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Stratification of *Camellia* Germplasm to Facilitate Construction of Core Collection; A Prerequisite for Tea Crop Improvement

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ABSTRACT

Majority of tea germplasm accessions have not been characterized adequately for agronomic traits. Hence, crop improvement programme has been practiced on a narrow genetic base. Construction of a core collection of tea germplasm would help in understanding the genetic diversity of germplasm collection and facilitate better use of genebank materials in tea breeding programme. This study was focused on the need for a broader approach, with logical progression from conservation, management and utilization of tea genetic resources in Sri Lanka to avoid inbreeding and narrow genetic base in future tea cultivars. Available information of 600 germplasm accessions conserved at the Tea Research Institute was collated. Accessions were first stratified into groups based on utilization and further divided into subgroups using available information on taxonomy, pedigree, geographical origin, and breeding history. It shows that more than 90% of tea accessions are beverage types which comprise *Camellia sinensis* species and can be further sub grouped as introductions, estate selections and improved cultivars. Non beverage types of other *Camellia* spp. and few non *Camellia* spp. are available in the collection. The study implies that though large numbers of diverse accessions present in the collection, majority of them make no contribution to the crop improvement programme. A stepwise, structured hierarchy developed to form genetically homogeneous small groups formed the diversity tree of tea genetic resources as an initial step towards assembling a core collection in tea germplasm in Sri Lanka.

Key words: *Camellia sinensis*, conservation, core collection, diversity tree, germplasm

INTRODUCTION

Tea, *Camellia sinensis* (L.) O. Kuntze, is a national commodity of Sri Lanka, covering over 222 000 ha of the country and providing 12.8% of the export earnings of the country's economy (Anon, 2008). Sri Lankan tea has very limited genetic diversity, as it is an introduced crop (Singh and Gunasekare, 2000). Several authors have shown that the genetic base of cultivated tea is very narrow due to the recurrent use of same parents for the tea breeding programme over the years (Singh *et al.* 2003). The limited genetic diversity of the crop bears with it several risks, including greater genetic vulnerability to pest and disease epidemics; lack of adaption to climate change related stresses; lack of genetic variation for specific quality traits; reaching performance plateaus (Haussmann and Parzies, 2009) and jeopardizes the potential for sustained genetic improvement in the long term.

The exploitation of genetic potential inherent in tea germplasm is hampered by the non-availability of characterized and evaluated data. Currently, about 600 accessions are being maintained in the field gene bank of the Tea Research Institute of Sri Lanka (TRI). These accessions consist of 'introductions', 'estate selections', improved cultivars and related species.

The core collection approach was developed by Frankel (1984) to deal with the documentation, conservation, multiplication, evaluation and characterization of large germplasm collections. A core collection is a set of accessions selected to represent the genetic diversity of a base collection with minimum redundancies (Frankel, 1984). This strategy was introduced with the intention of minimizing the cost of genetic conservation while ensuring representation of maximum genetic variation for rapid evaluation of germplasm, to provide better access to the base collection. A core collection is a starting point for screening of a base population in the search for desirable traits, allowing efficient use of land, time and money.

Traditionally, a core collection has been constructed on the basis of a variety of taxonomic, morphological, agronomic and eco-geographical criteria. The advent of DNA marker systems provided large numbers of polymorphic loci and demonstrated the suitability of DNA molecular markers as core selection criteria.

In China, a preliminary core collection of tea was constructed using place of origin, tree form and variety types (Li and Jiang, 2004), along with morphological and DNA molecular markers (Li *et al.* 2005). To construct a core collection, it is important to compile all available information, including morphological, pedigree, passport, biochemical and molecular markers. Singh *et al.* (2000) reviewed the status of tea genetic resources in Sri Lanka and stressed the need for conservation.

Gunasekare and Kumara (2005) initiated the documentation of available information on estate selections since the 1930s as an initial step towards systematic maintenance of tea germplasm resources. This paper aims to collate available information to systematize and construct a preliminary core collection concept for the tea genetic resources of Sri Lanka.

MATERIALS AND METHODS

Information was gathered on the 600 accessions of tea conserved in the genebank at TRI, Talawakelle. Accessions were first stratified into groups based on utilization. Then major groups were further divided into subgroups using available information on taxonomy, pedigree, geographical origin, and breeding history. Stratification of collection into distinct groups was achieved using a step-wise procedure to develop a hierarchy, which was graphically represented by a 'diversity tree'. The major divisions were then successively split into subgroups until the subgroups were genetically homogeneous and no further difference were available to justify further sub-grouping. The hierarchy was recorded as a descriptor called the 'path indicator', a series of ciphers that describe the subsequent divisions (Boukema *et al.* 1997).

RESULTS

The tea genetic resources in Sri Lanka can be broadly categorized into two groups: beverage types and non beverage types. It shows that more than 90% of tea accessions in Sri Lanka are beverage types and belongs to *Camellia sinensis* species whereas, non beverage types and wild types are poorly represented in the collection. Beverage types can be further sub-grouped into introductions, estate selections and improved cultivars, based on available pedigree data and breeding history. The strength of different types of accessions in the collection is given in Figure 1.

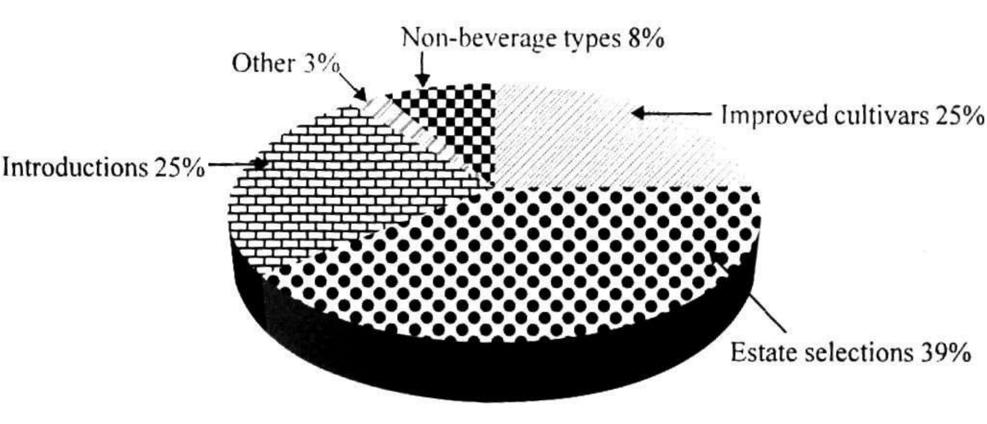

Figure 1. Strength of genebank collection showing major groups

Introductions

The first tea germplasm in Sri Lanka was introduced as tea seeds from India and China during the early stages of the industry, and became limited due to increased quarantine or political restrictions and cost of importation. Currently in the TRI germplasm collection, accessions from India, Indo- China, Japan, Korea and Russia are available. The continued exploration of exotic germplasm for cultivar development should not be overlooked, as many recommended tea cultivars in use were based on germplasm from introductions.

Introductions from India

The ancestry of the popular TRI 2020 series cultivars were traced back to 30 seeds of a single tree bearing the identification number 4/10 (currently designated ASM 4/10) introduced by Dr F.R. Tubbs in 1937 from Tocklai Experimental Station of the Indian Tea Association (now Tea Research Association) near Jorhat, Assam (Richards, 1965). Eight bushes were selected from 15 seedlings raised in 1946, and seven of these selections were released to the industry during 1959–1960, designated TRI 2020 to TRI 2027. Realizing the potential in the seeds of ASM 4/10, subsequent introduction of another batch was carried out in 1958. Fifteen TRI 62 series accessions were released in the 1960s (Richards, 1965), out of which three are recommended for commercial cultivation (Anon, 2002).

Fourteen TRI 3000 series cultivars originating from another batch of seeds from ASM4/10 are available as TRI germplasm. The date of introduction of the batch is unknown. The cultivars are TRI 3047, 3048, 3049, 3052, 3054, 3055, 3057, 3058, 3062, 3063, 3064, 3065, 3070 and 3071, with three of these being recommended for commercial planting (Anon, 2002).

The seedlings known as Stock 450, another introduction from India by Dr P. Sivapalan, in 1988 were evaluated in cultivar testing trials (Anandappa, 1990a) and 6 selected for further evaluation for release as potential cultivars. Currently 26 accessions are available at TRI germplasm.

Introductions from Indo-China

TRI 2043, an accession popular for production of 'Silver tips', is a TRI selection made by Dr Tubbs during the 1950s from Shan Bansang No 777 seeds received from Pho Ho Station, Indo-China. A similarly high quality cultivar, TRI 777, was a selection made at TRI from the seedling progeny of Shan Cho Long No 777 introduced from the same Station (Richards, 1965).

Introductions from Japan

Six seedlings of morphologically different 'Yabukita' plants from Japan are available at TRI. The appearance of these Yabukita germplasm resembles more towards the China type (Anandappa, 1990a). In 2001, further seed stock of Yabukita was received and they are available in the present collection.

Other Introductions

In 2001, two seed lots from Korea and China were received that included wild types, cultivated types and Oolong types. In addition, a seed lot from Azarbarjhan (Former Russian federation) was received in 2006, and a few unknown accessions of East African origin are available in the collection.

Estate selections

With the development of vegetative propagation techniques during 1931–1935, a clonal selection programme was started as a mean of developing improved planting materials. The selection programmes were extended to old seedling tea fields and accessions were selected based on their field performances and released as 'Estate selections' that can also be referred to as land races. Estate selections became available from the 1950s and continued to select such material thereafter. Estate selections are usually named with an acronym carrying sites or estates where they were originally selected.

According to Gunasekare and Kumara (2005), a total of 688 tea accessions were derived from old seedling tea populations from estates in ten agro-ecological regions. TRI currently hold 217 estate selections at Talawakelle. Accessions selected at the St Coombs Estate, where TRI is located, were given a 'TRI' designation.

Improved tea cultivars

The growers' ready and successful acceptance of cultivars of the TRI 2000 series encouraged plant breeders to exploit the inherent variability in open-pollinated seeds (Singh *et al.* 2003) and led to the development of the TRI 3000 series of cultivars during the 1980s. Accessions derived from open pollinated progenies are summarized in Table 1. Controlled hybridization of tea with the aim of creating variations was initiated at TRI during 1961–1962 (Singh *et al.* 2003). Crossing selected parents for best recombinants among the progeny appears to be the most fruitful line of work.

Early attempts in the controlled hybridization programme during the 1970s resulted in some of the TRI 3000 and TRI 4000 series of hybrid cultivars (Table 1). Simultaneously, non-conventional approaches to tea breeding were strengthened during this period to supplement the conventional tea breeding programme (Singh *et al.* 2003).

Polyploids

In Sri Lanka, natural and artificially induced polyploids have been reported by Sebastiampillai and Janikiram (1969). According to them, two cultivars (HS 10A and GF 5/01) available at TRI were triploids, and cultivar HR 1/8 was reported to be an aneuploid. All were estate selections made by early planters.

Five artificially induced tetraploid cultivars, namely TRI 2023, 2024, 2025, 2026 and DT 95 (Sebastiampillai, 1976) are available at TRI, and a tetraploid of TRI 2025 was released as a new cultivar, TRI 3069, for commercial planting.

Table 1.Origin and sources of estate selections and improved tea cultivars in Sri Lanka

Origin/ Source	No. of accessions
Estate selections (based on agro-ecological region)	
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)
Half- sib selections (Open pollinated)	
ASM 4/10	2 (1)
TRI 2000 series (TRI 2024, 2025, 2026)	20 (8)
Estate selections	2
Full-sib selections	
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

(Figures in parenthesis are number of recommended cultivars for commercial planting)

Non beverage types

Camellia sasanqua Thunb.

Two seed batches of *C. sasanqua*, an ornamental tea, collected from Shizuoka-Ken and Kagoshima-Ken, Japan, were received from the National Research Institute of Vegetables, Ornamental Plants and Tea (NIVOT), Japan in 1990 (Anandappa, 1990b). *C. sasanqua* or 'Sazanca' is a native Japanese species with high ornamental value and oil content. Currently, 40 *C. sasanqua* seedlings are available in the TRI germplasm collection (Table 2).

Other *Camellia* species

Currently, seventeen non-beverage types and ornamental cultivars are available in the R.G. Coombs Camellia garden at TRI, which was established in 1950 and later augmented with new plants in 1968. The origins of these ornamentals are unknown. Species available include *Camellia lutescens*, *Camellia rosaeflora*, and various *Camellia japonica* varieties (jubilee, red, albaplena, daitairin, elegans, white, donkelaari, herculea, yellow, sulphurea, diamond de bardi and old double pink) (Table 2).

Table 2. Non beverage types available in the collection

Species	No. of accessions
Other *Camellia* spp.	
Camellia sasanqua Thunb	40
Camellia japonica	13
Camellia lutescens	01
Camellia rosaeflora	01
Non *Camellia* spp.	
Pyrenaria barringtoniaefolia	01
Gordonia zeylanica cv. Elliptica	01

Non *Camellia* species

Two other non *Camellia* species belongs to the family *Theaceae*, such as *Pyrenaria barringtoniaefolia* and *Gordonia zeylanica* cv. Elliptica are also available in the collection. A stepwise, structured hierarchy developed to form genetically homogeneous small groups formed the diversity tree of tea genetic resources. With the information available on pedigree,

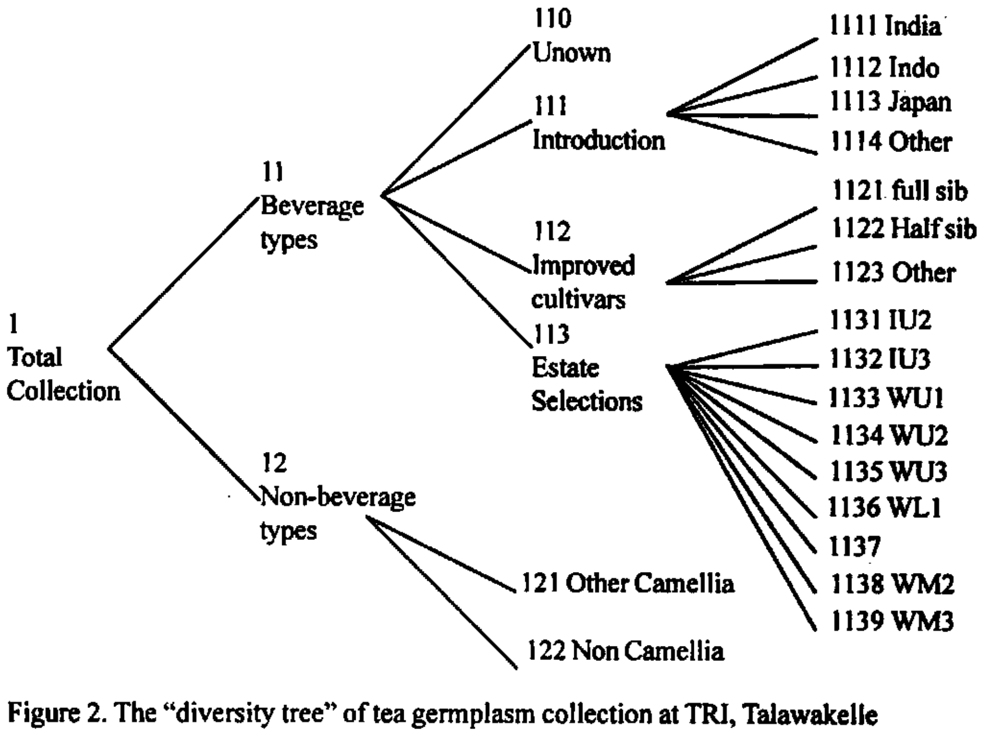

Figure 2. The "diversity tree" of tea germplasm collection at TRI, Talawakelle

Table 3. Structure of the "path indicator"

Path	Group	No of entries
1	Total collection	482++
11	Beverage types (*C. sinensis*)	437++
12	Non beverage types	57
110	Unknown origin	15
111	Introductions	70++
112	Improved cultivars	135
113	Estate selections	217
1111	India	62
1112	Indo China	2
1113	Japan	6++
1114	Other	
1121	Full sib selections	106
1122	Half sib selections	24
1123	Other	5
1131	Selected from IU2 zone	3
1132	Selected from IU3 zone	36
1133	Selected from WU1 zone	104
1134	Selected from WU2 zone	30
1135	Selected from WU3 zone	6
1136	Selected from WL1 zone	8
1137	Selected from WM1 zone	6
1138	Selected from WM2 zone	13
1139	Selected from WM3 zone	11
121	Other *Camellia* spp	55
122	Non *Camellia* spp.	2

breeding history, geographical origin and other parameters, the tea germplasm collection could be broadly categorized into the above mentioned groups. The resultant diversity tree of tea germplasm with its path indicator is illustrated in Figure 2 and the structure of the path indicator is given in Table 3.

DISCUSSION

It has been realized the importance of understanding the genetic diversity and relatedness of cultivars to avoid inbreeding and narrow genetic base in future tea breeding. As in many other crops the danger of narrowing genetic basis is increasing in most tea growing countries as a result of the popularity of a few cultivars for breeding and planting (Yao *et al.* 2008). Because of the common ancestry of early introductions from north India (Singh *et al.* 2003), subsequent crop improvement programmes were practiced on a narrow genetic base. The close genetic relationship of TRI cultivars was confirmed using isozymes (Liyanage *et al.* 2003) and RAPD markers (Mewan *et al.* 2005). Recent Coefficient of Parentage (COP) analysis of some tea cultivars revealed that most of the commercial tea cultivars are derived from two parental lines: Assam/Cambod introductions, and estate cultivar, CY 9 (Ariyaratne and Gunasekare, 2007). A survey revealed that over 55% of the tea extent is under clonal plantings, of which over 80% is limited to a few cultivars (Singh *et al.* 2000). Similarly narrow genetic diversity of tea cultivars reported in Kenya (Seurei, 1996), India (Hajra, 2001; Balasaravanan *et al.* 2003) and Japan (Kaundun *et al.*, 2000). A narrowing of genetic base increase the risk of natural hazards like pests, diseases, and climate changes and also impose limitations on selections and tea breeding.

The results from the study imply that there is a tremendous genetic potential available in the collection. Large portion of the collection (39%) comprises estate selections. The estate selections are derived from widely varying 10 agro ecological regions of the country and a large genetic variation among these land races could be expected. Introduced germplasm from different countries as open pollinated seedlings also undoubtedly offers great variability which could be utilized in tea crop improvement. Though non beverage types are poorly represented in the collection (8%), those are likely sources of new and valuable genes capable of increasing yield, resistance and adaptability to climatic changes in the future. However, greater efforts of pre-breeding approaches such as embryo rescue techniques may be resorted to produce inter specific derivatives carrying the gene(s) conferring desirable traits. Thus, it is a prerequisite to understand the genetic variation warehoused in germplasm repositories and utilize them effectively in crop improvement programmes.

To meet the challenges arising from tea genetic resources conservation and utilization, construction of a core collection could be the most attractive option. Core collections offer a way to improve access to germplasm collections by providing a highly diverse, representative subsample of the total collection. Further evaluation of core may help in identification of gaps in the collection for prioritization of exploration and collection activities of new material, even from related wild species. The core can also provide the optimum set of material for emergency situations, such as environmental disasters or human activities, where only a portion of the collection can be secured (van Hintum *et al.* 2000).

The present study is the first attempt towards a construction of a core collection in tea genetic resources conserved in Sri Lanka. The study generated the diversity tree of the tea genetic resources in the country which is the first step of such process. Accessions of identified diverse groups are being characterized for morphological, biochemical and molecular descriptors and evaluated for agronomical important traits. This could be further supplemented with more precise bio-geographical information through the use of Geographical Information Systems (GIS) linked to databases on climate, soils, pests and other information. Once the evaluations are completed it should be possible to construct a well characterized core subset of tea germplasm using a holistic approach that provides a proper working collection for the subsequent search for desired alleles for crop improvement programmes, and a point of entry to the entire germplasm collection.

CONCLUSIONS

The diversity tree developed from the study reflects the untapped genetic diversity of tea genetic resources, both beverage and non-beverage type, in Sri Lanka, and the potential of using them in tea crop improvement programmes in future. Systematically collated, comprehensive information system for tea genetic resources in Sri Lanka is now available for utilization.

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