Tea genetic resources in Sri Lanka: Collection, conservation and appraisal

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ABSTRACT: At the Tea Research Institute of Sri Lanka conservation aspects on tea germplasm in *ex-situ* gene bank as living collection was initiated in 1986. Currently, over 600 accessions are being conserved in field gene bank, and efforts have been taken to characterize, evaluate and utilize them in the tea breeding programme. This paper highlights the significant achievements in the areas of germplasm collection, conservation, characterization and evaluation and their use in tea breeding programme, giving prominence to the cost-effective complementary strategies adopted in germplasm conservation and holistic approaches followed in germplasm characterization to facilitate managerial activities. Over the years, the methods for detecting genetic diversity have expanded from analysis of discrete morphological variants to biochemical approaches and to co-efficient of pedigree analysis to methods based on DNA markers. This kind of an approach, where genetic diversity assessment is supplemented with a combination of criteria, had provided a better sound base towards efficient conservation, maintenance and utilization of existing genetic diversity in the collection, than restricting to a single criterion. Moreover, the biotechnological approaches in strengthening germplasm activities in using them in a rational manner is also discussed, and the importance of assembling a functional core collection of germplasm to enhance the practical applicability of tea genetic resources in current tea breeding programme to address the diverse needs of the growers and the consumer is also highlighted.

Keywords: Germplasm; conservation; characterization; appraisal; core collection; tea; *Camellia* species

Introduction

Tea is an introduced crop to Sri Lanka. Hence, no wild relatives or land races of tea available in the country. Imported seeds were the only source of planting material available during the early years of tea cultivation in Sri Lanka.[1] Seeds were imported mainly from India and China, and hence seedling populations were of mixed types containing jats of different sizes. Those seedlings were therefore highly heterogeneous.

Tea was first introduced to Sri Lanka (then Ceylon) from Kolkata Botanical Garden in India by Dr. Robert Bruce. The seeds brought were planted in Royal Botanical Garden in Peradeniya in 1824. The first commercial plantation of tea was, however, established by a Scottish planter, James Taylor in 1867 at Loolecondera Estate, Hewaheta, which is located in the mid country region. At the time, coffee was the mainstay of the country's economy and was facing a crisis owing to coffee rust disease caused by fungus, *Hemileia vastatrix*. As a result most of the coffee plantations were replaced with tea within a short period and has been grown with success since then.

Origin of Planting Materials used in the Country

By 1875, an extent of nearly 4000 ha was under tea cultivation which showed a steady increase over the years. Since the inception of tea industry until the 1950s, tea plants were raised from seed, mainly the China type. The advent of commercially viable vegetative propagation technique coupled with introduction of Tea Replanting Subsidy Scheme by the government in 1958, encouraged planting of vegetatively propagated tea cultivars. Thereafter, low productive seedling fields had been replaced gradually with high-yielding vegetatively propagated cultivars quite rapidly. At present, over 45% of the total tea acreage is under seedlings of highly mixed types and the balance extent is under vegetatively propagated cultivars. Much of the seedling tea is about more than 100-years old and some tea fields are no longer considered agriculturally productive. Those fields are being earmarked for replanting with new tea cultivars aiming at high productivity causing loss of these valuable genetic resources with time.

Threat for Genetic Erosion

The genetic base of cultivated tea is very narrow due to the recurrent use of same parents in the tea breeding programme over the years[2] and also use of few popular tea cultivars widely in tea plantations aiming to achieve high productivity. The limited genetic diversity renders

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tea plantations more vulnerable to stress conditions such as pests, diseases and drought. With the advent of successful vegetative propoagation method in tea, gradual replacement of old seedling tea that are less productive was steadily increased, thus causing reduced genetic variability in planting material used in tea estates. Fortunately, limited extents of old seedling tea fields are still retained in some tea estate in the country. Those seedlings posses countless of genetic variations. In the face of rapid genetic erosion due to uprooting of old seedling tea for replanting programmes, it has become important to collect and preserve those genetic resources before losing them forever.[3] Thus, adoption of appropriate and effective conservation strategies has received more attention over the years.

Collection and Conservation Efforts

Painstaking evaluation and characterization of germplasm to maximize the genetic diversity may be counter productive where the rate of erosion is so high that the diversity is lost while planning is in progress. Under such circumstances, undertaking a collecting expedition in a more accelerated pace is of prime importance. It has often become necessary to collect germplasm before it can be ascertained that it contains genes of genotypes not already present in the collection. Hence, strategic and complementary approaches have been adopted in conserving the germplasm.

Complementary Strategies in Germplasm Conservation

A new strategy has been introduced to conserve old seedling teas that are still in existence in many of the tea estates in Sri Lanka,[4] before they are uprooted and replanted with more uniform and improved cultivars. This approach is similar to *in-situ* or on-farm conservation, in which growing community plays a pivotal role. This also provides an opportunity for selecting potential tea cultivars from existing old seed tea populations that are well adapted for specific locations.[5] As it entails a large number of samples and recourses, initial planning is to follow the method described by Fu[6] and Park *et al.*,[7] that involves molecular marker analysis using pooled samples from bushes in each *in situ* site to identify the site(s) having individuals with high genetic diversity.[8]

Strength of the *Ex-Situ* Germplasm Collection

Conservation aspects on tea germplasm in *ex-situ* gene bank as living collection was initiated in 1986. Currently, about 600 accessions are being maintained in field gene bank at the Tea Research Institute of Sri Lanka. The tea genetic resources conserved in the field gene bank can be broadly categorized into two groups: (1) beverage type and (2) non-beverage type (see Fig. 1).

Beverage types comprise of all tea accessions, and can be broadly divided into adapted and unadapted germplasm as well as advanced breeding lines. Those materials can be further sub-divided as "Introductions", "Estate Selections" and "Improved Cultivars", primarily based on available pedigree data and breeding history.[9] "Estate selections" are the once that have been selected from old seedling populations on tea estates from various agro-ecological regions in the country. Accessions are maintained in gene banks at main station at Talawakelle and regional stations at Ratnapura, Passara and Galle, some accessions being maintained as duplicates in more than one location.

The systematic breeding activities undertaken in keeping with varied objectives have resulted in deriving improved cultivars, and obsolete varieties were also conserved in the genebank. In addition, some of the new elite germplasm generated through breeding but not entered into final stages of evaluation to release as potential cultivars have also been conserved as pre-breeding materials for future use.

Beverage Types

Introductions

Introduction of tea seeds from India and China was the only source of planting material during the early stages of the tea industry. Currently at TRI germplasm collection, accessions introduced from India, Indo China, Japan, Korea and Russia are also available.

Introductions from India

The ancestry of popular "TRI 2020 series' cultivars is traced back to seeds originated from a single tree at Tocklai Experimental Station of the Indian Tea Association (now Tea Research Association) in Assam.[10] Realizing the potential for exploiting genetic variability in the seeds of ASM 4/10, subsequent introductions of another batch was carried out in 1958 where 15 "TRI 62 series" accessions were released[10] in 1960s, out of which 3 are being recommended for commercial cultivation.[11] Fourteen "TRI 3000 series" cultivars also originated form another batch of seeds from ASM 4/10 are available at TRI germplasm.[12]

Introductions from Indo China

TRI 2043, which has popularity for production of "Silver tips", is a selection made at the TRI by Dr. Tubbs during 1950s from the progeny of seed of Shan Bansang No. 777 received from Pho Ho Station, Indo China. Similarly, high quality cultivar TRI 777 is also a selection made at TRI from the seedling progeny of Shan Cho Long No. 777 from the same Station.[10]

Introductions from Japan

Six seedlings of morphologically different "Yabukita" plants, seeds brought from Japan are available at TRI germplasm and the appearance more towards China type.[13] In 2001 another seed stock of "Yabulita" was received and nine genotypes of Yabukita are available in the present collection.

Other Exotic Types

In 2001 two seed batches from Korea and China were received. Four wild types, 40 cultivated type Korean seedlings and 14 Oolong type China seedlings are available in the present collection. Plants raised from a batch of seeds from Russia which was received in 2006 and some East African accessions with unknown origin are also available in the present collection.

"Estate Selections"

With the development of vegetative propagation technique during 1931–1935, clonal selection programme was started as a means of developing improved planting materials. Selection programme was extended to old seedling tea fields, and accessions selected based on their field performances were released as "Estate selections". Selections started from way back in 1950s and continue till date. Estate selections are named on the sites or Estates where they were originally selected. Through the long-term estate cultivars selection scheme adopted at the institute since 1905, a total of 688 estate selections were originated from old seedling tea populations, existing in various estates covering 10 agro-ecological regions.[14] At present TRI holds 217 Estate selections in the collection conserved at four different locations.

Number of Estate selections available at TRI germplasm based on their geographical origin is given in Table 1.

Table 1: Origin and sources of estate selections in Sri Lanka (based on agro-ecological region)

Origin/Source	No. of accessions
Up country intermediate zone2 (IU2)	3
Up country intermediate zone3 (IU3)	36 (1)
Up country wet zone1 (WU1)	104 (4)
Up country wet zone2 (WU2)	30 (1)
Up country wet zone3 (WU3)	6 (1)
Low country wet zone1 (WL1)	8 (3)
Mid country wet zone1 (WM1)	6 (1)
Mid country wet zone2 (WM2)	13 (1)
Mid country wet zone3 (WM3)	11 (2)

Note: Figures in parenthesis are number of recommended cultivars for commercial planting.

Improved Tea Cultivars

The growers' ready and successful acceptance of cultivars of the TRI 2000 series encouraged the plant breeders to exploit the inherent variability of these cultivars by allowing them to produce open pollinated seeds.[2] Later, the selections made out from the open pollinated progenies led to the development of some of the TRI 3000 series cultivars during 1980s. A number of accessions derived from open pollinated progenies are given in Table 2.

Controlled hybridization of tea with the aim of creating variations was initiated at the institute during 1961–1962 in order to produce superior cultivars.[2] Crossing of selected parents and selection of best recombinants among the progeny appears to be the most fruitful line of work at present. Early attempts by tea breeders during 1970s resulted releasing of TRI 4000 series and some of the TRI 3000 series cultivars as a result of controlled hybridization programme. Summary of accessions derived from controlled hybridization programme is indicated in Table 3.

Table 2: Sources of open pollinated (half-sib selections) improved tea cultivars in Sri Lanka

Origin/Source	No. of accessions
ASM 4/10	2 (1)
TRI 2000 series (TRI 2024, 2025, 2026)	20 (8)
Estate selections	2

Note: Figures in parenthesis are number of recommended cultivars for commercial planting.

Table 3: Sources of cross-pollinated (full-sib selections) improved tea cultivars in Sri Lanka

Parentage	No. of accessions
ASM 4/10 X DT 95	6 (3)
ASM 4/10 X TRI 777	7
ASM 4/10 X CY 9	11 (6)
TRI 777 X TRI 2026	21 (1)
TRI 2000 series X TRI 2000 series	36 (10)
TRI 2000 series X Estate selections	15 (3)
Estate selections X Estate selections	8 (2)
Other	2

Note: Figures in parenthesis are number of recommended cultivars for commercial planting.

Special Type of Germplasm

Though many cultivars developed and selected at the TRISL are diplods ($2n = 2x = 30$), there are some natural triploid cultivars which were selected from seedling tea populations existing on estates.[15] They are HS 10A and GF5 and are available in the germplasm collection at the main genebank.[16] Those were confirmed as triploids and cultivar HR 1/8 was reported as a suspected aneuploid. Moreover, TRI 3069 which is a colchicine-induced tetraploid from a diploid cultivars of TRI 2025, is also a special kind of germplasm available in the collection. A non-fermenting tea cultivar, TRI 9, which is found to be deficient in enzymatic copper[17] is a unique type of local germplasm available in Sri Lanka. TRI 2043, which is an exclusive germplasm having properties for producing "silver tips" is also another special type of germpalsm found only in Sri Lanka. Cultivar TRI 2043 is a cambod hybrid with dense pubescence in the unfurled bud and anthocyanin pigmentation in immature leaves. This tea gives a very pale straw-coloured liquor. This unique type of tea fetches premium prices in the Middle East market.

Allied Species—Non-Beverage Types

Non-beverage type and related species conserved in the genebank includes *C. sasanqua*, *C. japonica*, *C. rosaeflora* and *C. lutenscens*, which show promise in interspecific hybridization programme. The germplasm collection is, however, under-represented with allied species of tea. Nevertheless, acquisition of diverse and elite germplasm from exotic sources is critical in this regard.

Enhancing Access to Germplasm

The exploitation of genetic potential currently warehoused in germplasm is hampered by the limited availability of information on characterization and evaluation data of available accessions. However, adequate description about the germplasm conserved is an important aspect that determine its use in the crop improvement programmes. Because of the larger number of accessions, often problems are encountered in conservation, documentation, multiplication and evaluation. To overcome this problems and to facilitate utilization of germplasm, attempts were focused on assembling a core collection of germplasm with the aim of minimizing the cost of genetic conservation while ensuring representation of maximum genetic variation.

Complementary and Cost-Effective Strategies in Germplasm Management

The cost involved in the plant genetic resource management activities in a crop such as tea, where field gene bank as a living collection is the only resort, is a matter of concern with the limited financial budget allocation for research activities at the institute. Hence, complementary and cost-effective alternatives have been sought for sustaining the germplasm conservation and management programme in the long run. Although the core-collection concept, introduced by Frankel,[18] aims primarily in facilitating germplasm managerial aspects, it also greatly facilitates the detail characterization and evaluation and search for desirable new characters in the collection.

Traditionally core has been constructed by a variety of taxonomic, morphological, agronomic and ecogeographic criteria. However, it is necessary to combine morphological, pedigree, passport, biochemical data and molecular marker data to build a core collection to enhance its practical applicability. Therefore, it is important to compile available information on tea genetic resources and make use of them in assembling a core collection.

Facilitating Germplasm Utilization and Managerial Aspects by Assembling a Core Collection

Assembling a core collection of germplasm at TRISL was initiated with the main aim being to create more descriptive information regarding the accession. Iin 2005, Gunesekera and Kumara[14] initiated the documentation of the information available on Estate selections made from 1930s onwards as an initial step towards systematic maintenance of tea germplasm resources. Thereafter, efforts were focused on collating the scattered information available on diffuse tea genetic resources, systematize them and to construct a preliminary core collection of tea genetic resources.

Accessions in the whole collection were stratified to groups based on the available information on taxonomy, pedigree, geographical origin, domestication, distribution, utilization and breeding history. The division into distinct groups was achieved using a step-wise procedure (stratification) resulting in a hierarchy and constructing a "diversity tree", following a method proposed by Boukema *et al.*[19] Firstly, the important major divisions were made, followed by splitting these subgroups into smaller ones, *etc*. This splitting into subgroups was continued until the subgroups were genetically homogeneous or there was no information to base further groupings. The hierarchy was recorded in a descriptor called the "path indicator", a series of ciphers which describe the subsequent divisions. The results generated were of considerable importance to narrow down the breeding stock and towards assembling a core sub-sets of the germpalsm.[9]

Characterization and Evaluation of Germplasm

The systematic conservation and maintenance of genetic resources followed by their proper characterization and evaluation is a vital aspect in utilizing the genetic materials in crop improvement. Characterization of gernplasm can be done using morphological, biochemical and molecular descriptors following a relevant standard criterion included in the Descriptors for Tea.[20]

Morphological Characterization

In the past morphological characteristics have been used mainly for the purpose of cultivar identification (Richards & Sebastiampillai, 1964; Wickramaratne, 1981).21,22 Recently more systematic approach was adopted using tea descriptors developed by the International Plant Genetic Resource Institute20 (IPGRI, 1997) to arrive at an internationally accepted format for germplasm characterization, evaluation and documentation. Initial studies were focused on identifying minimum list of descriptors or highly discriminating descriptors relevant for rational characterization of local germplasm (Gunasekare et al., 2001; Gunasekare & Pieris, 2006; Piyasundara et al., 2006).23--25 Of the 35 vegetative descriptors proposed by the IPGRI, only 6 descriptors (viz. type of serration of leaf margin, waviness of the leaf margin, pigmentations in young leaf, pigmentations in leaf petiole, size of the leaf and leaf angle) were found adequate to define the phenotypic variation of the local collection and can be used in distinguishing accessions into phenotypically diverse groups. Slight modifications were also made in some vegetative descriptor scales to better suit the descriptors in charactering the collection (Piyasundara, 2008).26 Over 200 accessions have so far been characterized phenotypically and discriminated into discrete groups using standardized descriptors (Piyasundara et al., 2008; Piyasundara & Gunasekare, 2008).27,28

Figure 1. Phenotypic diversity in the present germplasm collection.

In another study, 65 exotic germplasm accessions were characterized using 31 vegetative traits and 8 floral traits . Multivariate analysis enables separating the accessions in to morphologically distinct 7 groups and hasve provided a reasonable foundation in discriminating those into categories. Biochemical characterization of the same exotic stock based on parameters such as chlorophyll content, total polyphenols and fermentation rate revealed that there is a considerable variation present in the exotic germplasm collection.[29]

Morphological diversity in the present collection depicted in Plate 1 (Figure 1) illustrates the rich variability present in the collection.

As reproductive traits are known to be less affected by the environment than the vegetative traits, some investigations were conducted to analysze the genetic diversity present in the collection using floral characteristics (Sundaravathany et al. 2005),30 especially the pistil traits (Ariyarathna & Gunasekare, 2008).31 It was revealed that the pistil traits such as number of arms in the style, length of style arms and length of the style column represents a wide variations in the germplasm accessions. The remarkably high variation in pistil morphology captured in the study also confirmed that the local germplasm collection was a result of an extensive inter-hybridization among major three tea taxa.

Biochemical Characterization

Biochemical characterization of tea germplasm was initiated with 35 accessions as a representative samples from tea germplasm. According to the IPGRI guidelines, thirteen biochemical descriptors were used to characterize germplam accessions. Wide variation was observed in important biochemical parameters such as total polyphenols, total catechins, amino acids and caffeine, among different accessions suggesting potential of selecting them in future breeding programmes. The results generated in this study enables separating the accessions into groups which are biochemically well defined (Kottawa-Arachchi et al, 2011).32

Isozyme Polymorphism and DNA-based Characterization

Phenotypic variation is positively associated with genetic diversity, yet also depends on environmental factors and the interaction between genotypes and environment. Hence, estimating genetic diversity using phenotypic markers have several limitations, in a perennial crop like tea which is also grown across diverse environments. As such, the attention was focused on using protein-based techniques. Polymorphism in isozyme variations was limited to very few isozymes with inadequate polymorphism (Liyanage et al., 1999).33 Due to paucity of isozyme loci, efforts have been shifted towards DNA-based marker systems. Work on this line was initiated primarily to measure the genetic diversity of 85 tea accessions to identify parents for future breeding programs using RAPD markers (Mewan et al, 2005; Goonatilake et al., 2006).34,35 The recommended cultivars and advanced breeding lines exhibited high degree of molecular similarity and the similarity was ascribed to their common pedigree. Few accessions such as TRI 777, TRI 2016, China and Yabukita, proved genetically diverse from the rest of the accessions studied. Results also revealed that estate cultivars, which were derived from seedling tea populations in 1960s, were more diverse than the cultivars developed during latter breeding programs in 1980s from open pollinated and controlled hybridized progenies.

Genealogical Approaches in Genetic Diversity Analysis

Genealogical approaches for measuring genetic diversity combined with statistical methods have not been carried out so far for woody perennials such as tea locally or internationally. The first report on Co-efficient of Parentage (COP) analysis in tea based on pedigree information to measure the genetic diversity among commercial cultivars and their parental lines, revealed that ASM 4/10 and CY9 were the main ancestral lines that contributed for the cultivated genepool (Ariyarathna & Gunasekare, 2006).36 Two major COP groups identified provide the base for categorizing cultivars into genealogically distinct groups, thus promoting effective utilization of germplasm in future breeding programs. It further highlighted that there is an appreciable amount of genetic potential to be tapped from some of the existing commercial cultivars and also unadapted germplasm.

Biotechnological Approaches in Germplasm Conservation

Tea is propagated vegetatively and therefore, conserved as a living collection as whole plants in field gene bank. However, there are several constraint related to managerial aspects of field gene bank, such as exposure to natural disasters, attacks by pests and pathogen and high maintenance cost. Alternatively, in vitro preservation of germplasm can be used as safe and complementary option to field gene banks. Among the cold storage methods, cryopreservation has proven to be a potential method for long term preservation as it requires minimum space, labour and maintenance.

Long-term storage of seeds derived from controlled hybridization program proves valuable and need some kind of storage methods until they are included in the cultivar evaluation program. Research carried out at TRISL enabled establishing a protocol for encapsulation of zygotic embryonic axes using alginic acid37 (Seran et al., 2005) and efficient plant recovery was obtained after 3 months storage at low temperature (Seran et al., 2006).38 It was revealed that there is a potential for using such material for in vitro preservation while maintaining the genetic integrity. Research activities are also focused on using somatic embryos for encapsulation to use them in cold storage.

Evaluation of Germplasm for Agronomically Important Traits

Though concerted efforts have been made to characterize and studying the genetic diversity of the collection, systematic evaluation of the germplasm for desirable agronomic traits, mainly the unadapted and exotic types, remains an unaccomplished task in the past breeding program (Gunasekare, 2007b).5 The quest for increasing yield per se in cultivars developed during 1960s has overlooked the other valuable traits present in the germplasm. In situation where there is evidence that the pool of available variation for biotic and abiotic stress-resistance genes within the recurrently used adapted lines are limited, search for novel sources of resistance is required. In such a situation, a wide range of accessions, whether they are adapted or unadapted, must be screened to find adequate levels of resistance to major pests and diseases (Gunasekare, 2007b).5 Furthermore, with restrictions of acquiring exotic genetic resources, evaluation of the available germplasm to maximize its use in the breeding program has been received much attention in the present tea breeding program.

The germplasm is being extensively evaluated for various important traits by a multi-disciplinary team. Evaluation for pest, shot-hole borer (SHB) has already been commenced39 (Walgama et al, 2008) and results revealed that adequate amount of resistance to SHB can be tapped from unadapted germplasms than from the adapted breeding lines. It is planned to extent the evaluation for other biotic stresses too to find accessions possessing multiple pests and diseases resistance to be included in the breeding program.

Documenting Information on Germplasm Descriptions

When allocating priorities for germplasm activities, often not enough time or resources have been assigned to documenting descriptions, including their evaluated characters. This will inevitably lead to an ineffective utilization of genetic resources, though information was generated regarding them. Collating and documenting passport and collection descriptors of accessions originating from estate selections made since 1930s onward have been accomplished. Accessions were stratified by agro-ecological region and presented according to the estate from which they were selected, to be able to access the information in a usable form (Gunasekare & Kumara, 2005).14 In that, a map indicating spatial distribution and origin of the "estate selections" were prepared. According to the information, a total of 688 tea accessions have been originated from old seedling populations existed on various estates covering 10 agro-ecological regions. A comprehensive document was prepared incorporating information on all evaluated traits and the same was used to formulate a database using Microsoft Access for easy retrieval of information in a more user-friendly manner.

Germpalsm Utilization

The role of germplasm in crop improvement, though well recognized, yet the lack of sufficient information on the performances of germplasm collection, has led to its limited use. There is a growing apprehension that planting large areas with few popular cultivars may lead to genetic uniformity and consequently to genetic vulnerability of the crop to biotc and abiotc stresses. Since 1960, the year which controlled hybridization program commenced at the Institute, only 22 parents have been used recurrently until late 1990 (Figure 2). With the growing concern of narrow genetic base in the cultivated gene pool, the attention was focused on using diverse parents in the breeding program rather than using limited progenitors recurrently over several years in the breeding program. Rational selection of progenitors was made possible due to availability of information generated over the recent years with regard to characterization and evaluation. The main feature of the recently performed crossing program was to use genetically diverse parents while eliminating the parents used over many years recurrently in the breeding program. As such utilization of germplasm in breeding has been enhanced considerably and 46 new progenitors have been utilized in the breeding program

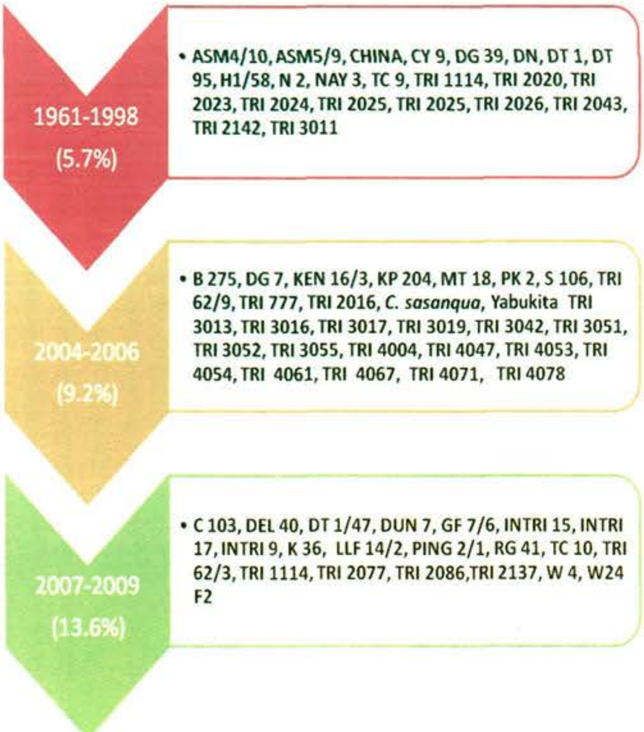

Figure 2. Germpalsm utilization in tea breeding from 1961–2009 (figures in parenthesis indicates the percentage utilization of the germplasm in tea breeding).

at TRISL since 2004 (see Fig. 2). Many new progenies have been generated from those crosses and have been entered into the current cultivar evaluation programme.

Germplasm Enhancement and Pre-Breeding

In the past, breeders needed only 1 or 2 elite traits to be incorporated in their new cultivars. Nowadays, materials with more desirable traits have become important due to changing climate, agronomic and cultural practices. Narrow genetic base of the cultivars at commercial plantations is an unavoidable consequence of successful plant breeding. In keeping with the above concerns, genetically more diverse parents, such as 'Yabukita" and extreme "China" type have been included in the breeding program.

Allied species of tea can be used as donors of gene or gene combinations required for further improving an existing well adapted genotypes lacking in one or more desirable traits. Furthermore introgressing new traits to the cultivated genepool needs pre-breeding which also would contribute to germplasm enhancement and broadening the genetic base. This is currently receiving attention. The limited exotic germplasm has been evaluated and C. sasanqua was identified as one of the potential non-tea species for introgression breeding to incorporate improved resistance to blister blight disease as well as low caffeine trait. Few inter-specific hybrids have already been recovered using in vitro techniques and they are being multiplied in vitro to raise clonal lines (Gunasekare, 2007c).40 Current pre breeding efforts are mainly focused on enhancing fruit set and retention in inter-specific hybrids with different Camellia species.

Though utilization of allied species and brining unadapted genetic background to adapted genetic background has certain limitations, it is hoped that these issues could be dealt with in future when the molecular markers linked to those traits are available for marker assisted introgression breeding.

Any Other Issues Related to the Germplasm

The emergence of new Intellectual Property Right (IPR) regimes, in relation to tea genetic resources necessitates working out modalities for benefit sharing between countries concerned. As the genetic resources of a particular crop are not distributed uniformly all over the world, no country can claim self-sufficiency in tea genetic resources. Even with most efficient and management programs, no single country can assemble resources, which would satisfy all present and future needs. Therefore, it is timely to foster collaboration at national and international levels for the acquisition and conservation of the tea germplasm, sharing of information and technologies, and a mechanism to access and safe movement of genetic resources and sharing of benefits arising from their utilization facilitated with appropriate regulatory mechanism. This would certainly help fostering crop improvement programs on tea not only at national level but also internationally to serve the mankind.

Future Trends, Aims and Goals of Germplasm Activities

In the face of emerging challenges in the tea industry locally and internationally, breeding and crop improvement of tea need to be focused on diversified breeding objectives to cater the needs of end-users, both growers and consumers as a whole. In this regard, metabolite profiling of germplasm has been identified as a major activity using the available collection to increase the practical application of germplasm in tea breeding to face the emerging challenges in the tea industry. Although most of the earlier attempts concentrated on studying single metabolite variation in tea cultivars, work on measurement of a broad range of metabolites is relatively limited. Therefore, recent attempts were focused on the use of high throughput metabolite profiling to evaluate the natural variations present in metabolite composition of Sri Lankan tea germplasm. This line of research would enable generating a wealth of data towards understanding the genetic diversity in germpalsm collection and also to establish correlations between groups of compounds that permit stratification of the collection in to diverse clusters. Furthermore, information on economically important individual metabolites such as caffiene, theanine etc. enables identification of candidate parents for crossing programmes and commercial exploitation of germplasm accessions suitable for making diverse products of tea, such as black tea, green tea, white tea, red tea, *etc*.

Many of the improved tea cultivars often are morphologically similar and hence, cause difficulties in discriminating among them using morphological traits alone. Thus, cultivar specific molecular markers need to be developed for fingerprinting of improved cultivars. Although plant genotyping using molecular techniques has an immediate practical application, this has not been fully explored in tea. Fingerprinting need to be used as a tool to resolve problems in mis-identification of germplasm and certification of clonal cultivars to control quality as well as to facilitate plant variety protection laws even before such regulatory framework is in place in the country.

References

1. Tubbs FR, 1939, The improvement of planting material. Tea Quarterly 11: 38-49
2. Singh ID, Shanmugarajah V, Gunasekare MTK, Ratnayake M, Sritharan U & Gunadasa SW, 2003, Tea Breeding In Sri Lanka (Chapter 3), In: Modder WWD (ed) Twentieth Century Tea Research in Sri Lanka. The Tea Research Institute of Sri Lanka, pp 37- 46.
3. Singh ID & Gunasekare MTK, 2000, Conservation of tea genetic resources, TRI Update 5(1): 1.
4. Gunasekare MTK, Ratnayake M & Ratnagoda BA, 2003, Tea reserves: Preserving the old seedling tea. TRI Update 8(1): 5-6
5. Gunasekare MTK, 2007b, Current status and future directions in breeding tea, In: Gunasena HPM, Girihagama PC (ed) Current status and future directions of plant breeding research in Sri Lanka. Sri Lanka Council for Agricultural Research Policy, pp 111-124.
6. Fu YB, 2000, Effectiveness of bulking procedures in measuring population pair-wise similarity with dominant and co-dominant genetic markers. Theoretical and Applied Genetics 100: 1284-1289
7. Park YG, Kaundun SS & Zhyvoloup A, 2002, Use of bulked genomic DNA-based RAPD methodology to assess the genetic diversity among abandoned Korean tea plantations. Genetic Resources & Crop Evolution, 49: 1-7
8. Gunasekare MTK, 2007a, Application of molecular markers to the genetic improvement of Camellia sinensis L. (tea): A Review. Journal of Horticultural Sciences and Biotechnology 82(2): 161-169
9. Ranatunga MAB & Gunasekare MTK, 2008, Assembling of preliminary core collection of tea (Camellia sinensis (L.) O. Kuntze) genetic resources in Sri Lanka. Plant Genetic Resource Newsletter (Rome), 155: 41-45
10. Richards AV, 1965, The origin of the popular TRI clones. Tea Quarterly 36: 183-187.
11. Anon 2002, The suitability of tea clones for the different regions. TRI Advisory Circular, No - PN1, December 2002
12. Anandappa TI, 1992, New tea clones. Tea Bulletin, 12: 28-33.
13. Anandappa TI, 1990, Report of the Plant Breeding division Tea Research Institute Annual Report. pp 93—100.
14. Gunasekare MTK, Kumara, JBDPA, 2005, Tea genetic resources in Sri Lanka: Genetic resources originating from Estate selections. Sri Lanka Journal of Tea Science 70 (2): 69-81.
15. Anandappa TI, 1973, Annual Report. Tea Research Institute of Sri Lanka, pp. 38-39
16. Gunasekare MTK & Ranatunga MAB, 2003, Polyploidy in tea (Camellia sinensis L.) and its application in tea breeding: A Review. Sri Lanka Journal of Tea science 68(2): 14-26.
17. Ramaswamy MS, 1960, Copper in Ceylon tea. Tea Quarterly 31: 76-81.
18. Frankel OH, 1984, Genetic perspectives of germplasm conservation. In: WK Arber, K Limensee, WJ Peacock and P Starlinger (Eds), Genetic Manipulation: Impact on Man and Society. Cambridge University Press: Cambridge, pp. 161-170.
19. Boukema IW, Hintum JL & Astley D, 1997, Creation and composition of the Brassica oleracea core collection. Plant Genetic Resource Newsletter 111: 29-32.
20. IPGRI, 1997, Descriptors for tea (Camellia sinensis), International Plant Genetic Resources Institute, Rome, Italy.
21. Richards AV & Sebastiampillai AR, 1964, A note on the identification of some TRI clones. Tea Quarterly 35: 168.
22. Wickramaratne MRT, 1981, Variations in some leaf characteristics in tea (Camellia sinensis L.) and their use in the identification of clones. The Tea Quarterly 50: 183-198.
23. Gunasekare MTK, Arachchi JDK, Mudalige AK & Peiris TUS, 2001, Morphological diversity of tea (Camellia sinensis L.) genotypes in Sri Lanka. In: Proceedings of 57th Annual Session of Sri Lanka Association for the Advancement of Science (SLAAS), Part I, p. 83.
24. Gunasekare MTK & Pieris TUS, 2006, Phenotypic variation in germplasm accessions of tea (Camellia sinensis L.) in Sri Lanka. Plant Genetic Resource Newsletter (Rome) 146: 39-42
25. Piyasundara JHN, Gunasekare MTK, Peiris TUS & Wickramasinghe IP, 2006, Phenotypic diversity of Sri Lankan tea (Camellia sinensis L.) germplasm based on morphological descriptors. Tropical Agricultural

Research 18: 237-243.

26. Piyasundara JHN, 2008, Systematic characterization of tea (Camellia sinensis L.) germplasm using morphological descriptors, Master of Philosophy Thesis. Post Graduate Institute of Agriculture, Peradeniya, Sri Lanka.

27. Piyasundara JHN, Gunasekare MTK & Wickramasinghe IP, 2008, Characterization of tea germplasm in Sri Lanka using morphological descriptors. Proceedings of 2nd Symposium on Plantation Crop Research, 16-17 October, 2008, Colombo, Sri Lanka, pp. 389- 395.

28. Piyasundara JHN & Gunasekare MTK, 2008, Morphological comparison of tea (Camellia sinensis L.) germplasm originating from old seedling tea populations in different agro-ecological regions in Sri Lanka, In: Proceeding of National Symposium 2008, University of Ruhuna, Sri Lanka, 23-24 October 2008, 30 pp.

29. Silva KWKI, Ranatunga MAB, Gunasekare MTK & Wickramasinghe IP, 2010, Characterization and evaluation of exotic tea germplasm stocks for utilization in the breeding program. In: Proceeding of Undergraduate Research, Department of Agricultural Biology, 1: 34-37.

30. Sundaravathany A, Kumara AP & Gunasekare MTK, 2005, Characterization of tea germplasm using reproductive traits. Journal of Jaffna Science Association 12; 19p.

31. Ariyarathna HACK & Gunasekare MTK, 2008, Pistil related morphological traits reflect genetic diversity of tea in Sri Lanka, In: Proceedings of 2nd Symposium on Plantation Crop Research, 16-17 October, 2008, Colombo, Sri Lanka: pp. 383-388.

32. Kottawa-Arachchi JD, Gunasekare MTK, Ranatunga MAB, Jayasinghe L & Karunagoda RP, 2011, Analysis of selected biochemical constituents in black tea (Camellia sinensis L.) for predicting the quality of tea germplasm in Sri Lanka. Tropical Agricultural Research 23: 30-41.

33. Liyanage AC, Fernando WMU & Pathirana KPSK, 1999, Determination of genetic diversity of tea cultivars in Sri Lanka using isozyme polymorphisms. Report on FAO/IAEA seminar, Philippines, 1999. pp. 85-87.

34. Mewan K, Liyanage A. Jayamanne E, Gunasekare MTK & Karunanayake E, 2005, Studying genetic relationship among tea (Camellia sinensis L.) cultivars in Sri Lanka using RAPD markers. Sri Lanka Journal of Tea science 70(1): 42 – 53.

35. Goonatilake WAS, Priyantha C, Mewan KM & Gunasekare MTK, 2006, Genetic diversity in tea (Camellia sinensis (L.) O. Kuntze) as revealed by RAPD-PCR markers. In: Proceedings of International Symposium on the Issues and Challenges of the 21st Century, 4-8 July 2006, Sabaragamuwa University of Sri Lanka, p. 28.

36. Ariyarathna HACK & Gunasekare MTK, 2006, Genetic base of tea (Camellia sinensis L.) cultivars as revealed by pedigree analysis. Journal of Applied Genetics 48(2): 125-128.

37. Seran T, Hiriburegama K & Gunasekare MTK, 2005, Encapsulation of embryonic axes of Camellia sinensis L. (tea) and subsequent in vitro germination. Journal of Horticultural Science and Biotechnology 80(1): 154-158.

38. Seran T, Hiriburegama K & Gunasekare MTK, 2006, Short-term storage of encapsulated zygotic embryonic axes of tea at low temperature. Tropical Agricultural Research 18: 358-366.

39. Walgama RS, Gunesekera MTK, Jayathilake MM, Kottawaarchchi JD & Liyanage DD, 2008, Evaluation of tea (Camellia sinensis L.) germplasm for host-plant resistance to shot-hole borer, Xyleborus fornicatus Eichh. (Coleoptera: Scolytidae). Sri Lanka Journal of Tea Science 73: 29- 38.

40. Gunasekare MTK, 2007c, Section on Plant Breeding. Tea Research Institute Technical Report, 2007.