

REVIEW ARTICLE

Review on Morphological and Molecular Characterization for Tea (*Camellia sinensis* [L.] O. Kuntze var. *sinensis*) Breeding

M.M.N.T. Bandara¹, E.M.A.P. Nabadawewa¹, L.M.H.R. Alwis¹, N. Dahanayake^{2*}

¹Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Badulla, Sri Lanka

²Department of Agricultural Biology, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

Abstract: Tea (*Camellia sinensis*) is the world's second most consuming beverage and one of the major exporting plantation crops in Sri Lanka. Tea yield is highly influenced by weather conditions, shoot density, shoot weight and genetic factors which inherited by tea plants. Development of vegetative propagated cultivars has led to a genetic bottleneck condition in tea plantation. Therefore, it is important to understand the genetic variability through proper characterization for a successful breeding program. Morphological characterization is a cost-effective method and molecular characterization based on molecular markers such as RAPD and ISSR give more reliable data and it is a perfect method for the conservation of genetic resources. The genetic diversity of tea can be conserved ex-situ and in-situ in the fields and gene banks. These approaches will reduce the time taken for conventional breeding of tea and aid in development of superior cultivars.

Keywords: Breeding, Genetic diversity, Molecular characterization, Morphological characterization, Tea.

Introduction

Overview of the Tea Industry

Tea (*Camellia sinensis*) is an evergreen, woody, and perennial plant with a life span of over 100 years (Xia et al., 2020). It normally grows for about 30 meters but for the plantation purpose, it is maintained as a shrub by a suitable pruning system. Tea is considered as the world's second most consuming beverage which is originated in the forests of Southwest China (Wang et al., 2020). Availability of numerous types of beverages from the same tea plant such as black tea, green tea, oolong tea, white tea etc. (Xia et al., 2020) is an advantage to become the most famous beverage in the world next to water. Tea contains polyphenols as its major chemical constituent which gives many health benefits as anti-oxidant activity that fights against cancer, reduces obesity and lowers blood cholesterol level (Teshome, 2019).

Recently Tea has been planted in 58 countries on all 5 continents along with Asia which has the largest area of tea cultivation in the world, while Africa is in the place of second (Wang et al., 2020). Tea was first cultivated as a commercial plantation in South Asia by the influence of being British colonies. However, now the cultivation has spread to Asia, Africa, and some parts of the Middle East because tea can be cultivated in regions where the temperature, rainfall, soil conditions and other affecting environmental factors are favourable for the growing of tea (Jayasinghe and Kumar, 2020). The major tea-producing countries of the world are China, India, Kenya, Sri Lanka and Indonesia.

Tea is maintaining huge beverage market worldwide (Conway, 2021). Therefore, it can be concluded that the tea is having a sound share of the global beverage market with numerous varieties. Furthermore, value added tea products such as tea wines, tea instant beverages and tea cosmetics are also emerging into the market and thereby market share becomes more competitive. Therefore, the tea industry must rise

*corresponding author: nilanthi@agri.ruh.ac.lk

 <https://orcid.org/0000-0001-5631-2423>



This article is published under the Creative Commons CC-BY-ND License (<http://creativecommons.org/licenses/by-nd/4.0/>). This license permits commercial and non-commercial reuse, distribution, and reproduction in any medium, provided the original work is not changed in any way and is properly cited.

against all those challenges to be fitted for a stable market share (Kithsiri et al., 2020).

Botanical classification of tea and its growth

As in the Botanical Classification, commercially cultivating tea plant is scientifically known as *Camellia sinensis* (L.) O. Kuntze var. *sinensis*. Among the 82 named *Camellia* spp. tea *Camellia sinensis* (L.) O. Kuntze var. *sinensis* is the most important in taxonomically as well as commercially (Handbook on Tea, 2008). Most of other *Camellia* sp. are used as ornamental plants. Therefore, it is an important necessity to obtain distinctive classification of tea as it is much important to improve this economic plant for its conservation and better utilization (Xiao and Li, 2002).

However, some complexities in tea taxonomy make it a challenge still. Proper attention is needed for the tea taxonomy. A low priority is given even in the research studies even though accurate taxonomic information of *Camellia* may aid in identifying genotypes which are having high productive potentials. Then, an accurate identification could be used to improve genetically with a proper breeding system.

The commercial tea cultivars have three different taxon which are: *C. sinensis* (L.) O. Kuntze or China type, *C. assamica* or Assam type and *C. assamica* sub spp *lasiocalyx* or Cambod or Southern type (Nathaniel, 2008). Among them *Camellia sinensis* and *Camellia assamica* are known to be distinct species (Paul et al., 1997). Present day, most commercial plantations around the world are planted with hybrid clones between *C. assamica* and *C. sinensis*. However, according to their morphological closeness to the main taxon, some hybrids are still known as Assam, China or Cambod type (Devarumath et al., 2002).

During commercial tea cultivation pruning is done to maintain the growth of the tea bush. During the normal growth behavior of the tea, it grows as a plant which will grow up to a height about 30m Pruning is done generate more shoots and facilitate good plucking table.

Usually, tea flowering starts after the growth of the third flush in the tea bush. Tea flowers are white in color with central, yellow-colored stigmas and anthers. According to Tea flowers are obligate outbreeders. Self-pollination is not observed in tea as they have identified with late acting self-incompatibility (Mukhopadhyay et al., 2016).

The tea seeds are spherical in shape and flattened on the side of the hilum. Generally, only one seed per locule can be seen, but 2 -3 may also be developed in a locule.

There are thousands of chemical compounds available in a tea leaf and many other chemicals are generated during tea processing. These chemical compounds highly contribute on the aroma and flavor of the tea. (Ho et al., 2015).

Tea Yield and Breeding Program

Tea is a crop that is highly affected by the weather conditions, especially droughts. Tea is known to be a rain fed cultivation and irrigation is rarely used in tea plantations. Therefore, drought conditions create irreparable losses for the yield. Also, increased rainfall conditions affect the tea plantations adversely by eroding topsoil and wash away the fertilizers and useful chemicals in the field (Willson and Clifford, 2012). The optimum temperature for tea cultivation is about 22°C and the reduction of monthly rainfall by 100 mm could reduce the productivity by 30 - 80 kilograms of Made Tea ha⁻¹ (Nathaniel, 2008). Thus, it can be concluded that the extended drought conditions have a negative impact on the productivity of tea yield. The droughts occurred in Sri Lanka and reduction of tea yield during 1983 and 1992 are best examples to indicate such scenario (De Costa et al., 2007). However, the research by Tuwei and Corley (2021), to find the drought tolerance for tea by comparing yields of selected clones in drought and non-drought years were able to find that tea clones vary in drought tolerance. The drought tolerance index developed by them, and drought symptom scored are core related with yield loss. These factors were found to be heritable so that they can be used in breeding cultivars for drought tolerance.

Tea yield is also greatly influenced by the shoot density and mean shoot weight per bush. These two parameters are considered as the yield components of tea which determines the potential productivity of a crop (Wijerathne, 2001). A study has shown that high yielding plant types should have high active and banji shoot fresh weight along with high shoot density and these traits could be effectively used in tea cultivar selection programs (Neranjana et al., 2014). Furthermore (Babu, 2007) conducted an analysis using 34 diverse tea genotypes in India to interpret nature of interrelationship among yield and yield related traits and revealed that dry and fresh weight of shoots, shoot density and leaf breadth are important yield determinants in tea. There is positive correlation

between size of the leaf and the yield in tea. Therefore, producing polyploidy tea cultivars with bigger leaves may be effective in obtaining a higher yield (Gunasekera and Ranatunga, 2003). In a recent review by Zakir and Addisu,(2020) has mentioned that natural polyploidy in tea either occurred by spontaneous chromosome doubling in somatic tissues or by occurrence of unreduced gametes. They have further mentioned that artificial induction of polyploidy can be done by chemicals such as Colchicine. The tea production could be increase by 40 -60% by induced polyploidy (Hasnain et al., 2015). Polyploidy increases the genetic materials in tea plant contributing to larger tree size and higher yields. Therefore, polyploidy can be used in tea breeding to cope with the environmental changes and to get a better high-quality yield (Zakir and Addisu, 2020).

Favorable weather conditions, Adoption of better management practices in the corporate sector after privatization of the management of estates previously managed by the state, proliferation of small holders mainly who are planting vegetatively propagated (VP) varieties and replacement of low yielding seedling tea with high yielding VP varieties can be listed as the major contributors towards the growth of production. The same study further mentioned that the yield of tea is also depends on the genetic factors which are inherited by the tea plant. But the fact that introduction of VP varieties narrows the genetic diversity of tea.

The success of a breeding or genetic conservation program is dependent on the genetic variation present in the gene pool. Therefore, it is important to understand the variation properly for a successful breeding program (Paul et al., 1997). Conventionally tea is propagated either through seeds or cuttings. Till the beginning of 19th century seedling teas was the commercial method for propagation (Chen et al., 2012). The most attractive feature about a field planted with tea raised from seed is the enormous variation observed. Since tea is generally cross-fertilized, seed tea is extremely heterogeneous with great diversity in growth, vigor, and morphological characters (Wijerathne, 2001). In seed propagation it is necessary to establish limited and separate areas of tea to make seed gardens. Mass selection based on morphological features was carried out on nursery seedling for selecting the parental plants for seed gardens, and tea growers began to select broad-leaf Assam types for planting so that some amount of uniformity was achieved (Nathaniel, 2008). Presence of tap root system in seedling teas as compared to VP tea could be capitalized so that seedlings have the potential of producing acceptable yield at least that are comparable to VP cultivar (Piyasundara et al., 2008). With the

wide distribution of the industry, it needs some rapid multiplication of tea plants. The first attempt to vegetative propagated tea was done in Indonesia by budding and grafting. Then faster propagation by single leaf cutting was developed in India, Sri Lanka and Indonesia (Chen et al., 2012).

There are two types of tea breeding systems mainly; they are hybridization and selection. Hybridization can be done by natural or artificial pollination. Selection method is much famous among these two methods. Majority of the tea clones have been developed through selection. The advantage of this approach is better isolation of characters with the help of molecular characterization. This can be done through marker assisted selections for a particular trait and recommend suitable plants for a better breeding program (Gunasekare et al., 2012) .

Producing cultivars with high yield, high quality and resistant against biotic and abiotic stresses are extremely recommended in the breeding strategies of tea breeding as they are the major objectives of the successful breeding program. In order to incorporate those desirable characteristics into the existing cultivars, research is continuing to create new progenies through controlled hybridization among known cultivars having important characteristics (Ariyaratne and Gunasekare, 2009). Therefore, it is necessary to preserve the genetic variability in tea. This is very important because the tea plantation all over the world has undergone a larger replanting of old seedling population (Gunasekare et al., 2012). Development of molecular markers for assessment of tea diversity is researched enough. But their utilization in marker assisted selections such as QTL mapping, trait introgression by molecular breeding and genetic improvement of tea are yet to be validated.

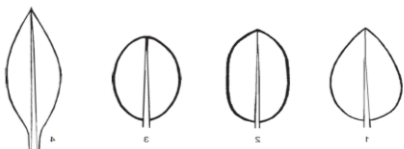
Morphological Characterization of Tea

For the identification of superior tea plants for a successful breeding program which is aimed to obtain a high-quality cultivar, a proper characterization should be performed. This depends on the information available on characterization and the evaluation of the accessions (Piyasundara et al., 2008). Use of morphological characters is cost effective with compared to the use of biochemical and molecular level characterization for preliminary characterization of large number of accessions to identify morphologically similar groups and for simple varietal identification of phenotypically distinguishable cultivars. Earlier selection of high-yielding genotypes

was done based on the visual estimations. These visual estimations include plucking point, size of flush etc. thereafter its being able to identify a positive correlation between yield and traits such as shoot density, shoot weight, internodal length, etc. (Neranjana et al., 2014). International Plant Genetic Resource Institute (IPGRI) and the International Union for the Protection of New Varieties of Plants (UPOV) have supplied the descriptor lists for standard ways of plant characterization ((International Plant Genetic Resources Institute, 1997b, a).

Leaf architecture (Table 01), growth habits, and floral biology are some of the important criteria in the morphological characterization of tea.

Table 01: Leaf-based descriptors used in Tea classification (IPGRI, 1997)

Descriptor	Explanation
Leaf Shape	 1: Ovate, 2: Oblong, 3: Elliptical, 4: Lanceolate
Leaf Base	 1: Acute, 2: Rounded, 3: Blunt
Leaf Margin	 1: Entire, 2: Wavy, 3: Serrulate, 4: Biserrate, 5: Denticulate

Morphological characterization is the most famous method of characterization among the tea breeding community even though there are some disadvantages. Due to the higher phenotypic variation of tea germplasm, those characteristics are much useful in

breeding programs. Exploitation of such variability by careful selection and thereby can be utilized for developing improved planting material (Vijayan et al., 2013). There are number of research where this morphological characterization was used to identify the desired characteristics to use in tea breeding around the world and in Sri Lanka. Research in India, the morphological characterization of seedling teas was documented, and identified a significant divergence among them. The immature leaf color of the population showed a considerable variation (Vijayan et al., 2013). Furthermore, in the western Himalayas, out of 121 accessions studied due to continuous variation of morphological characteristics the qualitative morphological characters failed to show any significant variation between cultivars, and among the quantitative morphological characters leaf petiole length and anther length did not show any variation. Leaf length and leaf width showed a higher Eigen value which can be used to identify the cultivar types and yield characteristics (Rajkumar et al., 2010).

A morphological analysis, which was conducted in Vietnam was performed based on 21 morphological traits related to stem, leaf, shoot, and flower, grouped 15 tested tea accessions into two principal clusters, of which the China type and the hybrids related to the China type were separate from the Shan and Assam type (Phong et al., 2016). This result is in accordance with the conventional classification of tea taxa, and similar to the study of genetic diversity of teas grown in Vietnam based on leaf characters where Seventeen tea accessions were morphologically described at Phu Tho Province in Vietnam and assessed for their diversity. Results proved the diversity of investigated tea morphologies based on morphological screening (Pandolfi et al., 2009).

Great variation of morphological characters was observed within 87 accessions of five taxa selected in the Yunnan province of China. Among them, quantitative characters of all the leaves (leaf length, leaf shape, leaf color, leaf texture, leaf margin serration, etc.) and most of the flower characters (except the characters of the period of flowering and petal length) showed a significant difference and fruits characters (fruit set, fruit color, etc.) did not show a significant difference. Seven of the thirty-three qualitative characters (life form, bud color, petal texture, pubescence on the ovary, style number, stamen location, and locule per fruit) showed a significant difference (Chen et al., 2005).

When considering phenotypic diversity based on morphological characters of Sri Lankan tea, in a study where 13 morphological descriptors were analyzed

using the Principal Component Analysis method used the 4 Principal Components (PC) with a higher Eigenvalue. It showed a significant contribution to the variation existing in the genotype studied. Furthermore, they accounted for approximately 78% of the total variation. A dendrogram constructed using cluster analysis for the first four PCs can be clearly differentiated into three well-recognized clusters. Out of 13 descriptors used except mature leaf petiole length and the appearance of leaf upper surface, all other 11 descriptors significantly contributed to the phenotypic variation of the germplasm. Of the 11 descriptors leaf width, leaf shape, leaf pigmentation, and petiole pigmentation were found to be highly discriminating descriptors (Piyasundara et al., 2008).

A further study in Sri Lanka where 203 accessions of tea germplasm were characterized using 20 morphological descriptors have shown that it has differentiated into 7 major clusters based on Principal Component analysis. As the first systematically recorded tea germplasm characterization using morphological descriptors, this study has provided the base for more advanced morphological characterization, where it facilitates the cultivar identification program of Sri Lankan tea cultivation (Piyasundara et al., 2009).

Serrations of the leaf margin, waviness of the leaf lamina, pigmentation in the young leaf, pigmentation in leaf petiole, size of the leaf, and leaf angle were found to be the most morphological discriminating descriptors for effective characterization of tea germplasm in Sri Lanka (Piyasundara et al., 2008). Further, these descriptors significantly contribute to the total phenotypic variation present in the germplasm and are useful in characterizing the tea germplasm (Piyasundara et al., 2009). There are 52 standard lists of descriptors proposed by IPGRI, but the study carried out by Piyasundara et al., (2008), using 203 germplasm accessions to represent the ancestry and origins of tea could be clustered into seven clusters with regards to 20 morphological characters. It further concludes that the minimum number of descriptors that can be used for the characterization of tea germplasm in Sri Lanka. So, these will be useful in future cultivar identification programs.

Neranjana et al., (2014) has mentioned that fresh and dry weight of banji shoots, shoot density and fresh and dry weight of active shoots showed very high positive correlation with yield. The active and banji shoot density as well as the shoot density of a tea bush could be effectively used in tea cultivar selection programs. The presence of considerable genetic diversity was

observed in a morphological study of clonal teas in southwestern. However they have suggested conducting a molecular study for the confirmation of the morphological results (Zakir and Addisu, 2020, Zakir and Dawid, 2020) .

Floral and leaf characteristics have been considered during the characterization process. When considering floral characteristics a wide variation was found in relation to the stigma positioning and style splitting (Ariyaratna et al., 2007). Further, it has revealed that there is a remarkably high variation in pistil morphology which was a result of an extensive inter-hybridization among major three tea taxa namely, Assam, China, and Cambodia. So, it confirms the Sri Lankan germplasm is rich with genes from all the three tea taxa (Ariyaratne and Gunasekare, 2009). Once considering the pistil-related morphology, remarkably high variation in pistil morphology is shown and it conclude that the Sri Lankan tea germplasm was a result of an inter-hybridization of major three taxa. This confirms the richness of germplasm from all the three taxa (Ariyaratna et al., 2007). Another study on floral characterization of tea exotic varieties in Sri Lanka has found that the collection was predominantly represented by China type followed by Cambod type. This was achieved by the characterization of fifty-four exotic accessions using 14 floral traits. The research has further mentioned that China-type accessions in the exotic germplasm can be useful in future tea breeding programs since the Sri Lankan tea germplasm is mostly represented by the accessions of Cambod type followed by Assam type (Kottawa-Arachchi et al., 2017).

Furthermore, a study conducted regional adaptability of US tea germplasm with 32 domestic individuals. They have first conducted a molecular analysis and then selected nineteen individuals for the morphological assessment of seven leaf traits, two floral and leaf yield to identify the plant's regional adaptability. The result of the study gives precise identification of plant materials and ability to choose more diverse accessions for breeding improved version of tea for the region (Clarke et al., 2023).

Molecular Characterization

Molecular characterization means the characterizing at the molecular level without any effect of environment or development or physiological state of the organism. Therefore, DNA-based markers are called as molecular markers (Avisé, 2012). Molecular Characterization is a very important procedure for the

determination of genetic identity in the accessions of a germplasm collection. Plant genetic resources could be properly utilized with a correct characterization. Characterization is basically studied either according to morphological traits, biochemical markers or molecular markers. Morphological characterization has some limitations in the precise identification of the accessions. Therefore DNA-based techniques are very helpful to overcome these limitations in both morphological characterization and the biochemical markers. DNA markers have the ability to identify polymorphism at the DNA sequence level. Consequently, they are independent of environmental influence in monitoring the whole genome (Ovesna et al., 2002).

The molecular marker-assisted characterization is a perfect method for the conservation of genetic resources. Molecular characterization could be used as an aid for the determination of breeding behavior of species, individual reproductive success, existence of gene flow, and movement of alleles within and between populations of the same or related species (Prasad, 2014). The Tea Research Institute of Sri Lanka was initiated in 1986, where tea germplasm conservation has been done since then. The genetic diversity of tea is detected and improved from analysis of morphological traits to biochemical approaches methods based on DNA markers which results in efficient conservation, maintenance, and utilization of existing genetic diversity and application of tea genetic resources in current tea breeding program (Gunasekare et al., 2012).

Plant DNA markers are essential tools for determining the diversity of plant species accurately. There are many marker techniques available for plant genetic resource characterization including RAPD, AFLP, SSR, SNP, etc. The DNA molecular markers provide a reliable and informative approach to estimate the genetic diversity and genetic relationship of tea cultivars. RAPD, AFLP, RFLP, and ISSR markers have been used in tea resource research for analyzing genetic diversity, phylogenetic analysis, and germplasm resource identification (Chen et al., 2005). Recently, more attention has been given to the use of different molecular markers such as Restriction Fragment Length Polymorphism (RFLP) (Matsumoto et al., 2002, Kaundun and Matsumoto, 2003), Random Amplified Polymorphic DNA (RAPD) (Wachira et al., 1995, Kaundun et al., 2000) and Amplified Fragment Length Polymorphism (AFLP) (Paul et al., 1997).

Molecular markers have been extensively used in the characterization of plant genetic resources. Many ways for analyzing the data from these

characterizations are used according to the specific interest of the study. All those analyses are generally started with a binary matrix of the presence or absence of DNA bands. The maintenance of a core set that could represent the genetic diversity of an entire germplasm collection is very useful in genetic studies. Therefore, a number of core collections have been successfully established with the aid of molecular markers (Rana, 2015).

The development of Amplified Fragment Length Polymorphism (AFLP) reveals significant levels of DNA polymorphism, indicating that AFLP is a reliable genetic molecular marker assay (Vos et al., 1995, Balasaravanan et al., 2003). AFLP markers were successfully used to investigate the diversity and genetic differentiation among Indian and Kenyan populations of tea where it could discriminate all of the tested tea genotypes from India and Kenya, even the genotypes which were unable to distinguish on the basis of morphological and phenotypic traits. Seventy-three unambiguous polymorphic amplified DNA fragments were revealed in this study (Paul et al., 1997).

According to a study carried out for South Indian tea by using Random Amplified Polymorphic DNA (RAPD) markers for the assessing of the ability of RAPD markers to characterize the variation of selected accessions of tea germplasm, a very high proportion of DNA diversity exhibited showing 65% polymorphism (Vijayan et al., 2013). To identify the genetic variation among the 18 tea clones of Bangladesh by reproducible amplification of DNA through RAPD analysis, 737 polymorphic bands out of 755 were identified (Boonarjee et al., 2013). Even though RAPD is able to overcome the technical limitations of RFLP, RAPD is much sensitive to experimental conditions with low reproducibility (Paul et al., 1997). A RAPD marker-assisted study of genetic relationships among Sri Lankan tea cultivars has shown that cultivars can be subdivided into two main groups as TRI cultivars and estate-selected cultivars. According to the RAPD analysis carried out, the study further described that TRI 2016, TRI 4006, China and Yabukita have been identified the most genetically distinct cultivars (Mewan et al., 2012). In an assessment of genetic relationships in vegetatively propagated and old seedling teas in Sri Lanka, the dendrogram obtained was able to divide eighteen tea accessions into three major groups as old seedlings and estate cultivars, cultivars derived from Cambodia hybrids and cultivars developed from controlled hybridization (Goonetilleke et al., 2007).

Inter-Simple Sequence Repeat (ISSR) markers have been widely used in tea characterization due to their nature of practicability and feasibility (Liu et al., 2009). The ISSR markers were first used for tea plants to assess the diversity of 25 clonal cultivars in India, and the results recommended that the ISSR-PCR method has the potential to be useful for genetic fingerprinting and molecular taxonomic classification of tea genotypes (Mondal, 2002). Also, ISSR markers were used to determine genetic relationship among tea cultivars from China, Japan, and Kenya in providing valuable information to assist parental selection in advanced tea breeding. In a study conducted by Tea Research Institute, China, 99.7% polymorphic bands were scored providing valuable information for the parental selection in current and future tea breeding programs by using ISSR markers (Yao et al., 2008). Also, 93 polymorphic bands out of 110 bands were identified by DNA using ISSR markers in a study conducted in Yunnan province (Liu et al., 2009).

Microsatellites or simple sequence repeats (SSRs) has become one of the most popular genetic markers recently. But there are only a few SSR markers are available for tea (*Camellia sinensis*) which is a major issue for applying genetic analysis, gene mapping, and marker assisted breeding in Tea (Ariyaratne and Gunasekare, 2009). In a study, novel 112 Tea Unigene-derived microsatellite (TUGMS) markers were found and thereby leading to widen the SSR marker resources in tea (Sharma et al., 2011). There are only about 212 SSR markers for tea, including genomic and EST-based SSRs.

In the first-ever genomic and EST-SSR-based framework of tea which has been reported has been mentioned that mapping of SSR loci will be of great value for comparative mapping with related species. Seventy-four novel polymorphic EST-SSR markers for the tea plant have been identified in China and they have shown high polymorphism and transferability in tea plants. They have also concluded that these markers could be used as an additional tool for cultivar identification, clustering, for parental selection in controlled hybridization in breeding program, for population structure and evaluation analysis (Quing et al., 2014). EST-SSRs were developed in Sri Lanka based on tea cultivars named TRI 2023, TRI 4023, and DT 1 (Mewan et al., 2012).

Unigene Microsatellites (UGMS) markers identified and characterized in a study conducted by the Institute of Himalayan Bioresource Technology could indicate the abundance and distribution of SSR in the expressed genome of *C. sinensis*. The identification and validation of 61 new UGMS markers will be much

helpful for the enriching of limited microsatellite marker resources in tea (Sharma et al., 2011).

In a study conducted by the Institute of Himalayan Bio Resource Technology 21 microsatellite markers were used to evaluate genetic diversity and DNA fingerprinting of 15 common tea accessions. Each accession had a unique marker profile, revealing that the microsatellite markers were useful for the differentiation of individuals among the tea collections. The polymorphism information content (PIC) ranged from 0.2 (CamsinM1) to 0.60 (TUGMS12), with an average of 0.359 (Bharadwaj et al., 2013).

In another study which was done by University of Cambridge, 15 microsatellite loci were developed for tea. These microsatellites have been evaluated for polymorphism in selected tea clones for the determination of their usefulness for authentication purposes. The majority of the developed microsatellites were known to be highly polymorphic (Freeman et al., 2004).

Genetic diversity and domestication origin of *C. taliensis*, were evaluated by using 14 nuclear microsatellite loci. In the study, these were evaluated using 587 individuals from 25 wild, planted, and recently domesticated populations and *C. taliensis* showed a moderately high level of overall genetic diversity ($H_s = 0.597$) (Zhao et al., 2014).

Eleven novel microsatellite primer pairs were developed for the wild tea, *C. sinensis* in Taiwan. These SSR markers were tested using 24 samples collected from wild tea populations and in cultivars and *C. japonica*. The number of alleles ranged from 4 to 18. The expected heterozygosity (HE) and observed (HO) heterozygosity were from 0.687 to 0.946 and from 0.042 to 0.792, respectively (Hung et al., 2007). Another study was carried out for genetic mapping by characterizing QTLs for catechin content in the tender shoots of tea using 406 simple sequence repeat (SSR) markers (Jian-Qiang et al., 2014). A study by Kottawa-Arachchi et al., (2021) where SSR markers for the characterization of exciting genetic diversity of Sri Lankan tea were able to group all accessions into three major groups and further cluster analysis gives three major genetic groups such as China types with Korean origins, Assam types with ASM4/10 ancestral relationship and mixture of different origins.

Another study conducted using SSR markers to find whether the genetic variation of newly improved clones in East Africa has stressed that there is

sufficient genetic diversity among the studied clones (Leonida et al., 2013).

Analysis of sixty-six clonal tea cultivars of China using thirty-three long core motif SSR markers gives the polymorphic information content (PIC) of the core SSR markers was >0.5 , with ≤ 5 alleles in each marker containing 10 or fewer genotypes (Wang et al., 2016).

An assessment conducted on genetic similarity among embryo culture tea plants using morphological and RAPD markers has confirmed that both can be effectively used in combination to assess the genetic similarity of embryo-cultured plants (Vithyashini et al., 2014).

Conclusions

The crop improvement program has been advanced with the integration of a genomic approach by simplifying the selection process. Therefore, the use of molecular markers in germplasm selection and tea breeding has become popular.

Selecting tea was a straightforward process in the early days, and it was based on morphology. But with the increase of the grower's demand; The cultivar development program has determined that resistance to biotic and abiotic stresses, along with tea quality, are important characteristics in addition to yield. This leads to the genetic bottleneck in tea diversity. Finally, with the large-scale use of vegetatively propagated cultivars further narrowing of genetic diversity was observed. Therefore, the characterization of tea germplasm for genetic improvement is important. The morphological characterization will give a background knowledge about the clones to be examined and molecular characterization will further confirm the genetic diversity.

These approaches will reduce the time taken in conventional breeding methods. Thereby, the breeders can generate superior cultivars incorporating genetic diversity available in the existing tea germplasm to new hybrid varieties. Selected tea varieties that have had their morphological and molecular characteristics verified can be utilized as primary sources to develop those in breeding programs.

References

- Ariyaratna, H., T. Arachchi, and M. Gunasekera. (2007). Floral Biology and Breeding System of Tea [*Camellia sinensis* (L.)]: Implications on the Tea Breeding Programme. Sri Lanka Journal of Tea Science **72**:31-43.
- Ariyaratne, H., and M. Gunasekare. (2009). Pistil related morphological traits reflect genetic diversity of tea in Sri Lanka. Sri Lanka Journal of Tea Science **74**:1-8.
- Avise, J. C. (2012). Molecular markers, natural history and evolution. Springer Science & Business Media.
- Babu, S. (2007). Path Analyses of Yield and Yield Related Traits of Thirty Four Diverse Tea (*Camellia* L. sp.) Genotypes. Asian Journal of Plant Sciences **6**:441-443.
- Balasaravanan, T., P. K. Pius, R. Raj Kumar, N. Muraleedharan, and A. K. Shasany. (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.
- Chen, J., P. Wang, Y. Xia, M. Xu, and S. Pei. (2005). Genetic diversity and differentiation of *Camellia sinensis* L. (cultivated tea) and its wild relatives in Yunnan province of China, revealed by morphology, biochemistry and allozyme studies. Genetic Resources and Crop Evolution **52**:41-52.
- Chen, L., Z. Apostolides, and Z.-M. Chen. (2012). Global tea breeding. Springer. Berlin.
- Clarke, C., B. S. Richter, and B. Rathinasabapathi. (2023). Genetic and morphological characterization of United States tea (*Camellia sinensis*): insights into crop history, breeding strategies, and regional adaptability. Frontiers in Plant Science **14**.
- Conway, J. (2021). Production of tea worldwide 2006-2019, by country.
- De Costa, W., A. J. Mohotti, and M. A. Wijeratne. (2007). Ecophysiology of tea. Brazilian Journal of Plant Physiology **19**:299-332.
- Devarumath, R., S. Nandy, V. Rani, S. Marimuthu, N. Muraleedharan, and S. Raina. (2002). RAPD, ISSR and RFLP fingerprints as useful

- markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* ssp. *assamica* (Assam-India type). *Plant Cell Reports* **21**:166-173.
- Freeman, S., J. West, C. James, V. Lea, and S. Mayes. (2004). Isolation and characterization of highly polymorphic microsatellites in tea (*Camellia sinensis*). *Molecular Ecology Notes* **4**:324-326.
- Goonetilleke, W., P. Priyantha, and K. Mewan. (2007). An assessment of genetic relationships in VP and old seedling teas in Sri Lanka using RAPD markers. *Sri Lanka Journal of Tea Science* **73**:39-48.
- Gunasekare, M., M., J. Ranatunga, Piyasundara, and J. Kottawa-Arachchi. (2012). Tea Genetic Resources in Sri Lanka: Collection, Conservation and Appraisal. *International Journal of Tea Science* **8**:51-60.
- Gunasekera, M., and M. Ranatunga. (2003). Polyploidy in tea (*Camellia sinensis* L.) and its application in tea breeding. a review. *Sri Lanka Journal of Tea Science*. **68**:14-26.
- Hasnain, A., M. Razaq, and Salahuddin. (2015). Induced Polyploidy as a Tool for Increasing Tea (*Camellia sinensis* L.) Production. *Journal of Northeast Agricultural University (English Edition)* **22**:43-47.
- Ho, C.-T., X. Zheng, and S. Li. (2015). Tea aroma formation. *Food science and human wellness* **4**:9-27.
- International Plant Genetic Resources Institute. (1997a). Descriptors for Tea (*Camellia Sinensis*). Bioversity International.
- International Plant Genetic Resources Institute. (1997b). Descriptors for Tea (*Camellia Sinensis*). Bioversity International.
- Jayasinghe, S. L., and L. Kumar. (2020). Climate Change May Imperil Tea Production in the Four Major Tea Producers According to Climate Prediction Models. *Agronomy* **10**:1536.
- Jian-Qiang, M., M.-Z. Yao, C.-L. Ma, X.-C. Wang, J.-Q. Jin, X.-M. Wang, and L. Chen. (2014). Construction of a SSR-based genetic map and identification of QTLs for catechins content in tea plant (*Camellia sinensis*). *PloS one* **9**:e93131.
- Kaundun, S. S., and S. Matsumoto. (2003). Identification of Processed Japanese Green Tea Based on Polymorphisms Generated by STS-RFLP Analysis. *Journal of Agricultural and Food Chemistry* **51**:1765-1770.
- Kaundun, S. S., A. Zhyvoloup, and Y.-G. Park. (2000). Evaluation of the genetic diversity among elite tea (*Camellia sinensis* var. *sinensis*) accessions using RAPD markers. *Euphytica* **115**:7-16.
- Kithsiri, K. K., V. Jayamanna, and L. Abewickrama. (2020). Evaluation of competitiveness of Ceylon tea in the world market. *Sri Lanka journal of Agriculture and Ecosystems*. **2**:89-98.
- Kottawa-Arachchi, J., S. Dilrukshi, M. Piyasena, and M. Ranatunga. (Year) of Conference. Floral characterization of exotic tea (*Camellia sinensis* L.) germplasm in Sri Lanka. Pages 43-45 in *Proceedings of the International Symposium on Agriculture and Environment*.
- Kottawa-Arachchi, J., M. Ranathunga, R. Sharma, A. T. Amarakoon, M. Gunasekare, H. Chaudhary, and R. Attanayake. (Year) of Conference. Molecular characterization of Sri Lankan tea [*Camellia sinensis* (L.) O. Kuntze] genotypes with diverse origins using SSR markers for future breeding programmes. Page 2 in *International Conference on Applied and Pure Sciences, Faculty of Science, University of Kelaniya, Sri Lanka*.
- Leonida, C., S. Kamunya, A. Alakonya, M. Solomon, M. Uwimanna, and O. Phillip. (2013). Characterization of 20 clones of tea (*Camellia sinensis* (L.) O. Kuntze) using ISSR and SSR markers. *Agricultural Science Research Journal* **3**:292-302.
- Liu, B., Liu BenYing, W. L. Wang LiYuan, L. Y. Li YouYong, T. Y. Tang YiChun, H. W. He Wei, C. H. Cheng Hao, and W. P. Wang PingSheng. (2009). ISSR markers for

- discriminating tea germplasm resources from Yunnan Province. *Journal of Tea Science* **29**:355-364.
- Matsumoto, S., Y. Kiriwa, and Y. Takeda. (2002). Differentiation of Japanese green tea cultivars as revealed by RFLP analysis of phenylalanine ammonia-lyase DNA. *Theoretical and Applied Genetics* **104**:998-1002.
- Mewan, K., I. Abeysinghe, M. Saha, P. Y. Pang Yongzhen, E. Karunanayake, T. Tirimanne, J. Everard, and R. Dixon. (2012). Development of genomic and EST microsatellite markers and their usefulness in genetic conservation and improvement of tea (*Camellia sinensis* L.). *Sri Lanka Journal of Tea Science*, **77**:22-44.
- Mukhopadhyay, M., T. K. Mondal, and P. K. Chand. (2016). Biotechnological advances in tea (*Camellia sinensis* [L.] O. Kuntze): a review. *Plant Cell Reports* **35**:255-287.
- Nathaniel, R. K. (2008). *Hand Book on Tea*. Tea Research Institute of Sri Lanka. Talawakele, Sri Lanka.
- Neranjana, T. B. K. H., M. Ranatunga, J. Kottawa-Arachchi, and K. Ranaweera. (2014). Correlation and Path Coefficient Analysis of Yield and Yield Related Traits in Tea: Implications on Cultivar Selection. *Sri Lanka Journal of Tea Science* **79**:1-11.
- Ovesna, J., K. Poláková, and L. Leišová. (2002). DNA analyses and their applications in plant breeding. *Czech Journal of Genetics and Plant Breeding* **38**:29-40.
- Pandolfi, C., S. Mugnai, E. Azzarello, S. Bergamasco, E. Masi, and S. Mancuso. (2009). Artificial neural networks as a tool for plant identification: a case study on Vietnamese tea accessions. *Euphytica* **166**:411-421.
- Paul, S., F. N. Wachira, W. Powell, and R. Waugh. (1997). Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. *Theoretical and Applied Genetics* **94**:255-263.
- Phong, N. H., W. Pongnak, K. Soyong, S. Poeaim, and A. Poeaim. (2016). Diversity of tea (*Camellia sinensis*) grown in Vietnam based on morphological characteristics and inter-primer binding sites (iPBS) marker. *International journal of Agriculture Biology* **18**:385-392.
- Piyasundara, J., M. Gunasekare, and I. Wickramasinghe. (2008). Characterization of tea (*Camellia sinensis* L.) germplasm in Sri Lanka using morphological descriptors. *Sri Lanka Journal of Tea Science* **74**:31-39.
- Piyasundara, J. H. N., M. T. K. Gunasekare, and I. P. Wickramasinghe. (2009). Characterization of Tea (*Camellia sinensis* L.) Germplasm in Sri Lanka using Morphological Descriptors. *Sri Lanka Journal of Tea Science* **74**:31-39.
- Rajkumar, S., S. Karthigeyan, R. K. Sudived, R. Rajkumar, N. Muraleedaran, S. C. Das, M. Hazarika, and A. P.S. (2010). Genetic Diversity of Indian Tea (*Camellia sinensis* (L.) O. Kuntze) Germplasm Detected Using Morphological Characteristics. *Journal of Cell and Plant Science* **1**:13-22.
- Rana, M. K. (2015). *Molecular Characterization of Plant Genetic Resources*, New Delhi.
- Sharma, H., R. Kumar, V. Sharma, V. Kumar, P. Bhardwaj, P. S. Ahuja, and R. K. Sharma. (2011). Identification and cross-species transferability of 112 novel unigene-derived microsatellite markers in tea (*Camellia sinensis*). *American Journal of Botany* **98**:e133-e138.
- Teshome, K. (2019). Effect of tea processing methods on biochemical composition and sensory quality of black tea (*Camellia sinensis* (L.) O. Kuntze): A review. *Journal of Horticulture and Forestry* **11**:84-95.
- Vijayan, D., J. Thomas, K. M. John, P. Pius, and N. Muraleedharan. (2013). Characterization of selected south Indian tea (*Camellia* spp.) germplasm using morphological traits and RAPD markers. *Keanean Journal of Science* **2**:45-52.
- Vithyashini, L., M. Ranatunga, K. Ranaweera, and I. Wickremasinghe. (2014). Assessment of Genetic Similarity Among Embryo Cultured *Camellia Sinensis* L.(Tea) Plants Using

- Morphological and Rapd Markers. Proceedings of the Peradeniya Univ. International Research Sessions. **18**:557.
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. v. d. Lee, M. Hornes, A. Friters, J. Pot, J. Paleman, and M. Kuiper. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**:4407-4414.
- Wachira, F. N., R. Waugh, W. Powell, and C. Hackett. (1995). Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. *Genome* **38**:201-210.
- Wang, R. J., X. F. Gao, X. R. Kong, and J. Yang. (2016). An efficient identification strategy of clonal tea cultivars using long-core motif SSR markers. *SpringerPlus* **5**:1152.
- Wang, X., H. Feng, Y. Chang, C. Ma, L. Wang, X. Hao, A. I. Li, H. Cheng, L. Wang, P. Cui, J. Jin, X. Wang, K. Wei, C. Ai, S. Zhao, Z. Wu, Y. Li, B. Liu, G.-D. Wang, L. Chen, J. Ruan, and Y. Yang. (2020). Population sequencing enhances understanding of tea plant evolution. *Nature Communications* **11**:4447.
- Wijerathne, M. A. (2001). 'Shoot Growth and Harvesting of Tea', . Tea Research Institute of Sri Lanka, Thalawakelle.
- Willson, K. C., and M. N. Clifford. (2012). *Tea: cultivation to consumption*. Springer Science & Business Media.
- Xia, E.-H., W. Tong, Q. Wu, S. Wei, J. Zhao, Z.-Z. Zhang, C.-L. Wei, and X.-C. Wan. (2020). Tea plant genomics: achievements, challenges and perspectives. *Horticulture Research* **7**.
- Xiao, P., and Z. Li. (2002). Botanical classification of tea plants. Pages 17-34 in Yong-Su. Zhen, editor. *Tea: Bioactivity and Therapeutic Potential. Medicinal and Aromatic Plants—Industrial Profiles*. Taylor and Francis, London. CRC Press.
- Yao, M. Z., L. Chen, and Y. R. Liang. (2008). Genetic diversity among tea cultivars from China, Japan and Kenya revealed by ISSR markers and its implication for parental selection in tea breeding programmes. *Plant Breeding* **127**:166-172.
- Zakir, M., and M. Addisu. (2020). Estimation of Genetic Variance on Tea (*Camellia sinensis* (L.) O. Kuntze) Clones based on Morphological Markers in Southwestern Ethiopia. *International Journal of Plant Breeding* **7**:641-647.
- Zakir, M., and J. Dawid. (2020). Polyploidy and it's Applications in Tea (*Camellia sinensis* L.) Breeding; Review. *International Journal of Research Studies in Science, Engineering and Technology* **7**:30-34.
- Zhao, D.-w., J.-b. Yang, S.-x. Yang, K. Kato, and J.-p. Luo. (2014). Genetic diversity and domestication origin of tea plant *Camellia taliensis*(Theaceae) as revealed by microsatellite markers. *BMC Plant Biology* **14**:14.