutamine synthase gene of theanine biosynthesis pathway has been cloned (Mohanpuria *et al.,* 2011). Similarly, many other studies related to proteomics, transcriptomics and metabolomics have been thoroughly studied.

Proteomics studies in tea correlate the potential protein modifications to particular phenotypes through techniques such as high-performance liquid chromatography, mass spectrometry, sodium dodecyl sulfate polyacrylamide gel electrophoresis, two-dimensional gel electrophoresis, *in silico* protein modeling, and matrix-assisted laser desorption/ionization. Li *et al*. (2008) first performed a proteomics analysis of tea pollen and identified the differentially expressed proteins of pollen that are associated with cold stress. Proteomic analysis of albino leaf identified 61 proteins during the three developmental stages of an albino cultivar of tea. Important proteins that play crucial roles in the periodic albinism has been identified (Li *et al.,* 2011). Abscisic acid (ABA) is an important phytohormone responsible for drought resistance and in order to develop drought-tolerant tea genotypes, understanding of ABA effects on tea plant under drought stress is essential and proteome analysis is a major tool for addressing this. Among the protein spots identified by MALDI-TOF MS, some are downregulated and a couple upregulated by exogenous ABA application. The upregulated proteins have roles in glycolysis and photosystem II stabilization and some of them are responsive to drought stress, carbohydrate and nitrogen metabolism, control of reactive oxygen species (ROS), defense, signaling or nucleic acid metabolism. Combination of exogenous ABA and drought has shown time dependent upregulation after initiation of drought stress. It has also been revealed that ABA pre-treatment is a valuable approach that mitigated drought stress in tea plants under laboratory conditions (Zhou *et al.,* 2014).

In spite of some inherent limitations, EST sequencing has long been the basis for reference transcript discovery. Large-scale RNA sequencing (RNA-seq) provides a reliable approach to generate datasets for functional genomic analysis. The transcriptome from poly $(A)^+$ RNA of tea has been analyzed at an unprecedented depth to obtain large number of reads that are, assembled into unigenes consisting of contig clusters and singletons. This technology produced 10-fold higher unigenes than existing tea sequences deposited in GenBank. Targeted searches identified the majority of genes associated with flavonoid, theanine and caffeine biosynthesis pathways, important to tea quality, have been discovered. Unigenes related to theanine and flavonoid synthesis are validated and expression patterns in different organs of the tea plant are analyzed (Shi *et al.,* 2011). RNA-Seq also paves the way to uncover the genetic components underlying the biosynthesis of characteristic metabolites in tea compared to oil tea. Several unigenes for tea and oil tea has been detected based on their differences at the transcriptomic level. A potential connection has been observed between gene expression and content variation for catechins, theanine and caffeine in tea and oil tea and the metabolism has been activated during the accumulation of characteristic metabolites in tea, which are present at low levels in oil tea reflecting differential regulatory mechanisms underlying secondary metabolic pathways in tea versus oil tea (Tai *et al.,* 2015). Catechins not only contribute to tea quality but also serve important functions in the anti-stress regulation but the percentages of various catechins are different among tea cultivars. Understanding the transcript changes in cell type, developmental stage or stress response is an impressive approach (Mondal and Sutoh, 2013). Transcriptomes from leaf tissues of four tea plant cultivars has been sequenced followed by *de novo* assembly and reversed-phase high-performance liquid chromatography (RP-HPLC). The analyses of transcriptome profiles and physiological indicators not only identified the putative genes involved in the flavonoid biosynthetic pathway but also provided some novel insights for the mechanisms of catechins biosynthesis (Wu *et al.*, 2014).

Metabolomics is an important study in tea, since tea quality is a combination of quite a lot of metabolites of different natures. Metabolic char-acteristics associated with climatic variables have been investigated by nuclear magnetic resonance spectroscopy in South Korean tea and thus Lee *et al.* (2010) assessed the quality in green tea. Ku *et al*. (2010) detected differential metabolites expressed in shade-grown tea plants and that aided to realize the consequence of shade on tea growth. The dependence of the global green tea

metabolome on plucking positions has been investigated through NMR analysis (Lee *et al.,* 2011) that correlated the plucking shoots with quality.

High-throughput sequence-driven research

High-throughput sequencing technologies are a fast and cost-effective approach to generate genome-scale sequence resources, and thus have increasingly been employed in numbers of crop plants to decipher important genes and genetic pathways. Mostly to those plants without a sequenced reference genome or non-model species, it provides an efficient and prior way to investigate patterns of gene expression, discover novel genes, and obtain a large number of genetic markers (Niedringhaus *et al.,* 2011; Wei *et al.,* 2015; Zhang *et al.,* 2015). Presently, three second generation sequencing platforms, including Roche 454, Illumina Genome Analyzer and Life Technologies SOLiD, are available and they can generate massive sequence reads at an extraordinary depth for high-throughput transcriptome or genome analysis (Niedringhaus *et al.,* 2011). Using Illumina HiSeq 2000 platform, Zhang *et al.* (2015) reported the first large-coverage transcriptome datasets for *Camellia taliensis*, one of the most important wild relatives of cultivated tea. They generated approximately 241.5 million high-quality paired-end reads, accounting for ~24 Gb of sequence data from tender shoots, young leaves, flower buds and flowers which yielded a set of 67,923 transcripts. These transcripts are differentially regulated at different developmental stages. There were considerable nucleotide variation within the genes involved in important secondary metabolic biosynthetic pathways, of which flavone synthase II gene (FNSII) is the most variable between *Camellia sinensis* and *Camellia taliensis* species. This study also reported that *C. taliensis* shows a remarkable expansion of LEA genes, compared to *C. sinensis*, which might contribute to the observed stronger stress tolerance of *C. taliensis*. Using the 454 GS-FLX sequencing platform, Xia *et al.* (2014) generated approximately 600,000 RNA-Seq reads from four tissues of *C. oleifera*, an important oil-rich plant. These reads were trimmed and assembled into 104,842 non-redundant putative transcripts with a total length of 38.9 Mb. Based on the BLAST similarity searches, nearly 42.6 per cent transcripts could be annotated with known genes, conserved domains, or gene ontology (GO) terms. Comparisons with the cultivated tea tree, *C. sinensis*, identified 3,022 pairs of orthologs, of which 211 exhibited the evidence under positive selection. Majority of the genes are potentially related to lipid metabolism. They revealed that evolutionary analysis of omega-6 fatty acid desaturase (FAD2) genes among 20 oil-plants unexpectedly suggests that a parallel evolution may occur between *C. oleifera* and *Olea oleifera*. Moreover, more than 2,300 simple sequence repeats (SSRs) and 20,200 single-nucleotide polymorphisms (SNPs) were also reported in the *C. oleifera* transcriptome. Tai *et al.* (2015) uncover the genetic components underlying the biosynthesis of characteristic metabolites in tea using RNA-Seq analysis of transcriptome in *C. oleifera* and *C. sinensis*. They reported that 3,787 and 3,359 bud genes, as well as 4,042 and 3,302 leaf genes, were up-regulated in tea and oil tea, respectively. Moreover, high-performance liquid chromatography (HPLC) analysis revealed high levels of all types of catechins, theanine and caffeine in tea compared to those in oil tea. Their comparison of the transcriptomes and related metabolites has revealed differential regulatory mechanisms underlying secondary metabolic pathways in tea versus oil tea. Paul *et al.* (2014) dissected the molecular processes operating in the leaves during the period of active growth and winter dormancy through transcriptome analysis using Illumina Genome Analyzer IIx (Illumina, USA), to understand non-deciduous nature of tea plant. The analysis of 24,700 unigenes obtained from 57,767 primarily assembled transcripts revealed the operational mechanism of winter tolerance, down-regulation of genes involved in growth, development, protein synthesis and cell division, and inhibition of leaf abscission due to modulation of senescence related processes during winter dormancy in tea.

Future prospects

Till date significant progress has been made in tea biotechnology (Mondal *et al.,* 1998, 2000b, 2001a; Mondal 2002b) and other area of molecular biology such as construction of cDNA libraries, generation and annotation of ESTs, analysis of gene expression profiling, construction and use of cDNA microarrays, development and utilization of simple sequence repeat, microsatellite and short tandem

repeat markers and the cloning and expression of genes involved in secondary metabolism, disease and pest resistance (Chen *et al.*, 2009). Though markers have been developed and used mainly to study the genetic diversity yet, none of them have been utilized for mapping of agronomically important QTLs, marker assisted breeding (MAB) for gene introgression which has a tremendous role in genetic improvement of tea. In the genus *Camellia*, developing biparental mapping population is difficult and hence association-mapping approaches could be a better alternative. Unfortunately, such an approach has remained hitherto unexplored for identifying the agronomically important genes. Although several aspects of tea molecular biology work can be intended, yet priority should be given to, (i) undertake a *de novo* whole genome sequencing for development of reference genome, and (ii) re-sequence popular trait-specific cultivars for generating large scale SNPs markers and their utilization for association mapping, gene introgression and high density linkage map constructions.

References

Afridi, S.G., Ahmad, H., Alam, M., Khan, I.A. and Hassan, M. 2011. DNA landmarks for genetic diversity assessment in tea genotypes using RAPD markers. *African Journal of Biotechnology* **10**: 15477-15482.

Ariyaratne, P.N.K., Mewan, K.M., Goonetilleke, W.A.S.N.S.T. and Attanayake D.P.S.T.G. 2009. Study of genetic relationships of tea (*Camellia sinensis* (L.) O. Kuntze) using SSR markers and pedigree analysis. Proceedings of 9th Agricultural Research Symposium 293-298.

Bakkappa, S., Talebi, E. and Subramanya, G. 2011. Role of molecular markers (RAPD & ISSR) in silkworm conservation. *International Journal of Advanced Biological Research* **1**: 01-07.

Balasaravanan, T., Pius, P.K., Kumar, R.R., Muraleedharan, N. and Shasany, A.K. 2003. Genetic diversity among south Indian tea germplasm (*Camellia sinensis*, *C. assamica* and *C. assamica* spp. Lasiocalyx) using AFLP markers. *Plant Science* **165**: 365-372.

Bandyopadhyay, T. 2011. Molecular marker technology in genetic improvement of tea. *International Journal of Plant Breeding and Genetics* **5**: 23-33.

Bano, Z., Rajaratnam, S. and Mohanty, B.D. 1991 Somatic embryogenesis in cotyledon culture of tea (*Thea sinensis* L.) *Journal of Horticultural Science* **66**: 465-470.

Bhardwaj, P., Sharma, R.K., Kumar, R., Sharma, H. and Ahuja, P. S. 2014. SSR marker based DNA fingerprinting and diversity assessment in superior tea germplasm cultivated in Western Himalaya. *Proceedings of Indian National Science Academy* **80**: 157-162.

Biao, X., Kuboi, T., Xu, J. and Yongyan, B. 1998. Effect of polyphenol compounds on tea plant transformation. *American Society of Plant Physiology,* pp. 314.

Boonerjee, S., Nurul Islam, M., Hoque, M.I. and Sarker, R.H. 2013. Genetic diversity analysis of eighteen tea (*Camellia sinensis* L.) clones of Bangladesh through RAPD. *Plant Tissue Culture and Biotechnology* **23**: 189-199.

Borchetia, S., Das, S.C., Handique, P.J., and Das, S. 2009. High multiplication frequency and genetic stability for commercialization of the three varieties of micropropagated tea plants (*Camellia* spp.). *Scientia Horticulturae* **120**: 544-550.

Chen, L., Gao, Q., Chen, D. and Xu, C. 2005. The use of RAPD markers for detecting genetic diversity, relationship and molecular identification of Chinese elite tea genetic resources [*Camellia sinensis* (L.) O. Kuntze] preserved in a tea germplasm repository. *Biodiversity and Conservation* **14**: 1433-1444.

Chen, Z. and Liao, H. 1982. Obtaining plantlet through anther culture of tea plants. *Zhongguo Chaye* **4**: 6-7.

Chen, S., Qi, G., Li, H., Zou, Y. and Shan, H. 2012. PCR-RFLP analysis of cpDNA in tea cultivars (*Camellia sinensis* L.) in Sichuan of China. *Journal of Agricultural Science* **4**: 25-30.

Hara, Y., Luo, S.J., Wickremasinghe, R.L. and Yamanishi, T. 1995. Special issue on tea. *Food Reviews International* **11**: 371-545.

Das, A., Das, S. and Mondal, T.K. 2012. Identification of differentially expressed gene profiles in young roots of tea [*Camellia sinensis* (L.) O. Kuntze] subjected to drought stress using suppression subtractive hybridization. *Plant Molecular Biology Reporter* **30**: 1088-1101.

Freeman, S.J., West, C.J., Lea, V. and Mayes, S. 2004. Isolation and characterization of highly polymorphic microsatellites in tea (*Camellia sinensis*). *Molecular Ecology Notes* **4**: 324-326.

Forrest, G.I. 1969. Studies on the polyphenol metabolism of tissue culture derived from the tea plant (*C. sinensis* L.). *Biochemical Journal* **113**: 765-772.

Gupta, S., Bharalee, R., Bhorali, P., Das, S.K., Bhagawati, P., Bandyopadhyay, T., Gohain, B., Agarwal, N., Ahmed, P., Borchetia, S., Kalita, M.C., Handique, A.K. and Das, S. 2013. Molecular analysis of drought tolerance in tea by cDNA-AFLP based transcript profiling. *Molecular Biotechnology* **53**: 237-248.

Hung, C.Y., Wang, K.H., Huang, C.C., Gong, X., Ge, X.J. and Chiang, T.Y. 2008. Isolation and characterization of 11 microsatellite loci from *Camellia sinensis* in Taiwan

using PCR-based isolation of microsatellite arrays (PIMA). *Conservation Genetics* **9**: 779-781.

Jain, S.M. and Newton, R.J. 1990. Prospects of biotechnology for tea improvement. *Proceedings of the Indian National Science Academy* **56**: 441-448.

Ji, P.Z., Li, H., Gao, L.Z., Zhang, J., Cheng, Z.Q. and Huang, X.Q. 2011. ISSR diversity and genetic differentiation of ancient tea (*Camellia sinensis* VAR. *assamica*) plantations from China: Implications for precious tea germplasm conservation. *Pakistan Journal of Botany* **43**: 281-291.

Kantety, R.V., La Rota, M. and Matthews, D.E. 2002. Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Molecular Biology* **48**: 501-510.

Kato, M. 1985. Regeneration of plantlets from tea stem callus. *Japanese Journal of Breeding* **35**: 317-322.

Kato, M., Mizuno, K., Crozier, A., Fujimura, T. and Ashihara, A. 2000. Caffeine synthase gene from tea leaves. *Nature* **406**: 956-957.

Katsuo, K. 1969. Anther culture in tea plant (a preliminary report). *Study of Tea* **4**: 31.

Kaundun, S.S. and Matsumoto, S. 2003. Identification of processed Japanese green tea based on polymorphism generated by STS-RFLP analysis. *Journal of Agricultural and Food Chemistry* **51**: 1765-1770.

Konwar, B.K., Das, S.C., Bordoloi, B.J. and Dutta, R.K. 1998. Hairy root development in tea through *Agrobacterium rhizogenes* mediated genetic transformation. *Two and a Bud* **45**: 19-20.

Lee, J.E., Lee, B.J., Chung, J.O., Hwang, J.A., Lee, S.J., Lee, C.H., and Hong, Y.S. 2010. Geographical and climatic dependencies of green tea (*Camellia sinensis*) metabolites: a ^{1}H NMR-based metabolomics study. *Journal of Agricultural and Food Chemistry* **58**: 10582-10589.

Lee, J.E., Lee, B.J., Hwang, J.A., Ko, K.S., Chung, J.O., Kim, E.H., Lee, S.J. and Hong, Y.S. 2011. Metabolic dependence of green tea on plucking positions revisited: a Metabolomic Study. *Journal of Agricultural and Food Chemistry* **59**: 10579-10585.

Li, J., Chen, J., Zhang, Z. and Pan, Y. 2008. Proteome analysis of tea pollen (*Camellia sinensis*) under different storage conditions. *Journal of Agricultural and Food Chemistry* **56**: 7535-7544.

Li, Q., Huang, J., Liu, S., Li, J., Yang, X., Liu, Y., & Liu, Z. 2011. Proteomic analysis of young leaves at three developmental stages in an albino tea cultivar. *Proteome Science* **9**: 44-56.

Liu B.Y., Cheng, H., Li, Y.Y., Wang, L.Y., He, W. and Wang P.S. 2012. Fingerprinting for discriminating tea germplasm using inter-simple sequence repeat (ISSR) markers. *Pakistan Journal of Botany* **44**: 1247-1260.

Luo, Y.Y. and Liang, Y.R. 2000. Studies on the construction of Bt gene expression vector and its transformation in tea plant. *Journal of Tea Science* **20**: 141-147.

Matsumoto, S. and Fukui, M. 1998. *Agrobacterium tumefaciens* mediated gene transfer in tea plant (*Camellia sinensis*) cells. *Japan Agriculture Research Quarterly* **32**: 287-291.

Matsumoto, S., and Fukui, M. 1999. Effect of acetosyringone application on *Agrobacterium*-mediated transfer in tea plant (*Camellia sinensis*). *Bulletin of the National Research Institute of Vegetables, Ornamental plants and Tea* **14**: 9-15.

Matsumoto, S., Takeuchi, A., Hayastsu, M. and Kondo, S. 1994. Molecular cloning of phenylalanine ammonia-lyase cDNA and classification of varieties and cultivars of tea plants (*Camellia sinensis*) using the tea PAL cDNA probes. *Theoretical and Applied Genetics* **89**: 671-675.

Mishra, R.K., Chaudhary, S., Ahmad, A., Pradhan, M. and Siddiqi, T.O. 2009. Molecular analysis of tea clones (*Camellia sinensis*) using AFLP markers. *International Journal of Integrative Biology* **5**: 130-135.

Mishra, R.K. and Sen-Mandi, S. 2001. DNA fingerprinting and genetic relationship study of tea plants using amplified fragment length polymorphism (AFLP) technique. *Indian Journal of Plant Genetic Resources* **14**: 48-149.

Mohanpuria, P., Rana, N.K. and Yadav, S.K. 2008. Transient RNAi based gene silencing of glutathione synthetase reduces glutathione content in *Camellia sinensis* (L.) O. Kuntze somatic embryos. *Biologia Plantarum* **52**: 381-384.

Mohanpuria, P., Kumar, V., Ahuja, P.S. and Yadav, S.K. 2011. Agrobacterium-mediated silencing of caffeine synthesis through root transformation in *Camellia sinensis* L. *Molecular Biotechnology* **48**: 235-243.

Mondal, T.K. 2000a. Studies on RAPD markers for detection of genetic diversity, in vitro regeneration and Agrobacterium-mediated genetic transformation of tea (*Camellia sinensis*). Ph.D. thesis, Utkal University, India.

Mondal, T.K. 2002b. *Camellia* biotechnology: a bibliographic search. *International Journal of Tea Science* **1**: 28-37.

Mondal, T.K. 2011. *Camellia*. In: *Wild Crop Relatives: Genomic and Breeding Resources,* (Ed.) C. Kole, Heidelberg: Springer-Verlag, pp.15-40

Mondal, T.K., Bhattacharya, A. and Ahuja, P.S. 2001a. Induction of synchronous secondary embryogenesis of tea (*Camellia sinensis*). *Journal of Plant Physiology* **158**: 945-951.

Mondal, T.K., Bhattacharya, A., Laxmikumaran, M. and Ahuja, P.S. 2004. Recent advances in tea biotechnology. *Plant Cell Tissue and Organ Culture* **75**: 795-856.

Mondal, T.K., Bhattacharya, A., Sharma, M. and Ahuja, P.S. 2001c. Induction of *in vivo* somatic embryogenesis in

tea (*Camellia sinensis*) cotyledons. *Current Science* **81**: 101-104.

Mondal, T.K., Bhattacharya, A., Sood, A. and Ahuja, P.S. 1998. Micropropagation of tea using thidiazuran. *Plant Growth Regulation* **26**: 57-61.

Mondal, T.K., Bhattachraya, A., Sood, A. and Ahuja, P.S. 1999. An efficient protocol for somatic embryogenesis and its use in developing transgenic tea (*Camellia sinensis* (L.) O. Kuntze) for field transfer. In: *Plant Biotechnology and In Vitro Biology in 21st Century* (Eds.) Altman A. *et al.*, The Netherlands, Dordrecht: Kluwer Academic Publishers, pp. 101-104.

Mondal, T.K. and Chand, P.K. 2002. Detection of genetic instability among the miocropropagated tea (*Camellia sinensis*) plants. *In Vitro Cellular and Developmental Biology - Plant* **37**: 1-5.

Mondal, T.K., Singh, H.P. and Ahuja, P.S. 2000a. Isolation of genomic DNA from tea and other phenolic rich plants. *Journal of Plantation Crops* **28**: 30-34.

Mukhopadhyay, M. 2012. Biochemical, physiological and molecular changes in tea under zinc, boron and aluminium stresses. *Ph. D Thesis,* University of Kalyani, Kalyani, India.

Mukhopadhyay, M., Mondal, T.K. and Chand, P.K. 2015. Biotechnological advances in tea (*Camellia sinensis* [L.] O. Kuntze): A review. *Plant Cell Reports* doi: 10.1007/s00299-015-1884-8.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473-497.

Nagoor, B.H. 2009. Performance of India's tea exports: A comparative study of major tea exporting countries of the world. IGIDR Proceedings/Project reports series. Retrieved November 20, 2015 from http://www.igidr.ac.in/pdf/publication/PP-062-21.pdf.

Ndunguru, J., Kyallo, M., Kavia, F. and Erasto, J. 2014. Characterization of clonal tea (*Camellia sinensis* (L.) O. Kuntze) from Tanzania using SSR markers. *International Journal of Research in Plant Science* **4**: 85-91.

Niedringhaus, T.P., Milanova, D., Kerby, M.B., Snyder, M.P. and Barron A.E. 2011. Landscape of Next-Generation Sequencing Technologies. *Analytical Chemistry* **83**: 4327-4341.

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

Paul, A., Jha, A., Bhardwaj, S., Singh, S., Shankar, R. and Kumar, S. 2014. RNA-seq-mediated transcriptome analysis of actively growing and winter dormant shoots identifies non-deciduous habit of evergreen tree tea during winters. *Scientific Reports* **4**: 5932. doi: 10.1038/srep 05932, 2014.

Paul, S., Wachira, F.N., Powell, W. and Waugh, R. 1997. Diversity and genetic differentiation among population of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. *Theoretical and Applied Genetics* **94**: 255-263.

Rajashekaran, P. 1997. Development of molecular markers using AFLP in tea. In: *Molecular Approaches to Crop Improvement*. (Ed.) Varghese, J.P. *Proceedings of National Seminar on Molecular approaches to Crop Improvement*. Kerala, India: Kottayam, pp. 54-58.

Ramkumar, S., Sureshkumar, P., Mandal, A.K. A., Rajaram, K. and Mohankumar, P. 2011. Identification of superior varieties of tea (*Camellia sinensis* (L.) O. Kuntze) in the selected UPASI germplasm using biomarkers. *International Journal of the Physical Sciences* **6**: 727-743.

Sahu, J., Sarmah, R., Dehury, B., Sarma, K., Sahoo, S., Sahu, M., Barooah, M., Modi, M.K. and Sen, P. 2012. Mining for SSRs and FDMs from expressed sequence tags of *Camellia sinensis*. *Bioinformation* **8**:260-266.

Shi, C.Y., Yang, H., Wei, C.L., Yu, O., Zhang, Z.Z., Jiang, C.J., Sun, J., Li, Y.Y., Chen, Q., Xia, T. and Wan, X.C. 2011. Deep sequencing of the *Camellia sinensis* transcriptome revealed candidate genes for major metabolic pathways of tea-specific compounds. *BMC Genomics* **12**: 131.

Singh, M., Saroop, J. and Dhiman, B. 2004. Detection of intra-clonal genetic variability in vegetatively propagated tea using RAPD markers. *Biologia Plantarum* **48**: 113-115.

Tai, Y., Wei, C., Yang, H., Zhang, L., Chen, Q., Deng, W., Wei, S., Zhang, J., Fang, C., Ho, C. and Wan, X. 2015. Transcriptomic and phytochemical analysis of the biosynthesis of characteristic constituents in tea (*Camellia sinensis*) compared with oil tea (*Camellia oleifera*). *BMC Plant Biology* **15**: 190.

Takeuchi, A., Matsumoto, S. and Hayatsu, M. 1994. Chalcone synthase from *Camellia sinensis* isolation of the cDNAs and the organ specific and sugar-responsive expression of the genes. *Plant Cell Physiology* **35:**1011-1018.

Tanaka, J.I., Sawai, Y. and Yamaguchi, S. 1995. Genetic analysis of RAPD markers in tea. *Japanese Journal of Breeding* **45**: 198-199.

Tanaka, J. and Taniguchi, F. 2002. Emphasized-RAPD (e-RAPD): A simple and efficient technique to make RAPD bands clearer. *Breeding Science* **52**: 225-229.

Taniguchi, F., Fukuoka, H., and Tanaka, J. 2012. Expressed sequence tags from organ-specific cDNA libraries of tea (*Camellia sinensis*) and polymorphisms and transferability of EST-SSRs across *Camellia* species. *Breeding Science* **62**: 186-195.

Tanksley, S.D., Yong, N.D., Paterson, A.H. and Bonierbals, M.W. 1989. RFLP mapping in plant breeding: New tools for an old science. *Biotechnology* **7**: 257-264.

Taylor, S.J. and McDowell, I. 1993. Tea: types, production and trade. In: *The Encyclopedia of Food Science, Food Technology and Nutrition* (Eds.) Macrae R. *et al.*, Academic Press, London, pp. 4521-4527.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J. and Kuiper, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* **23**: 4407-4414.

Wachira, F.N., Waugh, R., Hackett, C.A. and Powell, W. 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* **38**: 201-210.

Wei, Y., Jing, W., Youxiang, Z., Mingming, Z., Yan, G., Hua, D., Lijun, P. and Dingjin, H. 2015. Genome-wide identification of genes probably relevant to the uniqueness of tea plant (*Camellia sinensis*) and its cultivars. *International Journal of Genomics*. http://dx.doi.org/10.1155/2015/527054.

Wu, Z., Li, X., Liu, Z., Xu, Z. and Zhuang, J. 2014. *De novo* assembly and transcriptome characterization: novel insights into catechins biosynthesis in *Camellia sinensis*. *BMC Plant Biology* **14**: 277.

Wolfe, A.D. and Liston, A. 1998. Contributions of PCR based methods to plant systematics and evolutionary biology. In: *Plant Molecular Systematics II.* (Eds.) Soltis D.E. *et al.,* Chapman Hall, New York. pp. 43-86.

Xia, E.H., Jiang, J.J., Huang, H., Zhang, L.P., Zhang, H.B. and Gao, L.Z. 2014. Transcriptome analysis of the oil-rich tea plant, *Camellia oleifera*, reveals candidate genes related to lipid metabolism. *PLOS One* **9**: 8. e104150.

Zehra, M., Banerjee, S., Mathur, A.K. and Kukreja, A.K. 1996. Induction of hairy roots in tea (*Camellia sinensis* L.) using *Agrobacterium rhizogenes*. *Current Science* **70**: 84-86.

Zhang, H.B., Xia, E.H., Huang, H., Jiang, J.J., Liu, B.Y. and Gao, L.Z. 2015. *De novo* transcriptome assembly of the wild relative of tea tree (*Camellia taliensis*) and comparative analysis with tea transcriptome identified putative genes associated with tea quality and stress response. *BMC Genomics* **16**: 298, doi: 10.1186/s12864-015-1494-4.

Zhao, L.P., Liu, Z., Chen, L., Yao, M.Z. and Wang, X.C. 2007. Generation and characterization of 24 nobel EST derived microsatellites from tea plant (*Camellia sinensis*) and cross species amplification in its closely related species and varieties. *Conservation Genetics* **9**: 1327-1331.

Zhou, L., Xu, H., Mischke, S., Meinhardt, L.W., Zhang, D., Zhu, X., Li, X. and Fang, W. 2014. Exogenous abscisic acid significantly affects proteome in tea plant (*Camellia sinensis*) exposed to drought stress. *Horticultural Research* **1**: 14029.

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