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# **ACRONYMS**

ABS	Access and benefit sharing					
AFLP	Amplified fragment length polymorphism					
CBD	Convention on Biological Diversity					
срDNA	Chloroplast DNA					
DUS	Distinctness, uniformity, and stability					
EST-SSR	Expressed sequence tag – simple sequence repeat					
GBS	Genotyping by sequencing					
ITPGRFA	International Treaty for Plant Genetic Resources for Food and Agriculture					
MLS	Multilateral System					
NBPGR	National Bureau of Plant Genetic Resources (India)					
QTL	Quantitative trait locus					
SMTA	Standard Material Transfer Agreement					
SNP	Single nucleotide polymorphism					
SOP	Standard operating procedure					
SSR	Simple sequence repeat					
TRA	Tea Research Association (India)					
TRICAAS	Tea Research Institute, Chinese Academy of Agricultural Sciences					
TRISL	Tea Research Institute of Sri Lanka					
UPOV	International Union for the Protection of New Varieties of Plants					



## **EXECUTIVE SUMMARY**

After water, tea is believed to be the most widely consumed beverage in the world. It has a very long, global history of production and consumption. Some degree of tea production is recorded in 62 countries today, although the top 10 countries account for about 93% of tea grown. Some countries produce and consume much of their own tea while others are primarily producers or consumers. The strong interdependence of countries with respect to tea production and consumption has led to its high value in the international marketplace – and to the need for significant investment in breeding new cultivars. The development of new tea cultivars with desirable characteristics and a wide genetic base is, in turn, dependent upon breeders having access to as much genetic diversity as possible.

Tea genetic resources are being conserved *ex situ* and *in situ* in a number of tea-producing countries. The main center for diversity, in terms of rare alleles and heterozygosity, is South and Southwest China. There are also important sources of diversity in Northeast India and in the northern border areas of Myanmar, Thailand, and Vietnam, adjacent to China.

Additionally, diversity still exists in old seedling gardens in many parts of the world that needs to be better conserved and used. This diversity offers unique populations that were a product of the movement and hybridization that occurred when tea was planted from various sources of seed during the early establishment of tea production in China, Japan, Korea, India, Sri Lanka, Malawi, Kenya, Madagascar, and Indonesia. These seed derived

populations have adapted to local conditions and can serve as sources of more specific adaptation. There is general recognition that these old seed gardens are important sources of genetic diversity for the future.

Tea genetic resources have been effectively utilized in the past, but only a small amount of the diversity has been evaluated or used. A survey of users has indicated that genetic resources for tea are important for breeding, research, and direct use in production fields. The genetic diversity of cultivars that are released or in farmers' fields are not viewed as adequate for future genetic enhancement or production. Yet despite constraints, these users are finding traits of interest, especially for research on tea. The development and availability of core subsets and the wider scale genotyping of collections offers many opportunities to increase the evaluation and use of conserved genetic resources by all types of users.

Tea and its genetic resources are vulnerable to challenges such as climate change, biotic threats, land use changes, fluctuating market prices, local labor costs/shortages, increased uprooting/replanting with clonal cultivars, and other changes in the tea sector. Mitigating the loss of tea genetic resources will require much greater collaboration by collection holders, producers, processors, national tea boards, national governments, and the global tea sector. Currently, this does not seem to be a priority in the few global forums for the tea sector, such as the FAO Intergovernmental Group on Tea or the Ethical Tea Partnership. There is no platform

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for internationally sharing collection information at the accession level or for collaborating on issues related to conservation and use. There is very limited exchange of germplasm internationally, and no current multilateral discussion on policy options to facilitate greater international exchange. There is recognized value in the greater use of genetic resources, but currently there are significant barriers to the effective use of conserved diversity in tea breeding programs beyond institutes' own limited collections.

Respondents to the survey of conservers of tea genetic resources mainly report conserving local landraces, cultivars, and breeding material from Camellia sinensis var. sinensis and C. sinensis var. assamica. The majority of collections conserve accession that were collected or acquired from their own institutes. The respondents conserve their accessions in field collections, typically at one site. There is very limited use of complementary conservation options such as cryopreservation. The survey identified some key risks facing ex situ collections of tea genetic resources. Mitigating these risks will require staff succession planning and training on conservation as well as stable, adequate annual funding for routine operations to secure collections for the long term. Effort needs to be made urgently to secure these collections through safety duplication in other field sites or through alternatives such as cryopreservation.

Visits were made to seven institutes that conserve tea genetic resources. There were a number of opportunities identified for more global collaboration on issues such as an approach to safety duplication; common guidelines for costeffective routine operations; common descriptors for characterizing accessions; and the development of a common platform to link databases and share accession level information. Most of the institutes have links with, and interest in, conservation of the diversity still present in old seedling fields, in wild stands, or in forests. In some countries, this local diversity has a market and local value that currently secures its conservation. In other countries, the diversity is at risk of loss with the uprooting of old seedling fields for planting new clonal cultivars or with conversion of the land to new crops. In these cases, institutes are involved with estates to secure parcels of these seedling fields for conservation. There have also been efforts to collect this diversity, but most of the institutes visited have limits to the land available for germplasm gardens. Thus, developing genomic tools to assess diversity still held in the fields and rationalizing it for conservation is a critical need they all share for the future. The development and use of a globally

agreed genotyping platform is a key action proposed for the global system. This would allow for global level evaluating of diversity; identification of key gaps to fill through collecting; rationalization of ex situ collections as well as in situ sites; and the opportunity to contribute to a global platform for sharing accession level information for conservation and use.

In summary, the background review, the survey of conservers and users, and the visits to the sites of seven key collection holders confirmed that the current conservation system for tea genetic resources is nationally focused with the primary aim of conserving local diversity and securing some key introductions for institutes' breeding programs. There is an appreciation of the value of conservation, and it is often an institutional and national priority. There are no formal global links between these nationally based institutes, however, and no mechanism for international collaboration. This has resulted in very little knowledge of the diversity conserved or its use outside a given institute or country. This is not the secure, rational, cost-effective, or sustainable system needed to ensure long-term conservation or use. This is very unfortunate when the risks facing tea production systems and tea forests are considerable. Thus, there was an agreement among all the institutes visited that there is value in a more global conservation and use system. A significant impact of the global system would be to secure conservation, as well as to increase access and use of tea genetic resources for the long term.

It is clear that the current conservation system for tea genetic resources lacks key enabling investments to facilitate the development of a more secure global system. The current system is built upon national conservation and use with a few bilateral international exchanges and collaborations. Thus, these key priority investments are required to build the global system:

- As governments, industry, farmers' organizations, and NGOs consider the future needs for tea sector development, securing the global conservation and use of tea genetic resources needs to be considered as a priority.
- Greater global dialog and collaboration among collection holders and with users will be facilitated by a global meeting among conservers and key users.
- 3. Resources need to be made available to support key global actions and collaboration to address the priority needs.



# **BACKGROUND**

The drinking of tea can be traced back thousands of years in ancient China. Tradition holds that it was first brewed as a medicine by the prehistoric sage ruler Shennong. The earliest physical evidence yet identified is in dried plant bundles from funerary sites of the Western Han Dynasty, 207 B.C.-9 A.D. (Lu et al, 2016). Production developed rapidly in China starting in the Tang Dynasty (618–907 A.D.). However, tea has achieved popularity in other parts of the world only since the middle of the seventeenth century. There have been numerous publications on the history of tea making and production, such as The True History of Tea (Mair and Hoh, 2009); Tea: The Drink That Changed the World (Griffins, 2007); and Jane Pettegrew's World of Tea: Discovering Regions and Their Tea (Pettegrew, 2018). It is a history that has influenced our current cultures, agricultural systems, and economies.

Tea has mainly been domesticated for the use of its leaves in hot beverages, with or without initial processes of oxidation. In China, tea is classified into six basic groups according to the degree of oxidation of tea polyphenols: green tea, white tea, yellow tea, oolong tea, black tea, and dark (fermented) tea. Horie et al (2017) reported on the process used to produce four post fermented teas (where the initial oxidation is halted, and then microbes introduced to oxidize the tea further through fermentation) in Japan and their quality components. There are also other traditional uses, such as miang in Thailand, a fermented tea leaf that is chewed or eaten (Khanongnuch et al, 2017), or laphet in Myanmar, a fermented tea leaf eaten as food (Han and Aye, 2015).

Tea is now produced in 62 countries globally (American Specialty Tea Alliance, 2018). These include 18 countries in Africa, 22 in Asia, 2 in North America, 11 in South and Central America, and 9 in Europe and Eurasia. The top 10 countries for production in 2017 are given in Table 1 (FAO, 2018a). Table 1 also includes the estimated production for 2017 for black and green tea separately (FAO, 2018b). China and India account for 63% of total tea production globally in both years, while the top 10 countries together account for about 93% of production. The top 10 producers of black tea were projected to account for 90% of black tea production, while China, Vietnam, Indonesia, and Japan were projected to produce 98% of green tea globally for 2017.

China, India, and Pakistan are the top three markets for tea consumption. FAO (2018c) concluded that robust demand and the associated higher prices had stimulated substantial supply increases that resulted in significant growth in domestic consumption and trade. Export earnings from tea increased 75% in the last 10 years to 5.46 billion USD in 2016, contributing to improved rural income and household food security in the tea-producing countries, which are mainly in the developing world. Global tea production and consumption are projected to keep rising for the next decade. This is driven by rising demand in developing and emerging countries, many of which are also new tea producers. The increased demand will create new rural income and opportunities to improve food security in tea-producing countries. Black tea production is expected to rise annually by 2.2% in

Table 1. Estimated black and green tea production (tonnes [t]) for 2017 and total tea production (t) in 2017 for top producing countries.

Country	2017 estimate	ed production	2017 total production (ITC, 2018)
	Black tea	Green tea	
China	310,000 t	1,530,000 t	2.610,000 t
India	1,260,000 t		1.320,000 t
Kenya	440,000 t		440,000 t
Sri Lanka	310,000 t		310,000 t
Turkey	310,000 t		260,000 t
Vietnam	80,000 t	90,000 t	180,000 t
Myanmar			020,000 t
Indonesia	110,000 t	30,000 t	130,000 t
Argentina	80,000 t		80,000 t
Japan		80,000 t	80,000 t
Iran	30,000 t		20,000 t
Bangladesh	80,000 t		80,000 t
Global total	3,330,000 t	1,770,000 t	5,810,000 t

the next decade. This will reflect a major output increase for China, Kenya, and Sri Lanka. Green tea is forecasted to gain 7.5% annually for the next decade, largely driven by China, where production is expected to double by 2027. In the past, this has been driven by expanded area under production or increased production per unit area. In the future, with significant risks from climate change in the limited area of production for commercial tea growing and constraints to availability of suitable land, increasing supply in relation to increases in demand will be more difficult.

Carr (2018) did a short review of the tea production areas globally and made a number of observations on the status of production. He concluded that tea production had expanded away from its center of origin and production in Southeast Asia to many diverse habitats since the nineteenth century and that this process continues today. Tea consumption in the traditional tea regions has increased, such that countries that used to traditionally export tea now import tea. The result is that new tea growing countries have been able to meet the international demand by expanding production. In countries like Kenya, this has been a very rapid process driven by the expansion of smallholder producers. Traditional estates or plantations are declining in terms of the proportion of global tea production, but they are still seen as the standard-setters, although smallholders can also produce high-quality tea. Civil strife and disaster, such as the Chernobyl nuclear accident, have resulted in tea plantings being abandoned, but many of these can be brought

back into production. Many countries are facing a shortage of labor or increasing cost for labor, so there is an increasing focus on mechanization in tea production systems. In general, across producing countries, tea prices paid to producers are still low, but millions of people in developing countries still depend upon tea for their livelihoods.

Some countries produce and consume their own tea, whereas others are primary producers or primary consumers. The strong interdependence of countries with respect to tea production and consumption has led to its high value in the international marketplace, and the need for significant investment in breeding. The development of new tea cultivars with desirable characteristics and a wide genetic base is dependent upon breeders having access to as much genetic diversity as possible. Breeding programs may maintain their own collections, but often they acquire new materials from other collections or genebanks or from direct selection from farmers' fields. Traditional tea breeding is a complex process that involves many years of crossing, multi-site field evaluation trials, and quality assessment. Changing climatic conditions, new pest and disease pressures, and the production of tea with fewer chemical inputs to meet consumer demand will result in the need for new tea cultivars with higher levels of resistance to abiotic and biotic stresses. Meeting these future challenges will depend on the genetic diversity that is conserved in genebanks, gardens, abandoned fields, and in the wild being available for use in the long term.

# Why a global system to secure tea genetic resources?

Compared to other major crops, tea has lagged behind in the development of coordinated global or regional research and conservation programs. The global nature of production and consumption of tea means that there is also a global interdependence for genetic resources (Khory et al, 2015). This is a key rationale for the conservation of crop genetic resources as a global public good. This interdependence is recognized in the Second Global Plan of Actions for Plant Genetic Resources for Food and Agriculture (FAO, 2010) and was the basis of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).

In 2018, the Global Crop Diversity Trust (Crop Trust) collaborated with the Tea Research Institute, Chinese Academy of Agricultural Sciences (TRICAAS) on the development of a global strategy for conservation and use of tea genetic resources. The Crop Trust is an international organization working to safeguard crop diversity for the very long term. Since 2006 it has worked with crop conservation and use specialists to develop global ex situ conservation strategies for key global food crops and commodities. Global conservation strategies facilitate a transition from the current complex, fragmented, and independent crop conservation system to a more integrated, collaborative, and cooperative global conservation system. The aim of this strategy is to provide the evidence base to secure the long-term conservation and use of tea genetic resources as effectively and efficiently as possible. It will serve as a framework to bring together stakeholders at all levels – local, regional, national, and global - in building longterm support through greater awareness, increased capacity, greater community engagement, and sustained funding.

## Tea genetic resources

Botanically, tea plants belong to the order Theales, family Theaceae, genus Camellia L., section Thea (L.) Dyer. Theaceae is a family of trees and shrubs that consists of 10 genera and 254 species (Beech et al, 2017). Camellia is the most well-known genera, which includes the source of tea, Camellia sinensis (L.) O. Kuntze. There have been more than 10 classification systems applied to tea in the last 200 years. The characteristics of the flower and fruit are used as descriptors, and other traits such as tree type, leaf texture, size, shape, and pubescence are also important indicators for tea classification. Sealy (1958) proposed 12 series of Camellia sect. Thea, including five species and two varieties. Based

on investigations of wild tea plants in Southwest China, Chang (1981) proposed that Camellia sect. Thea could be grouped into four series containing 44 species and three varieties on the basis of style splitting and ovary and leaf morphology. Later, Ming (1992) further revised Chang's classification into 12 species and six varieties. Chen et al (2000) revised the classification into five species and three botanical varieties. Tea plants characterized with five ovary locules and style splitting of the flower were classified into three species: C. tachangensis F.C. Zhang, C. taliensis (W.W. Smith) Melchior, and C. crassicolumna Chang. Tea plants characterized with three ovary locules and style arms of the flower were classified into C. gymnogyna Chang and C. sinensis (L.) O. Kuntze (var. sinensis, var. assamica (Masters) Kitamura, and var. pubilimba Chang). Compared with Chang's and Ming's systems, this proposal seems more concise and functional, and was validated by molecular marker analysis (Chen and Yamaguchi, 2002; Yang et al, 2016).



The place of origin and domestication of tea is clearly in China, given the country's long history of the utilization of tea for medicinal purposes and as a hot beverage. China is also the place of origin of most of the close relatives, many of whose leaves can also be used for infusions. However, the actual origin of tea and its dispersal to other parts of China is not so clearly understood. It is assumed to have originated in Yunnan province in Southwest China, then spread to other southern parts of China and beyond. Yao and Chen (2012) described the history of a Chinese tea industry

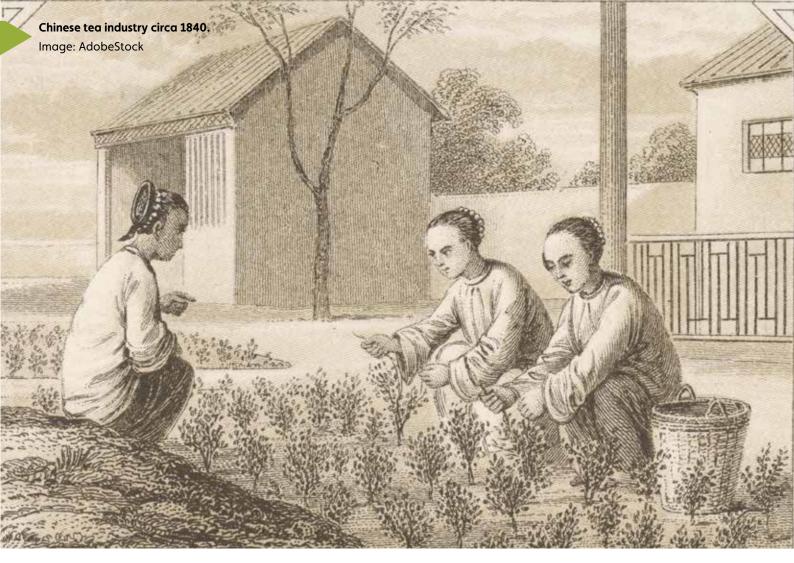
"that had been gradually developed from the Tang Dynasty (618–907 A.D.) to the Yuan Dynasty (1206–1368 A.D.), when fresh tea leaves were popularly processed to make commercial cake-like tea, named cake-tea. In those times, only noble people from the upper class enjoyed drinking tea. The complex and mysterious ceremony of tea drinking was formed and popularized in China. In 760–770 A.D., the first tea book, Tea Classics (Cha Ching), was written by Lu Yu. It is the first authentic literature on tea, which comprehensively described the origin of tea, cultivation, manufacture, drinking methods, history, culture, etc. At that time, tea seeds, cultivation, manufacture, and drinking methods were introduced into Japan and Korea by Buddhists. Subsequently tea was introduced to the other parts of the world. The cake-tea was replaced by diverse shapes and types of tea from the Song Dynasty (960–1279 A.D.) to the Qing Dynasty (1644–1910 A.D.), when more and more ordinary peoples embraced tea as a beverage and tea drinking became popular. In the 17th century, China began to supply tea products to Mongolia, Russia, Europe, and North America. The monopoly of tea exports from China slowly came to an end in 1886 when 81% of exported tea (approximate 134.1 kilotons) in the world was supplied by this country."

The global dispersal of tea cultivation followed a similar path. Mukhopadhyay and Mondal (2018) described how tea production spread from China to tropical and subtropical countries from 221 BC to Vietnam, Myanmar, Laos, and Thailand through migration during war time. Then during the fifth century, China established tea trade along the Silk Road and the Tea Horse Road. Zen Buddhist missionaries introduced tea from the Zhejiana province to Japan and Korea in the ninth century (805 AD and 828 AD respectively) as a medicine due to its meditation-enhancing properties. Tea consumption was introduced into Europe by the early sixteenth century. Tea cultivation and processing technology reached Brazil from China in 1812. In India, the indigenous variety assamica was discovered in 1823, although the tea plant was first

cultivated in 1834 from seeds brought from China. Tea cultivation in Transcaucasia started in 1883 using seeds from Hubei province of China, and after that, the tea plant first reached Turkey in 1924 (Ma and Chen, 2018). Other first cultivations of tea were recorded in Indonesia in 1684, Russia in 1833, Malawi in 1875, Iran in 1900, Kenya in 1903, and Argentina in 1924 (Ma and Chen, 2018; Chen et al, 2012c).

There have been a number of studies on the distribution of genetic diversity within C. sinensis accessions and cultivars in relation to their origin, notably by Yao et al (2008; 2012), Raina et al (2011), Wambulwa et al (2016; 2017), and Meegahakumbura et al (2016; 2018). Yao et al (2008) found greater genetic diversity among tea cultivars of China than Japan and Kenya using intersimple sequence repeat (ISSR) markers. This was consistent with other studies using morphological, biochemical, and other molecular markers. Overall, the Japanese tea cultivars clustered closely with the Chinese cultivars while the Kenyan cultivars were more distant. There was no differentiation of var. assamica or var. sinensis accessions in the study, which indicated the high degree of cross pollination between the two types in cultivars developed from local seed derived populations in farmers' fields and in Chinese tea breeding programs.

Yao et al (2012) evaluated genetic diversity among and within accession from 14 tea-producing provinces in China using expressed sequence tag - simple sequence repeat (EST-SSR) markers. The majority of rare alleles and higher diversity was found in accessions from Yunnan and its neighbors, Guangxi and Guizhou provinces. They concluded that the center of origin of tea in China was also the center of diversity. The genetic diversity not only varied significantly among regions but also between wild and cultivated accessions. The greatest diversity was found within wild accessions, then landraces, and finally improved cultivars with a much lower level of diversity. Wild accessions and landraces had a strong geographically based population structure, but the improved cultivars had a simple structure due to a history of frequent seed introductions, controlled hybridization, long-term selection, and clonal propagation. The wild and landrace accession were likely more geographically structured because of more spatially limited seed and pollen dispersal between distant populations. Thus, germplasm exchange occurred more frequently between nearby regions than more distant ones. The distribution of genetic diversity did provide evidence of the spread of the tea plant from the center of origin to other parts of China. The lowest level of diversity was in Northeast China in Henan, Jiangsu, Anhui, and Hunan provinces, where



there was greater selection for adaptation to the lower temperatures and droughts common there.

Raina et al (2011) concluded that "India hybrid tea" originated from the spontaneous hybridization of the Assam type tea (var. assamica) growing in the forest regions of Northeast India and the China type tea (var. sinensis) introduced around 1835 to Northeast India. The emergence of these hybrid types has resulted in enhanced diversity with no clear patterns of grouping within the various morphotypes in the collection held at the Tocklai Experiment Station (now the Tocklai Tea Research Institute) in Jorhat, Assam. These populations have been an important source of diversity for black tea production in many areas of the world, such as Sri Lanka and Kenya.

Wambulwa et al (2016) assessed the 193 accessions from the Tea Research Foundation of Kenya germplasm collection. These were mainly from a collection made from smallholder farms and tea estates in East Africa, as well as breeding and germplasm exchange programs. The individuals were classified into three types, var. sinensis, var. assamica, and Cambod type, or hybrids between the types. They reported higher level of genetic diversity over all accessions than expected from the short history of tea cultivation. The lowest level of

diversity was in accessions of var. assamica, possibly due to the narrow genepool introduced from India and the limited number of individuals that are used in cultivar development. These few individuals have contributed more genetic material to tea breeding programs than the other types. The tea accessions clustered according to their geographical origin, pedigree, and leaf pigmentation. The diversity in the germplasm collection was greater than that found in the field plantations.

Wambulwa et al (2017) further considered the origin of tea genetic resources in Africa. They utilized simple sequence repeat (SSR) and chloroplast DNA (cpDNA) markers to assess genetic relationships among accession from eight countries in Africa, China, India, and Sri Lanka. The study concluded that the current African germplasm is a result of multiple introduction routes into Africa. The relatively low amount of genetic variation among countries and the lack of any geographical structure in the groupings indicate that there has been frequent germplasm exchange between Africa and Asia, although var. assamica tea in Africa probably originated more from India or indirectly from Sri Lanka. The var. sinensis tea introduced into mainly Kenya came both directly from China (Zhejiang and Guangdong provinces) as well as indirectly from Japan, India, and Sri Lanka. Thus, germplasm

from India contributed more to existing tea genetic resources in Africa, except for Madagascar. Tea germplasm from Madagascar represented more direct introductions from China and there was no evidence of exchange between Madagascar and the rest of Africa. There was also evidence of six private haplotypes within African germplasm that may derive from wild closely related species such as C. irrawadiensis (a source of tea leaf anthocyanins in Kenya, it is incorporated into C. taliensis) and C. pubicosta from Northern Vietnam. The Cambod type tea was considered as an important resource for tea improvement due to its diverse genetic origins. They did find evidence of the presence of the use of Chinese var. assamica tea in Kenya and Sri Lanka but concluded that this limited exchange supported the need to expand its use in the future. They also concluded that the use of tea germplasm from China should be increased in the future given its limited past exchange and the importance of its characteristics for adaptation to low temperatures and drought.

Meegahakumbura et al (2016) reported on differentiation of the genetic diversity between var. *sinensis*, var. *assamica*, and Cambod type tea from China and India. They investigated the domestication history of cultivated tea and its center of origin using SSR markers. The study

Toklai India. Image: Paula Bramel/Crop Trust

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regrouped accessions into three groups; var. sinensis type tea, China var. assamica tea, and Indian var. assamica tea. There was also an admixture group that included the Cambod type. They concluded that the Cambod type originated from hybrids between tea types and thus should not be seen as a separate taxon. They also found the highest level of diversity within the Indian var. assamica types, likely due to extensive hybridization during its short breeding history. The var. sinensis types and the China var. assamica types were more closely related than was expected, with the greatest divergence between the China and the Indian var. assamica types. They concluded that var. sinensis, China var. assamica, and Indian var. assamica were likely domesticated independently in Southern China, Southwest Yunnan province of China, and the Assam region of India.

Meegahakumbura et al (2018) used the same SSR and cpDNA markers as Wambulwa et al (2017) in their assessment of diversity among cultivated tea types. They confirmed the existence of the three independent gene pools and their separate origins, but they found a further differentiation within the China var. assamica types. The var. assamica types from Southern Yunnan (Pu'er and Xishuangbanna) formed a distinct clad with haplotypes of C. talensis, while those from Western Yunnan (Lincang and Baoshan) grouped together with haplotypes of Indian var. assamica tea. They postulated that var. assamica tea from Western Yunnan and var. assamica tea from Assam arose from a single ancestral population from an area where Southwest China, Indo-Burma, and Tibet meet. The results still supported a separate domestication area for var. sinensis tea in Southern China, but its exact origin was still not clear. They recognized that the China var. assamica tea represents a valuable resource for future breeding, and thus the conservation of the ancient teas in situ in Yunnan province should be a high priority.

#### Ex situ conservation

Tea genetic resources are currently being conserved ex situ in many countries as field collections. As Chen et al (2012a) pointed out, "'in every research institute in China, there is a large or small tea germplasm garden". These tea germplasm gardens are field collections which are managed for conservation, research use, and production of tea. Chen et al (2012a) estimated that there were more than 10,000 accessions preserved in national and provincial tea germplasm gardens in China. A similar situation is found for many other production areas of the world, where national collections exist as well as regional, local, and even private collections.

Very little has been published on standard practices for secure conservation of tea in germplasm gardens. There are no manuals or best practice guides available. Yao and Chen (2012) concluded that there was a need for at least 10 plants to be established for clonal accessions and at least 20 plants planted for seed propagated accessions and natural tea populations in order to maintain genetic integrity and securely conserve tea accession in tea germplasm gardens. Otherwise, it would seem that field operations are very similar to that of tea in production, although not all germplasm gardens are pruned and/or plucked. In most institutes, tea genetic resources are maintained as part of an active tea breeding program. Thus, field collection maintenance is a long-term cost for the breeding program or the research station. Part of the cost for field maintenance can be offset with the sale of tea leaves harvested from the germplasm garden. In some institutes, annual funds are allocated especially for tea germplasm conservation by national governments, such as those of China (Yao and Chen, 2012) and India (Das et al, 2012).

One alternative approach for long-term conservation could be seed conservation. Tea is mostly self-incompatible, so for seed samples, only the maternal parent is known. As only half of the diversity being conserved is from the original accession, seed cannot be used to conserve individual accession level diversity, but it can efficiently conserve the diversity of populations for long-term conservation. Tea seed is recalcitrant and has a very short viability period that is related to its high moisture content. Patel et al (2018) did a review of the methods that had been researched to increase the storage time for tea seed. Overall, they found that storage methods that managed seed moisture, atmospheric temperature, relative humidity, microbial contamination, and insect infestation could extend seed storage from 9 months to 6 years. In general, there were no studies that identified methods to extend seed conservation as a viable long-term alternative to field collections.

Chen et al (2012a) also reported on the use of an *in vitro* conservation system where cultures were established from immature embryos as well as callus induced from nodal segments. Mondal et al (2004) reviewed micropropagation techniques and the effectiveness of *in vitro* regeneration techniques. Attempts have been made to develop low temperature *in vitro* storage approaches for encapsulated somatic embryos and nodal explants. They reported on a methodology using encapsulated nodal explants for successful storage for up to 45 days. The use of this approach for tea conservation could be considered for safe exchange



of germplasm or for embryo rescue for hand pollinated distant hybrids. Gunasekare et al (2012) described a technique for encapsulation of zygotic embryonic axis using alginic acid with an efficient recovery that they considered as having potential for long-term *in vitro* preservation.

Chaudhury and Malik (2004) reviewed the use of cryopreservation for plantation crops such as tea. There have been reports of high survival percentage from cryopreservation of embryonic axis, whole seeds, and shoot apices. Regeneration was possible directly from thawed tissue for tea. More research is needed to enhance the use of cryopreservation for long-term conservation of tea. Park and Kim (2007) concluded that cryopreservation of tea embryos was a viable and applicable method for long-term germplasm conservation. Yao and Chen (2012) discussed the need to consider in vitro systems for cryopreservation of tissues as well as cryopreservation of seed. The cryopreservation of tea seeds has been investigated but there is need for greater research. They concluded that future studies need to focus on the development of stable and effective cryoprotectants; rapid test to predict seed viability under cryopreservation; and methods for secure cryopreservation of tea seed tissues.

#### In situ conservation

Yu and Chen (2001) reviewed the classification and characterization of very large, old indigenous wild tea *Camellias* in various locations in China. Most of these trees were classified as *C. taliensis*, *C. tachangensis*, *C. crassicolumna* and *C. gymnogyna* as well as *C. sinensis* var. assamica and var. pubilimba. These massive wild tea *Camellias* have been used directly by local peoples, in a breeding program to derive cultivars, and evaluated for specific traits. Yang et al (2016) assessed the genetic divergence among *C. sinensis* and its wild relatives. In general, the lower heterozygosity of the wild accessions was postulated to be a product of their narrower distribution and small population sizes.

Zhao et al (2014) indicated that *C. taliensis* has been used locally to produce tea but only recently has it been recognized as a source of tea leaves outside local areas. In Western Yunnan, where *C. taliensis* is mainly found, it is called wild tea or local mountain tea. It makes a beverage that is similar to var. assamica, but it also has unique characteristics. The wild tea is either harvested from trees established from seed gathered from the wild and planted in tea gardens, or harvested from cleared and managed areas of the forest. The study assessed genetic diversity within wild, planted, and cleared populations. The genetic diversity within the planted

population was higher than that of the wild due to a reduction of the genetic diversity within the wild populations from human activities leading to habitat degradation. They found that the planted trees came from very few seed sources and were then dispersed artificially from the limited initial tea gardens. They concluded that both the wild and cleared population should be protected, especially from any further encroachment for production with clearing and management. The planted population should also be conserved since it represents an important source of diversity for the future.

This agreed with the results of Liu et al (2012) who found that fragmentation due to deforestation and over-exploitation of C. taliensis had restricted gene flow and seed dispersal in natural populations. This resulted in lower effective population size, reduced genetic variation, and greater differentiation among these isolated populations. They suggest the need to focus on habitat protection to maintain genetic diversity of the remaining populations through both gene flow and effective population size. A similar situation is found for Coffea arabica genetic resources in Ethiopia. Labouisse et al (2008) described the four major coffee production systems in Ethiopia as forest coffee, semi-forest coffee, garden coffee, and plantation coffee, and saw the forest coffee and semi-forest coffee systems as in situ genetic resources. In 2010, in an attempt to conserve



the last remaining coffee forests in Ethiopia and to halt the loss of biodiversity, the Yayu Biosphere Reserve and the Kafa Biosphere Reserve became part of the United Nations World Network of Biosphere under the Man and Biosphere reserve program. Gole (2003) identified constraints associated with establishing *in situ* reserves and action steps to be taken to develop a successful program.

Yang et al (2016) assessed the genetic divergence among C. sinensis and its wild relatives. Among the 18 accessions evaluated using genomic single nucleotide polymorphism (SNP) markers, there was a semi-wild landrace of C. taliensis that was clearly a hybrid between C. taliensis and var. assamica. It was intermediate in terms of genetic divergence between its two parents, but had the highest degree of heterozygosity among the wild accessions, likely due to its hybrid nature. The gene flow from var. assamica could be a threat to the integrity of the C. taliensis populations that will need to be managed for longer term in situ conservation. A similar situation is seen in apples. Feurtey et al (2017) concluded that wild populations of apples need to be protected from hybridization with domesticated apples by managing the risk for geneflow in the natural habitats and in seed nurseries set up for reforestation. EUFORGEN (2017) established technical guidelines for the conservation of Malus sylvestris in forest reserves. Something similar could be considered for the conservation of wild tea genetic resources in situ.

Yao and Chen (2012) concluded that both ex situ conservation in tea germplasm gardens and in situ conservation is supported by national and local government. There are plans and actions being taken to conserve famous tea landraces and wild tea plants in Fujian, Zhejiang, Guizhou, and Yunnan provinces. Recently, Guizhou provincial government and municipal governments in Yunnan issued local regulations to better protect the old tea plants and ancient tea plantations in their areas. In a proposal to support the designation of the Pu'er Tea Agricultural System in Yunnan as a Globally Important Agricultural Heritage System, the local tea production system described included old wild tea plants and their community in the forest, half domesticated tea plants, ancient tea plantations, and tableland tea plantations (People's Government of Pu'er City, Yunnan, 2012). The traditional systems are used by different indigenous communities to manage, harvest, and protect tea plants within natural forests, an ancient system known as tea forests. In Pu'er there are 26 tea forests extending over a combined 12,123 hectares, while old wild tea plants cover more than 33,333 hectares of Yunnan. There continues

to be threats to the wild tea plants as well as the ancient tea forests from economic development that has reduced the size of the tea plant population as well as degraded the ecosystem. This has been due to population growth, irrational harvesting of the wild trees, over-exploitation of tea forests, and large-scale elimination of tea plantations for grain and sugarcane cultivation, as well as the planting of new tea plantations around the old tea forests. An action plan was proposed to manage these risks for sustainable development that would conserve the wild tea and tea forests. One of the actions has been to establish a germplasm nursery that now conserves 1,100 germplasm accessions, including vegetatively propagated cuttings from precious and rare trees. There have been extensive inventories of wild tree populations as well as varieties found in the ancient tree forests. Additionally, the Ancient Tree Plantation on Jingmai Mountain in Pu'er has been nominated and tentatively listed as a UNESCO World Heritage site.

Liang et al (2013) described the outcome of multistakeholder assessment of the value and service of the tea forests in Manajing village, Yunnan. This area includes traditional tea forests as well as intensive tea plantation on terraces. In the past, there had been an increasing focus on establishing new tea terraces that was leading to a reduction in forest, including the tea forests. The multistakeholder assessment did lead to a more rational decision for land use and was reducing the threat to conservation of tea diversity in the tea forests and its many services. The comparative assessment of services rendered by the tea forests versus the tea terraces led to the restoration of traditional worship of the best ancient tea trees as hosts of tea spirits; increased visits to the tea forests that reduced encroachment by others; increased income from the harvest and sale of forest Abaina tea, with greater local management of the production and supply chain (described in Liang, 2010); reduced the establishment of new tea terraces; and was leading to the restoration of traditional systems in the tea forests and tea terraces. These outcomes were helped by the increased market value for local tea produced organically. The revival of the local tea culture and raising awareness of the value of healthy tea and the environment contributed to the appreciation of the tea forests at all levels of stakeholders. All of these efforts will result in increased in situ conservation of tea genetic resources in the wild and in the ancient tea forests. There are risks associated with this value-added market approach to in situ conservation, however, as it requires monitoring systems to ensure secure long-term protection.

#### Vulnerability of tea genetic resources

Tea and its genetic resources are vulnerable to challenges such as climate change, biotic threats, land use changes, fluctuating market prices, local labor costs and shortages, and other changes in the tea sector. When Tea 2030, a partnership of the tea industry and NGOs, produced a report in 2014 called The future of tea: A hero crop for 2030 (Forum for the Future, 2014), the report identified 10 critical factors that could greatly impact the future of tea production and consumption around the world. These included demographic change; resource constraints; climate change; competition for land; availability of labor and increases in mechanization; the balance of power across the supply chain; the emergence of new business models; sustainability leadership in emerging economies; improvements in wages and labor welfare in the supply chain; and consumer attitudes to the value of food. For example, increased competition for land could result in a reduction of land area under tea if it is converted to other plantation crops such as rubber or palm oil. Land competition could also lead to increased deforestation if new areas are developed for tea growing. Both of these are risk factors for the long-term productivity of tea due to negative environmental impacts, but they are also risks for tea genetic resources. Tea 2030 discussed the

implication of these various factors and concluded there was a need for a global focus on three main areas related to sustainable production: conserving and managing the environment and the communities where tea is grown; market mechanisms to provide increased value to all members of the supply chain; and engaging consumers to participate in the reduction of the detrimental social and environmental impacts related to tea consumption.

These are risks faced by many commodities globally that need to be mitigated in terms of local, regional, and global actions. Tea is a very important source of gross domestic product from exports and a source of rural livelihoods for many countries, in particular Kenya and Sri Lanka. There is a strong private sector interest in working with national governments and other private entities, including smallholder farmers, to mitigate and adapt the tea sector to these challenges. Carr (2018) concluded that the sustainability of the tea industry will depend upon a sustainable supporting service from research institutes, as well as extension and training. Ex situ tea germplasm collections are a key part of this supporting service. Conversely, any risk to the sustainability of the production system and the industry is a threat to conserved genetic resources that will need to be mitigated to reduce the loss of genetic diversity.

#### **Climatic threats**

FAO (2016a) presented a report of a working group on climate change that reviewed and assessed the vulnerability of tea production in India, Sri Lanka, Kenya, and China. They considered the impact of global warming on tea production, particularly the increased frequency of extreme weather events and the loss of predictability that complicates long-term management of production processes. The working group generally concluded:

"the assessment highlights disturbing trends of declining yields and productivity due to climate driven stresses (biotic and abiotic) in recent years in tea growing countries of the Working Group. Tea production is controlled by three broad elements: genotype; environment; and management. Tea bushes remain in the field for several years, resulting in severe deterioration of the growing environment due to repeated interventions in the form of: regular plucking/ pruning; cultural practices; addition of external inputs; and resulting field traffic. Climate change impacting local weather conditions (prominently changing rainfall trends resulting in frequent flood and droughts besides increase in temperature, change in relative



humidity and sunshine hours) further exacerbates the situation. The possible fallouts of the climate change are already witnessed in the loss of yields and increased management costs for developing coping strategies. Therefore, any spatial and/or temporal changes in the regional climate pattern will directly affect the regional economy and consequently the well-being of the region."

The working group presented a comprehensive review of the evidence for climate change, the predicted impact, and the adaptation strategies needed to maintain tea production and quality. They developed a summary set of recommendations for actions to be taken by tea growers, local research institutes, and policy makers. One key recommendation was to develop new cultivars resistant or tolerant to drought, heat, frost, pests, and diseases. This will be dependent upon the use of the diversity of the tea genetic resources conserved ex situ and those that are still maintained in nature or on farm. The changes the working group predicted with climate change will also increase risk of loss of ex situ field collections.

#### **Biotic threats**

Lehmann-Danzinger (2000) and Chen et al (2012b) reviewed pests for tea plants and the role of resistance in the integrated management of pests in tea production systems. Tea is a perennial plant that grows in warm and humid climates. This climate results in a relatively stable microclimate for the growth of insects, pathogens, and other pests. In fact, more than 1,000 insect and mite pests and more than 500 fungal, bacterial, and nematode diseases have been described for the tea plant. A summary list of those of significance is given in Table 2. Generally, shoot and leaf pests and diseases are of greatest concern, since the focus in cultivation is the leaf.

In tea breeding programs, resistance to major pests is an important target, and many resistant varieties have been developed in the past (Chen et al, 2012b). The use of resistant and tolerant cultivars in tea production systems is the foundation for evolving effective integrated management options. It is clear that tea genetic resources have been and will continue to be key resources for the development of resistance through breeding. So, while pests and diseases threaten tea production systems, they are also risks for ex situ field collections. Fortunately, many of the threats are local in nature and can be controlled with integrated nutrient and pest management in tea germplasm gardens. The use of multiple sites will also reduce the risk. Unfortunately, these pests

Table 2. Major biotic threats to tea locally and globally (Mukhopadhyay and Mondal, 2018; Chen et al, 2012b).

Type of pest	Major pest species
Defoliating chewing insects	Bunch caterpillar (Andraca bipunctata) Looper caterpillar (Buzura suppresseria) Red slug caterpillar (Eterusia magnifica) Flush worm (Lespeyrasia leucostoma) Tea lopper (Ectropis oblique) Tea tussock (Euproctis pseudoconspersa) Tea small leafroller (Adoxophyes honmai) Tea leafroller (Homona magnanima) Tea tortrix (Homona coffearia)
Sucking insects	Tea mosquito bug (Helopeltis theivora) Jassid (Empoasca flavescence) Thrips (Scirtothrips dorsalis) Tea leafhopper (Empoasca vitis) Tea black spiny whitefly (Aleurocanthus spiniferus) Mulberry scale (Pseudaulacaspis pentagona) Tea mirid (Helopeltis schoutedeni) Tea aphid (Toxoptera aurantii) Tea weevils (Myllocerinus aurolineatus and Basilepta melanopus)
Stem-boring insects	Tea shot hole borer (Xyleborus fornicates)
Mites	Purple mite (Calacarus carinatus) Pink mite (Acaphylla theae) Scarlet mite (Brevipalpus phoenicis) Red spider mite (Oligonychus coffeae) Tea kanzawai mite (Tetranychus kanzawai) Broad mite (Polyphagotarsonemus latus)
Leaf and stem diseases	Blister blight (Exobasidium vexans) Red rust (Cephaleuros parasiticus) Black rot (Corticium theae and Corticium invisum) Tea anthracnose (Gloeosporium theae-sinensis) Brown blight (Guignardia camelliae) Phomopsis stem blight (Phomopsis theae) Tea red leaf spot (Phyllosticta theicola) Tea gray blight (Pestalotiopsis theae) Hypoxylon wood rot (Hypoxylon serpens) Stem canker (Macrophoma theicola)
Root diseases	Charcoal stump rot (Ustulina zonata) Brown root rot (Phellinus lamaensis) Red root rot (Poria hypolaterita) Violet root rot (Spherostilbe repens) Armillaria root rot (Armillaria heimii)
Nematodes	Root knot (Meloidogyne incognita and Meloidogyne javanica)

and diseases are also major risks in the exchange of germplasm that need to be managed in developing safe approaches for the distribution of accessions.

#### Replanting and replacement of seedling tea

Ellis (1997) concluded that the most important event in the evolution of commercial tea was the development of "India hybrid tea". Carr (2018) noted that the longevity of tea bushes means that many of the original plants from these hybrid seeds of unknown parentage are still in production in many areas of the world. Estates were established with seedlings from introduced seed populations (jats), either through direct transfer of seed populations from Northeast India or from indirect transfer of seeds from areas that had originally been established from these seed populations, such as Sri Lanka or South India. These original seed populations had a high level of heterogeneity due to their origin from intercrosses of Chinese var. sinensis and native var. assamica from Northeast India. Many of these were planted in the late nineteenth or early twentieth century. Subsequently, there has also been natural selection for specific adaptation within these old seedling fields. Gunasekare et al (2012) concluded that with national replanting schemes introduced into Sri Lanka in 1958, there has been a conversion of seedling-derived fields to vegetatively propagated cultivars. Currently, there are still old seedling gardens retained on estates, but these are being rapidly uprooted for replanting. This represents a risk of erosion of genetic diversity for an important source of improvement and adaptation in the future. There is also a risk of loss of old seedling fields through replanting in many

areas of past production such as Northeast India (Das et al, 2012) and Kenya (Kamunya et al, 2012). The proportion of tea area planted to clonal teas in the main producing countries ranges from 50–60% in China, India, Sri Lanka, and Kenya to over 92% in Japan (Chen et al, 2012c).

The replacement of heterogeneous seed derived fields with homogenous clonal cultivars also represents a risk to tea production due to the possibility that large areas will be replanted to a near monoculture of tea bushes. This risk is recognized by Carr (2018) and many others, as genetic diversity within tea fields is important for elasticity and resilience. Ni et al (2012) concluded that the genetic basis for elite clonal cultivars was generally very narrow. Thus, there is a need to enlarge this diversity in the development of new clonal cultivars with greater use of broader tea genetic resources.

To mitigate the risk to future gains in tea productivity and preserve future market opportunities for unique teas, it will be necessary to carefully consider the genetic diversity between clonal cultivars being recommended for replanting schemes, as well as to ensure the long-term conservation of the genetic diversity that is still maintained in existing old seedling gardens. Das et al (2012) described how the Tea Improvement Division at Tocklai Experiment Station approached the conservation and use of diversity in old tea plantings. Since 1972, an Estate Selection Scheme has been used to select bushes in old seed populations before uprooting. The old seed derived population with wide diversity are surveyed,

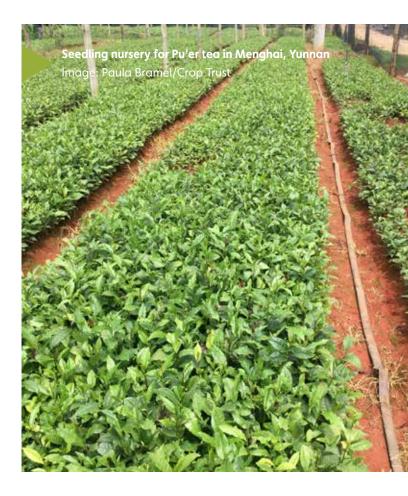


locally adapted elite plants are marked, and clonal accessions or cultivars are developed. The improved clones are released by the Tea Research Association as "TRA/Garden Series" cultivars that are location specific. Those with unique traits are maintained as accessions in the germplasm collection. This process results in both conservation of the phenotypic diversity as well as the availability of greater genetic diversity among released clonal cultivars.

Gunasekare and Kumara (2005) described the estate cultivar selection program that was initiated for Sri Lanka in about 1905. In the early years, cultivars were selected from seedling fields with the objective of identifying seed lots that were typical and to introduce new seed sources from China and India. In the 1920s, a shift was made to more intense selection for yield among estate teas in different agro-ecological zones. When vegetative propagation became available in the late 1950s, the selection program was accelerated and resulted in the development of a large number of estate selections or clonal cultivars. The process of intensified selection for yield resulted in a narrowing of the genetic diversity within all the initial estate selections. Thus, there is a need to further conserve the genetic diversity still found within the old seed fields on estates for use in the development of genetically diverse clonal cultivars for the future. One approach the national tea research program is taking is to do further collection from tea estates by initially working with the estate and community to conserve these old seedling teas in situ. Then they plan to utilize molecular markers to assess diversity among and within the in situ sites to identify sites and individuals for collection and conservation using the method described by Park et al (2002) for collection within old abandoned plantations in Korea. Carr (2018) concluded that the risk to production from the replanting of diverse seedling fields with clonal cultivars could be managed by planting diverse superior clones on estates and conserving the diversity found in the seed population ex situ on research stations.

## Utilization of tea genetic resources

Camellia sinensis is largely self-incompatible, making it almost impossible to produce inbred lines with predictable traits. However, tea is easily propagated by stem cuttings that are identical to the mother bush. This easy propagation from cuttings, combined with longevity of over 100 years, make the selection of elite mother bushes the best practice at present. Heritability studies on tea have been summarized by Corley and Tuwei (2018). Some traits like leaf color, leaf pose, and leaf



pubescence are highly heritable, while other traits like hair length, hair density, and oxalate crystals are inherited quantitatively.

Corley and Tuwei (2018) concluded that field selection for yield was effective but no good method has been identified to select for quality. A recent study by Koech et al (2018) has reported one to seven quantitative trait loci (QTLs) for caffeine, catechin, epi-catechin, epi-catechin gallate, epi-gallocatechin, epi-gallocatechin gallate, theaflavins-1, and theaflavin-2. Tea tasters' scores for color, briskness, brightness, astringency, and aroma were also linked to several QTLs. However, each QTL accounted for only 3-10% of the phenotypic variation. These QTLs were based on over 12,000 genomic markers. This illustrates that quality traits are multigenic and impossible to predict at present. However, new developments with SNP chips with 487,000 SNPs will probably improve our knowledge about the genes that control multigene traits. Improved phenotyping will meanwhile depend on better techniques for the mini-manufacture of green and black tea from individual tea bushes for evaluation by expert tea tasters. The selection of a new round of elite mother bushes, with a combination of desirable traits, will form the next generation of biclonal or polyclonal parents.



Tea genetic resources have been successfully used to develop clonal and seed cultivars. Yao and Chen (2012) listed the 123 national tea cultivars and 158 provincial tea cultivars released in China. These have been directly derived from traditional cultivars (both seedlings/jats and clones), individual selected clones from seedlings/jats, clones selected from open pollinated progenies, clones selected from controlled pollinated progenies, and in one case from mutation breeding. This demonstrates the wide diversity of breeding approaches that have been utilized successfully for tea in the past in China, but similar approaches have been used in many countries. Individual selection from local tea populations or open pollinated progenies is still being successfully used in China (Yao and Chen, 2012), and in India and Sri Lanka for estate teas (Das et al, 2012; Gunasekare et al, 2012). Other, more controlled, hybridization approaches include controlled hand pollination, biclonal seed orchards with two parents, and polyclonal seed orchards with multiple parents. Selections from the resulting progeny are then cloned for further testing and selection. Yao and Chen (2012) concluded that traditionally, it requires at least 20–25 years from individual selection to final release of a new cultivar. The use of controlled pollination and individual selection, along with selection with molecular markers and micropropagation, will reduce this timeframe.

The development, release, and adoption of successful clonal cultivars can narrow the genetic basis in tea production fields due both to wide scale adoption, such as that of Yabukita in Japan (Kaundun et al, 2000), and the prevalence of only a

few parents in all released cultivars, such as 75% of cultivars released from Tocklai, India, having been developed from Betjan, Cinnamura, and Cambod (Hajra, 2001). Thus, there is a need to increase the diversity of parents being used in tea breeding through greater use of tea genetic resources. Gunasekare (2012) reported that from 1961 to 1998, the Tea Research Institute of Sri Lanka had only used 22 parents recurrently in their controlled hybridization program. They recognized the need to increase their use of diverse parents in the breeding program, and since 2004 have utilized 46 new parents in crosses.

Enhanced use of ex situ collections depends upon sharing better documentation with users on the accessions in a collection. Gunasekare et al (2012) concludes that tea germplasm is not being used effectively due to the lack of documentation. Gunasekare and Kumara (2005) collated passport and characterization data on accessions originating from tea estates since the 1930s. This documentation allowed an assessment of the spatial origin of the accessions. Further documentation was done to include results from evaluations into a searchable database. Chen et al (2012a; 2012c) indicated that since 2005, the China National Germplasm Tea Repository has established a detailed database system based on 111 descriptors of accessions. It is not clear what the status of either of these databases is, nor of any others that might have been developed based on accession level passport, phenotypic and genotypic characterization, or evaluation data on currently held ex situ collections. The availability and sharing of this documentation in a form that can be used directly by any user has been recognized as an important step for a rational, cost-effective, sustainable global system for conservation and use. This step does not seem to have been a priority for the current conservers of tea genetic resource.

Sharing accession level documentation can also lead to greater use of large collections. Ni et al (2012) reported on an extensive review of the application of molecular markers in tea genetic resources as well as breeding. In general, they found that a wide diversity of molecular markers had been used in studies with tea. A number of these, including SSR and cpDNA markers, could provide robust tools for the study of genetic diversity, genetic variation, molecular identification or fingerprinting, molecular phylogenetics, genetic stability or integrity, and for linkage maps for marker assisted selection in tea breeding. The review demonstrated the current understanding of the degree and distribution of genetic diversity within and between locally collected germplasm in many countries. It also

indicated the vulnerability of narrowing diversity in some of the key producing countries, such as Japan, Kenya, India, and Sri Lanka.

Systematic assessment of genetic diversity within collections will also allow for sampling of this diversity for users into core collection or subsets that maintain the original diversity of the collection but in a size that facilitates the evaluation, use, and conservation of the collection. These core subsets have a known relationship back to the original collections, so resampling the groups of greatest interest or further subdivision is possible to identify further accessions for testing. FAO (2016b) reported that more than 1,000 trait-specific subsets of various crop collections had been developed by 2014. Many of these core sets have been identified to maximally sample the diversity but not to meet the needs of the crop breeder who is looking for more limited portions of the diversity with a high frequency of the trait of interest.

Raina et al (2011) identified a core subset of 105 accessions or clones that captured 98% of the diversity within the 1,587 accessions and 57 commercial clones from the Tocklai Experiment Station and the UPASI Tea Research Institute. This was based on amplified fragment length polymorphism (AFLP) markers and phenotypic details for 25 traits. Wang et al (2011) and Chen et al (2012a) reported that a core collection of Chinese tea germplasm had been identified, based on geographical origin, phenotypic traits, and EST-SSR markers. Ranatunga and Gunasekare (2009) described the development of a core for the collection held in Sri Lanka. Taniguchi et al (2014) identified a worldwide core collection based upon 23 SSR markers.

McCouch et al (2012) concluded that core collections, reference sets, or subsets could be used to prioritize sample sets for allelic mining approaches that involve more intensive genotyping. They hypothesized that combined use of phenotyping and genotyping data in genome wide association studies would identify regions of the genome that are associated with phenotypic variation for the trait of interest, especially for genebank accessions that are distantly related. McCouch et al (2012), Yu et al (2016), and Hickey et al (2017) all agree that genomic selection will allow for the exploitation of systematic assessments of diversity in genebank accession to calculate genomic estimated breeding values for the individual accessions. Yu et al (2016) outlined an approach for 'turbo-charging genebanks' through genetic selection. They suggested using the whole collection, rather than existing subsets,

to create a global assessment of genetic profiles of all accessions.

S. Tanksley (Nature Source Improved Plants) and J. de Silva (Unilever) are using genotyping by sequencing (GBS) to assess the genetic relationships between tea accessions sourced from China, India, Kenya, Turkey, Sri Lanka, and Malaysia (personal communication, November 2018). An initial evaluation of 826 accessions has led to the identification of 3,114 SNPs, with sufficiently wide genome coverage to enable the accurate prediction of traits of interest using genomic selection models. They conclude from the initial evaluation that GBS eliminates marker ascertainment bias commonly encountered in fixed SNP arrays and could be used as a standard genotyping platform for tea, to better focus longterm conservation and use on unique diversity.



Ni et al (2012) reviewed the literature on the use of molecular markers to estimate genetic relationships and for genetic diversity assessment in tea breeding and conservation. They concluded that while great advances had been made in the testing and application of molecular markers in tea genetic resources and in tea breeding, there was more to be done. They recommended four actions that needed to be made to enhance their use:

- Undertake a global tea germplasm diversity assessment and evaluation effort to globally enhance the use of conserved germplasm.
- Develop new and cheaper options for DNA markers that are better associated with traits, such as EST-SSR and SNP.
- Develop a high-density genetic linkage map with links to important QTLs to facilitate early selection and shorten the tea breeding cycle.
- Generate early selective markers for various abiotic and biotic stresses to revolutionize tea breeding with its lack of selection criteria for many of these traits and the long gestation period to evaluate traits phenotypically.

There is considerable potential genetic gain in plant breeding from the deployment of genomics in breeding programs (Hickey et al, 2017). This breeding approach utilizes global phenotypic and

Different types of tea in Sri Lanka Image: WikiCommons

genomic data that is anchored to the accessions conserved in genebanks. These accession level databases should deliver steep changes in the rate of genetic gain by more effective and targeted use of allelic diversity in landrace and wild accessions. Van Hintum (2016) concluded that the connection of different accession level data types to each other and to the germplasm itself is still a significant constraint to accessing data, conducting analysis, and interpreting findings to advise users on the breeding or research value of an accession. A review of the literature indicates that this interconnection of accession level information within and between collection holders does not exist for tea genetic resources. Sharing of accession level documentation will be a prerequisite for the effective use of the wide diversity of ex situ and in situ conserved genetic diversity for tea. There is no evidence of large scale, multi-institutional, multicountry collaborative projects focused on tea genetic resources or tea breeding.

#### Survey of users of tea genetic resources

As part of this study, a Users' Survey was sent out to 27 users of tea germplasm to better understand how genetic resources are being used, the key issues in relation to access from collection holders, and the main needs for genetic resources in the future. There were nine respondents to the survey. Six were users from research/breeding and three were from private companies. Of the private sector respondents, two were commercial plantations and one was a farmer-producer. The majority (63%) of the users obtained germplasm from their own collections and other collections in their country. Only two received germplasm from sources outside their own country. Only one user obtained germplasm directly from farmers.

All of the respondents concluded that genetic diversity in the clones or varieties that they currently use is not adequate or could be improved. They all concluded that genetic resources were important for cultivar improvement due to increased hybrid vigor from crossing or greater diversity for direct selection. The types of germplasm they had interest in accessing were mainly local landraces and cultivars or clonal cultivars. The majority of the respondents used genetic resources for specific traits involved in tea quality, resistance to pests, drought or heat tolerance, and for rare morphological traits such as purple or albino leaves. One user did indicate they had an interest in traits related to frost tolerance, ease of mechanical harvest, and adaptability to various growing conditions. One respondent described the use of diversity in their program as "insurance against

the vagaries of nature and to advance future developments in the tea sector".

The users' experience in germplasm exchange was mainly limited to within their own country, but the main constraints to use were related to inadequate documentation of the material available, not policy issues. Users are still finding traits of interest, but all respondents agreed that the genetic diversity of cultivars and in farmers' fields was not adequate for future genetic enhancement or production. They all had interest in greater sources of diversity for key local constraints and new opportunities.

#### Germplasm exchange

Genetic resources are subject to a number of international and national legal regimes that regulate access and benefit sharing (ABS). Germplasm exchange is also incentivized or discouraged by a variety of regulatory frameworks - the International Union for the Protection of New Varieties of Plants (UPOV) System, intellectual property and other proprietary regimes, biosecurity - as well as academic and public-private collaborative networks and organizations such as national agricultural research institutes, NGOs, and conservation entities. An expanded understanding of this wider ABS context for tea needs to be considered for the future. There is currently limited exchange of tea germplasm and it mainly depends upon institutional research agreements or national governments' bilateral agreements. An example of two recent germplasm exchanges is described in Kamunya et al (2012) for acquisition of improved cultivars from Japan and China by Kenya, utilizing bilateral government agreements.

The Convention on Biological Diversity (CBD) calls for the "fair and equitable sharing of benefits arising out of the utilization of genetic resources" as well as the conservation and sustainable use of these natural resources. It recognizes national sovereign rights over genetic resources and the need for access through prior informed consent on mutually agreed terms. Provider countries are encouraged to create conditions to facilitate access without imposing undue barriers to use. Prip and Rosendal (2015) describe the development and implementation of the Nagoya Protocol for ABS of genetic resources under the CBD.

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), which came into force in 2006, is recognized by the CBD as a sector-specific ABS regime. Many crops of global significance to food security are included in the Multilateral System (MLS) of the ITPGRFA, which



is the key to its ABS regime. Chiarolla et al (2013) concluded that decoupling of benefit sharing from access and use by a specific provider in ABS regimes such as the MLS is based upon recognition of the incremental improvement from multiple sources that characterizes plant breeding. Due to the high rate of global interdependence and exchange, countries gain more from having access to a global pool of plant germplasm and from addressing benefit sharing multilaterally (i.e. without any attribution of benefits to a specific provider country), rather than governing ABS bilaterally.

Although the scope of the ITPGRFA is all plant genetic resources relevant to food and agriculture, the MLS currently applies to 64 crops listed in its Annex I. Crops that are recognized in this MLS have global significance as well as global interdependence. Exchange of material in the MLS operates on an accession basis through a Standard Material Transfer Agreement (SMTA), in which the germplasm recipient is under an obligation to not claim ownership of the received material per se nor seeks intellectual property rights over the received material or its genetic parts or components. The recipient is entitled to claim intellectual property or other restrictions on a product (i.e. a new variety) that incorporates the received germplasm. If such claims result in a restriction of further research and

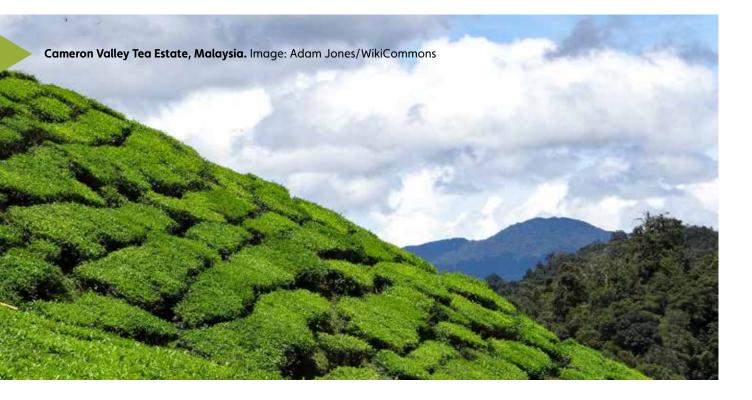
breeding on the received germplasm, the SMTA foresees monetary benefit sharing through the multilateral Benefit Sharing Fund. The SMTA also encourages non-monetary benefit sharing, in the forms of information exchange, access to and transfer of technology, and capacity building.

Halewood (2013) assessed the globally recognized effort to cooperate in the generation, pooling, conservation, and sharing of plant genetic resources and identified a number of options for recognizing the public, private, or common pool goods nature of plant genetic resources. The assessment identified many dilemmas and challenges for the inclusion of crops, such as tea, as a public good in the current systems, but concluded that if some of the weaknesses could be addressed, the ITPGRFA might be an option to facilitate ABS for crops such as tea. Tea is not included in the list of crops in Annex I of the ITPGRFA. Thus, the CBD/Nagoya Protocol provisions or national policy on ABS are assumed to apply for tea germplasm exchange. There are ongoing discussions to extend the scope of the MLS by increasing the number of crops on the list, or indeed to abolish it altogether.

Pisupati and Bavikatte (2014) argue that ABS should be approached as a business model that could incentivize a stream of revenue for conservation and sustainable use of biodiversity, rather than solely as a regulatory system for preventing biopiracy. Prip and Rosendal (2015) indicated that there was a need to do much greater research on how, and the extent to which, ABS is applied on the ground

and its consequences for equity and conservation to develop clear ABS regimes and alternative approaches for crops such as tea, especially if it continues to be excluded from Annex I.

Clearly, any movement of plant materials between countries and/or regions should be done very carefully so as not to introduce a new pest or a different strain of pest to an uninfected region. International movement of tea plant material is governed by national phytosanitary regulations that differ according to the risk assessed by each recipient country for each provider country. In some cases, this movement is not allowed due to significant risk to the local tea production systems. Many countries require quarantine measures to be taken as well. All of these regulations serve to further restrict the movement of tea genetic resources and their use for varietal development. One approach that has been used to manage the quarantine risk in plantation crops is to utilize in vitro cultures that have been cleaned of diseases and viruses. Bioversity International and CacaoNet developed Technical Guidelines for the Safe Movement of Cacao Germplasm (End et al, 2010). This manual provides general recommendations for movement of cacao germplasm with an analysis of various options such as seed, budwood, bareroot plants, in vitro, pollen and open flowers, and flower buds. They provide a summary of pest risks by principal pests and by country and description of all known pests of cacao. A similar manual should be developed for tea to enhance the movement and use of tea genetic resources.



# Summary of background and key issues for the future

Tea genetic resources are being conserved ex situ and in situ in a number of tea-producing countries. The main center for diversity, in terms of rare alleles and heterozygosity, is South and Southwest China. There are also important sources of diversity in the botanical varieties of C. sinensis as well as the wild tea plants in Northeast India and in the northern border areas of Myanmar, Thailand, and Vietnam adjacent to China. There is also still existing diversity in old seedling gardens in many areas of the world that needs to be better conserved and used. This diversity offers unique populations that were a product of the movement and hybridization that occurred when tea was planted from various sources of seed during the early establishment of tea production in China, Japan, Korea, India, Sri Lanka, Malawi, Kenya, Madagascar, and Indonesia. These seed derived populations have adapted to local conditions and could serve as sources of more specific adaptation. There is general recognition that these old seed gardens are still important sources of genetic diversity for the future.

There are limited options for the long-term ex situ conservation of tea genetic resources currently, mainly limited to field collection with its inherent risks and annual maintenance needs. There is a need to develop viable alternatives for long-term conservation.

There are efforts being made to secure tea genetic resources in situ in Southwest China, but more needs to be done, and this effort needs to be expanded to other key areas of diversity for the cultivated and wild species. There is a need to also consider in situ or on farm conservation for old seedling gardens in many countries. Long-term conservation using in situ approaches will also require the establishment of monitoring systems to manage the significant risks to these genetic resources in nature.

Tea and its genetic resources are vulnerable to challenges such as climate change, biotic threats, land use changes, fluctuating market prices, local labor costs and shortages, increased uprooting and replanting with clonal cultivars, and other changes in the tea sector. Mitigating these risks will require much greater global collaboration on genetic resource conservation and use to secure collections with greater accession level information sharing, secure monitoring systems, and mechanisms to respond to significant threats to key collections or centers of diversity.

Tea genetic resources are recognized as very important for addressing constraints as well as

opportunities for the future. The genetic resources have been effectively utilized in the past but only a small amount of the diversity has been used or evaluated. A survey of users indicated that genetic resources for tea are important for breeding, research, and direct use in production fields. This germplasm is mainly acquired from an institute's own collection or from other collections in its country. The genetic diversity of cultivars released or in farmers' fields were not viewed as adequate for future genetic enhancement or production.

There is very limited exchange of germplasm internationally. The main constraints to exchange seem to have been lack of knowledge of accessions held and not policy. Currently, there is no reported accession level sharing of passport, characterization, or evaluation data among collection holders or with users. There is also no reported organization or institution with an objective of facilitating regional or global collaboration among collection holders or among breeders/researchers. There may be formal mechanisms to facilitate collaboration and knowledge sharing within countries, but none have been found in the background review. Despite these constraints, users are finding their traits of interest, especially for research on tea. The development and availability of core subsets and the wider scale genotyping of collections offer many opportunities to increase the evaluation and use of conserved genetic resources by all the various users.

In conclusion, mitigating the loss of tea genetic resources will require much greater collaboration of collection holders, producers, processors, national tea boards, national governments, and the global tea sector. Currently, this does not seem to be a priority in the few global forums for the tea sector, such as the FAO Intergovernmental Group on Tea or the Ethical Tea Partnership. There is no platform for international sharing of documentation at the accession level or for international collaboration on issues related to conservation and use. There is very limited exchange of germplasm internationally and no current multilateral discussion on policy options to facilitate greater international exchange. There is a recognized value of greater use of genetic resources, but currently there are significant barriers to the effective use of conserved diversity in tea breeding programs beyond institutes' own limited collections. Thus, facilitating the development of a global system for the conservation and use of tea genetic resources that is rational, costeffective, sustainable, and secure for the long term is required.





# SURVEY OF COLLECTIONS OF TEA GENETIC RESOURCES

Most of the tea-producing countries have national, provincial, or local institutions that maintain collections of tea genetic resources. Private companies also maintain their own collections as part of their research programs. There is very little known about the status of the conservation of tea genetic resource collections globally, beyond what is in the country reports in Chen et al (2012c). Thus, a survey of the status of tea genetic conservation ex situ was carried out. A survey tool was developed and sent initially to 21 institutions in 15 countries in March 2018. These included national, regional, and local research institutions, universities, and the private sector. We obtained responses from 13 collection holders. The details of the institutions who responded are given in Annex 1.

Table 3 compiles assessments of the number of accessions of tea genetic resources held by institutions given in Chen et al (2012c), FAO (2018d), and the 2018 survey. In addition, Chen et al (2012c) estimated that the total number of accessions conserved in China by national and provincial tea germplasm repositories was at least 10,000. Taking all these estimates into account, there are currently nearly 26,000 accessions of tea conserved globally in ex situ collections. The survey respondents hold about 57% of these conserved accessions and the respondents represent major collection holders from China, Japan, India, Sri Lanka, and Kenya. The survey also included a sample of collection holders from all the major producing countries and thus was

a good representation for assessing the status of the conservation of tea genetic resources. The only significant gaps were from the Tea Research Institute in Vietnam and the Horticultural Research Institute in Thailand, given the potential uniqueness of genetic resources from those countries. We were also not able to determine whether there is any institution holding a collection of tea genetic resources in Myanmar, so this was also a gap in our assessment.

The oldest collection in the survey was established in 1911 and the youngest was just re-established in 2016. The total number of accessions held by all the institutions in the survey at their founding was 2,574, but today there has been more than a seven-fold increase in the number of accessions, mainly from local collection activities and not acquisitions from other collection holders.

## **Collection composition**

Survey respondents were asked to describe the composition of their collections in terms of the various species, botanical varieties, and species or variety crosses separately (Table 4). The classes included accessions that were local landraces in origin and mainly collected from seedling fields in the area; elite cultivars or clones; genetic stocks and breeding materials; and accessions that were something other than these three classes. Nine of the 13 respondents indicated the number of accessions within each class for each species or

Table 3. The number of accessions held in *ex situ* collections of tea genetic resources as reported in Chen et al (2012c), FAO (2018d), and in the 2018 survey.

Collection	Chen et al (2012c)	FAO (2018d)	Survey in 2018
Cocoa Research Institute of Nigeria	24		
Ataturk Tea and Horticultural Research Institute, Turkey	64		
Indonesian Research Institute for Tea and Cinchona	600		
Bangladesh Tea Research Institute	386	474	
National Institute of Horticultural and Herbal Science, Republic of Korea			631
Boseong Tea Experiment Station and Mokpo Experiment Station, National Institute of Crop Science, Republic of Korea¹	5,630		
Tea Research Institute, Vietnam	180	2,500	
Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization, Japan	4,000	7,312	6,500
Tea Research Institute, Kenya Agricultural & Livestock Research Organization	250		270
Tea Research Institute of Sri Lanka	500	560	540
Tocklai Tea Research Institute, Tea Research Association, India	2,200		
Tea Research Institute, UPASI Tea Research Foundation, India	1,250	567	
China National Germplasm Tea Repository <sup>2</sup>	3,000		2,806
Tea Research Institute, Fujian Academy of Agricultural Sciences, China			3,000
Tea Research Institute, Guizhou Academy of Agricultural Sciences, China			1,100
National Agricultural Technology Institute, Argentina		189	203
National Plant Genebank, Iran		50	
Citrus and Subtropical Fruit Research Institute, South Africa		28	
Horticulture Research Institute, Thailand		34	
Tea Research Foundation Central Africa, Malawi			184
Total	18,084	11,714	15,234

<sup>&</sup>lt;sup>1</sup> Estimated from the total numbers of accessions given in Jeong and Park (2012) for various periods of germplasm collection at the two experiment stations. <sup>2</sup> Estimated from the total given in Chen et al (2012c) for the Repository, which is maintained at the Tea Research Institute, Chinese Academy of Agricultural Sciences (TRICAAS) in Hangzhou. The tea genetic resources preserved in the Tea Research Institute, Yunnan Academy of Agricultural Sciences (TRIYAAS) in Menghai county, Yunnan is not included

Table 4. Number of accessions held by survey respondents of various species and botanical varieties.

Species, variety or cross	Wild	Local landraces	Cultivars or clones	Genetic stocks or breeding materials	Others	Total
C. sinensis var. sinensis		4,225	2,192	2,218	2,342	10,977
C. sinensis var. assamica		2,339	230	660	795	4,024
C. sinensis var. pubilimba		51	3	12	111	177
C. sinensis var. assamica ssp. lasiocalyx		168	49	179	0	396
C. sinensis var. assamica x var. sinensis		51	39	603	100	793
C. taliensis	138					138
C. crassicolumna	48					48
C. tachangensis	24					24
C. gymnogyna	26					26
C. irrawadiensis (C. taliensis)	7					7
Other Camellia species	340					340
Non-Camellia related species	56					56
Overall	639	6,840	2,513	3,672	3,348	17,006

botanical variety, while three respondents just indicated that they held accessions of this type.

Over all the collections, 39% were local accessions from *C. sinensis* var. *sinensis*, var. *assamica*, and var. *pubilimba*. The majority of accessions held were var. *sinensis* (65%) but only about 40% of those were local landraces. On the other hand, about 60% of the var. *assamica* accessions were local landraces. Cultivars or clones and breeding material accounted for about 37% of the accessions in these collections. Both var. *pubilimba* and the wild species would seem to be under-represented overall in the sample of collections surveyed.

The composition of the collections was also compared in terms of the number of institutes who conserved the various species, botanical varieties, and types of accessions (Figure 1). All of the survey respondents conserved accessions of var. sinensis, mostly as locals and breeding lines. Overall, 11 of the 12 respondents conserved var. assamica. Only one respondent conserved accessions of var. publimba. Wild species were only conserved by four of the respondents.

Respondents were asked about the source of the accessions that they conserved. The local nature of collections is evidenced when they are compared in terms of acquisitions coming from within versus outside the institute. For local landraces, four of the 11 institutes had only their own collections. Only one institute had the majority of its local

landrace accessions acquired from outside. For cultivars or clones, there was more germplasm exchange: two of the institutes only conserved their own collections, and three institutes had less than 2/3 of their cultivars or clones acquired from outside. For the tea related wild species, three of the institutes had only their own collection and one institute had only accessions acquired externally. The vast majority of the respondents conserved accessions that had been collected or acquired by their own institute, with the highest proportion of accession types acquired from others being in the wild species. Generally, the results of the survey indicated that there was little duplication or redundancy among the survey respondents.

The respondents were asked what part of the collection makes the collection important or unique. The responses included:

- Specific traits or elite accessions with high tea polyphenols content, high and low caffeine content, or high amino acids (theanine) content; white, yellow, or purple leaves; and morphological leaf traits like pubescence, size, and pose.
- Accessions of wild teas as well as local landraces, specifically from ancient tea plantings; original stands of var. assamica from Northeast India; and old seedling fields (jats), many of which have been uprooted and replanted with clones.

Figure 1. Number of survey respondents who conserve different classes of accessions in different species and botanical varieties.

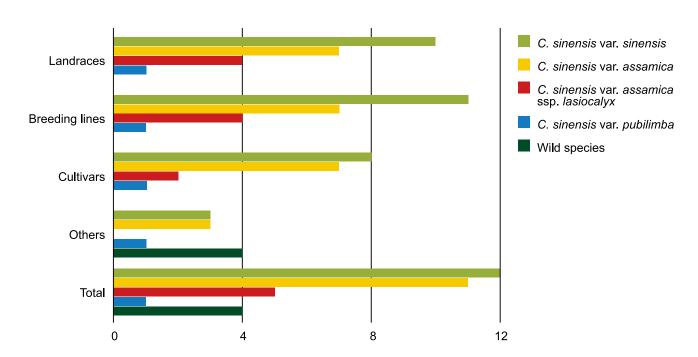


Table 5. Sources of accessions in 11 tea collections: internal (collected or acquired within the institute) and external (acquired from another institute)

Type of accessions	Institutes holding internally sourced collections	Of these, average share of accessions internally sourced	Institutes holding externally sourced collections	Of these, average share of accessions externally sourced
Local landraces	11	80%	7	21%
Genetic or breeding materials	11	96%	6	9%
Cultivars and clones	11	75%	9	33%
Tea related wild species	6	96%	3	58%
Non-tea related species	2	100%	2	100%
Duplicates from other collections	3	70%	6	30%
Others	1	100%	0	

They were also asked about gaps in their collection, and if they had plans to fill these in the future from acquisition or collection. All the respondents indicated they had plans to fill these gaps in the next 10 years, but a few indicated that this was dependent upon the availability of funds. The specific gaps identified were:

- More accessions of populations of wild tea species, especially from Yunnan and adjacent areas.
- ▶ More accessions from outside institutes.



- ▶ Abundant resources of species such as C. tachangensis, C. crassicolumna, C. taliensis and C. gymnogyna which need to be preserved and utilized.
- ► More var. *sinensis* type accessions.
- ▶ More collections of diversity in farmers' fields.
- Collections from seedling tea fields in tea estates in Malawi that have not been sampled.
- More genetic variability from the tea regions of Nilgiris (India) and St. Coombs (Sri Lanka).
- Old estates planted prior to 1950, which are being encouraged to maintain small patches of original tea when replanting rather than these being established in collections – but these have to be sampled in India, Sri Lanka, Kenya, and Malawi.
- ▶ Wild stands of var. assamica which need to be further sampled before they are lost in India.
- Original var. sinensis brought from China by original tea planters and established in fields in South India.
- Unknown diversity in old fields from original seed sources in Manipieri and Dahalli, India: specifically, encourage pluckers to identify bushes that have good traits such as drought tolerance to be cloned and conserved in germplasm gardens.

## Management of the collections

The total area allocated for tea genetic resource conservation by the research institutes surveyed ranged from 0.25 to 5 hectares. The annual budget allocation for the conservation of the accessions ranged from 800 to 80,000 USD, but some collection holders reported there was no specific separate allocation for conservation. All but one respondent indicated the collections were held 100% in field collections. That one respondent indicated that 4% of the collection was cryopreserved.

The respondents were asked which of their routine operations had established protocols (Figure 2). All the respondents had an established protocol for characterization, but only two had a protocol for safety duplication. In terms of key operations for conservation, the majority of the institutes had protocols established for clonal propagation, field maintenance, collection of new acquisitions, and pest/disease control. These results indicate that while there are established protocols for most of the key conservation operations in most institutes, there are significant gaps for operations such as distribution and safety duplication.

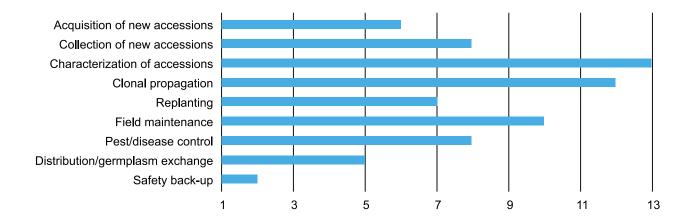
While there was little emphasis placed on safety duplication in terms of established protocols among the respondents, there were five institutes who maintained their collections at 2-4 distinct sites. There were various reasons for having multiple sites. For example, it allowed the collection holder to take advantage of better local adaptation. It was also a way to mitigate the risk of loss. In some cases, there was a lack of adequate availability of land at one site to maintain the entire collection. In other cases, the institute had relocated to another city but was maintaining the original site. There were historical reasons why the germplasm collection was maintained in multiple sites, but in one case, there was a shift going on to centralize the collection at a single site. The lack of focus on safety duplication and the use of a single field site is an indicator of insecurity for long-term conservation. Institutes need to consider mitigating these risks as a priority in the future. One option that needs to be better explored is cryopreservation, but also strategies for greater safety duplication.

To better understand these key operations for conservation, the respondents were asked about the frequency of the operation (Figure 3). The key routine operations for most of the institutes were to weed, prune, and fertilize the accessions in the field. Pest control and cloning were done routinely or occasionally in most of the institutes. Plucking and replanting were done rarely or never in half the institutes. From this assessment, it is clear that the key operations are mainly focused on ensuring normal growth and longevity of accessions in the field.

One other important issue for securing the accessions in the field was the number of plants established per accession. Maintaining an adequate number of plants in the field needs to consider mitigating the risk of loss of an individual accession as well as the loss of genetic diversity within the accessions. The survey respondents indicated they maintained between 2 and 25 plants per accession. In almost all cases, there was a constraint for land that did limit the number of plants they could maintain, and this was balanced with the need to secure the accession from being lost completely. Two respondents indicated that technical guidelines had been developed that recommended 20–25 plants for each accession, where five of the plants were left as natural growing trees. One respondent indicated that the number of plants per accession was based upon having sufficient numbers to allow for use in hybridization, characterization, and evaluation. So it seems that the number of plants per accession was mainly determined by institutional needs and land availability. In the future, greater consideration of maintaining diversity among and within accessions for the long term would benefit from technical guidelines developed by the global community.

Another key issue for securing accessions for the long term is replanting intervals. Replanting has an established protocol and is done routinely or occasionally by about 50% of the respondents. In some cases, replanting is done to fill gaps in

Figure 2. Number of the respondents who had established protocols for specific operations in the conservation of their collections.



accession plots or to establish a new field. The shortage of land could result in very little uprooting and replanting in the future. Thus, it could be a very important operation that needs to be considered for the future. The survey respondents were asked to describe the age of the trees in their accessions in terms of age distribution (Figure 4). Four collections had mainly young, newly established plants for 75–100% of their accessions. Only two collections had a notable share of plants older than 31 years - but these still accounted for less than 20% of their accessions. So while aging of plants could be an issue in the future, it is not one that needs to be urgently addressed. There is a need to establish technical guidelines for replanting that can be used for collection maintenance in the future.

The survey respondents were asked questions related to staffing and funding for the maintenance and management of the collections. For maintenance and distribution, there was a wide range of staff allocation across the respondents, from 3 to 10 staff at various levels. In some cases, the staff were not allocated fully to the tea germplasm gardens, but this was a routine task for the research program's staff. No respondent indicated that there was specific staff training in germplasm conservation or management. There were some issues with staff turnover for some of the respondents, but it was not clear whether this was due to inadequate funding, the short-term nature of research project funds, or the priorities of the institute. Staff retention, training, and succession

Figure 3. Numbers of respondents who categorized the frequency of specific operations as "routine", "occasional", "rare", and "never".

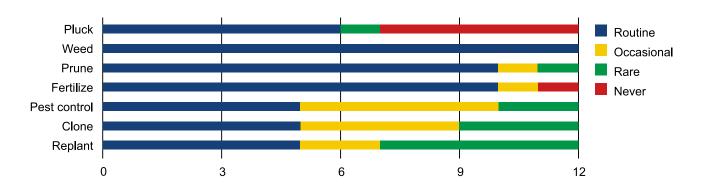
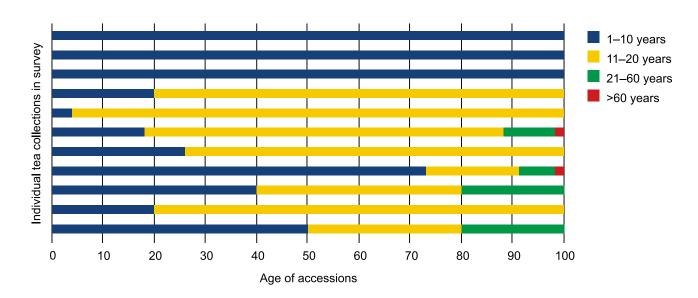


Figure 4. Proportion of accessions with tea plants from 1–10 years old, 11–20 years old, 21–60 years old, and greater than 61 years old for each respondent separately.



can be key sources of risk for long-term conservation since inadequate staffing, poorly trained staff, and new inexperienced staff with no clear handover of knowledge on the collection, its history, and its maintenance can lead to loss of accessions and knowledge for the future users. The status of staffing for conservation is not clear among these respondents and needs to be considered as a priority for securing the collections' sustainability.

Another source of risk for a collection can be inadequate annual funds to ensure routine maintenance and distribution of the accessions. Overall, most of the respondents indicated that the host institute provided for annual recurrent costs, although in some cases this was from research project funds. The budget was stable for eight of the 13 respondents, while two reported increased funds and one reported a decrease in funding. The respondents were asked about other sources of funds for the conservation of the collection. Six respondents indicated that there was no additional source of funds currently for the collection, while two respondents had funds from research projects, two respondents indicated that additional funds were allocated from their designation as the national conservation site for tea genetic resources, and only one respondent indicated that funds were raised from the sale of cuttings and plucked tea leaves. So while conservation is adequately funded for most of the collections, it does rely upon research projects and strong institutional support.

Assessing risk and taking careful actions to mitigate or reduce its likelihood or impact is a critical need for the long-term maintenance of tea in field collections. The survey respondents were asked questions related to risks to their collections and the actions being taken to mitigate these. The most frequently sited primary risks for the respondents were abiotic threats such as drought, high temperatures, and frost; pest and disease threats in the field; inadequate funding; and extreme weather events. Individual respondents identified unique primary threats such as loss of expertise from retirements of key staff, natural disasters, land use changes, the shift to clonal tea, the increased use of machine harvesting, and finally, damage from wild elephants and bison. Thus, the respondents were managing a number of threats that they could mitigate through management, but there were others that would require other options such as safety duplication in additional sites or through cryopreservation. For example, the respondents identified 21 pests and diseases that were threats. These were mainly being managed through chemical or biological controls with integrated pest management, such as sticky boards and light traps.

The most difficult biotic threats to manage for the germplasm gardens were those that cause stem death or tree senescence. Some of these would require relocation of the garden, and that was not always possible.

One key action to mitigate risk for conservation is safety duplication. There is very limited use of multiple sites or cryopreservation by these respondents. There is also very little redundancy or duplication among the accessions between the respondents. This could mean that there are many unique local accessions held as a single plot in one tea germplasm garden. This is a very risky strategy for the long term. If one tea germplasm garden is lost to one of the many threats, it could result in the complete loss of that diversity, especially given the status of old seedling fields and wild stands. Thus, effort needs to be made urgently to develop a global strategy to secure these collections through safety duplication in other field sites or through alternatives such as cryopreservation.



#### Utilization of the collections

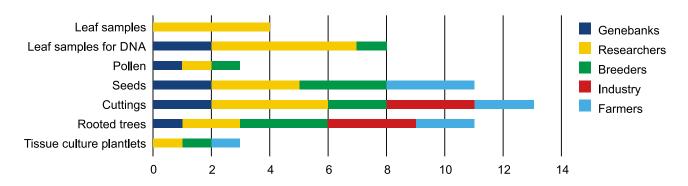
Conservation of tea genetic resources depends upon meeting the needs of users in terms of distribution of plant material and availability of relevant accession level information that allows users to select accessions for their own use. The relevant information should include phenotypic and genotypic descriptions as well as evaluation for important traits. To assess the status of the use of the collection, the survey respondents were asked questions related to distribution, information systems, genotyping, and evaluation. All the respondents indicated that they distributed accessions. Eight respondents did only internal distribution within the country, while two did external distribution to recipients in other countries. The reasons given for no external distributions included a lack of requests from users as well as lack of exchange or research partnerships outside the country. This could be due to the lack of knowledge about the collection and the accessions conserved.

There were a number of different types of plant material distributed by the respondents. These included tissue culture plantlets, rooted plants, cuttings, and seeds for establishment of bushes by the users (Figure 5). They also distributed pollen to be used for hybridization as well as leaf samples for DNA extraction and quality assessments. There were five groups of users for the conserved germplasm. These were other genebanks or collection holders for conservation; researchers and breeders; the private sector or industry; and farmers or farmer organizations. Only leaf samples were distributed to recipients (researchers) outside the country, and this was mainly to extract DNA for diversity assessments. Figure 5 illustrates a summary of distributions within the country of the different types of plant material across the respondents to the various user groups. The most frequent distribution was cuttings, then rooted plants and seeds. Cuttings and rooted

plants were distributed to all five user groups, but seeds had not been distributed to industry. Tissue culture plantlets was the least distributed form for establishment, and only to researchers, breeders, and farmers. Pollen and leaf samples for DNA extraction were only distributed to genebanks, researchers, and breeders. Leaf samples for quality assessments were only distributed to researchers. Thus, distribution within a collection is complicated by the number of different forms needed for the various users. This requires investment into propagation as well as field management for harvesting of cuttings and seed from the accession plots for direct distribution to meet diverse user needs.

The limited distribution of accessions to some user groups or to users outside the country could be due to constraints in accessing the accessions; availability due to germplasm health; or knowledge of accessions from a lack of information being available to help the users in selecting accession for use. In the survey, we assessed both the terms and conditions for access and the accession level information system. For all the respondents the institute was the legal owner of the collection, but in three cases the management involved another agency as well. Twelve institutes indicated that they did distribution within the country, and eight could theoretically do distributions outside the country. These external distributions were done or could be done under the terms and conditions of an institutional material transfer agreement (nine respondents), a research collaborative or consortium agreement (six respondents), the Nagoya Protocol (five respondents), and the ITPGRFA (seven respondents). There were a number of the respondents who were not familiar with the terms and conditions for distribution. Generally, there seemed to be a willingness to distribute plant material within and outside countries to users and there were many options available for accessing the accessions that could be used. The limited external

Figure 5. Number of respondents who distributed specific types of plant material to genebanks, researchers, breeders, industry, and farmers or farmers' organizations within the country.





distribution reported by the respondents would indicate that there is a lack of clear, transparent, and consistent ABS terms and conditions. This is an area of great concern for long-term secure conservation and use, given the importance of germplasm exchange globally to meeting the local challenges that tea producers will face in the future as well as the many new opportunities for tea sector development.

One key prerequisite for enhanced use of collections is the availability and ease of access to accession level information. This information could be in various forms, but it does need to consider the needs of the users as well. To ensure ease of access, it should be shared as widely as possible publicly, it should be in a searchable form, and it should be available on a global platform to allow users to access information across collections. One example of a global portal for sharing accession level information is Genesys (http://genesys-pgr.org), which currently hosts information on 3,883,328 accessions from 458 institutes across many crops. To assess the status of accession level information, the survey questions addressed issues related to availability, access, and the types of information taken on accessions. The respondents were asked about the availability of accession level information in searchable databases. Five of the respondents had passport data and six had characterization data. Three of the respondents (from China, Japan, and Argentina) had information available publicly to both internal and external users on the internet. This is less than a quarter of the collection holders, although six of the respondents

did indicate that their information was available internally, from a catalog, or from the expertise of the curator. The information on accessions included passport (four respondents), taxonomy (four respondents), characterization (five respondents), images (two respondents), and genotype (two respondents). So, there is limited availability and sharing of accession level information currently for the respondents to the survey. This should be a priority for enhancing the use of the collections in the future, and would be a key benefit from global actions.

For most crops, users are interested in knowing about the value of an accession to their current research or breeding program as well as the production system. There is also an interest in knowing about diversity at the molecular level, especially if this can be linked to specific genes or traits. All of this information increases the probability of utilizing an accession for improving specific traits or increasing hybrid vigor in the resulting progeny or in the field. This will also lead to greater gains and reduced time in developing new cultivars to meet future challenges and opportunities. Investments into evaluation, genotyping, and the sharing of this information can have benefits for the local as well as global community. The survey requested information on the status of evaluation of accession in the collection. Nearly all the respondents (92%) indicated that they were evaluating accessions for specific traits. These traits included germination period in early spring, yield, pest resistance, quality, harvestability, shoot color, drought tolerance, heat tolerance, and frost tolerance. In

most cases, a small number of accessions were being evaluated. Field observation and scoring in the field was the most common method used for the pest resistant and tolerance to abiotic stresses. Yield was determined by taking green leaf weight from regular plucking. The quality assessment involved sensory evaluation of cup tea, HPLC (High Performance Liquid Chromatography) and biochemical analysis for specific metabolites.

All the respondents indicated that they had ongoing genotyping with molecular markers, although some were still pending implementation until funds were available. The most frequently used markers were SSR, AFLP, and SNP. A number of the assessments have been published. The total number of accessions genotyped was 3,253, or about 20% of the accessions held by the respondents in the survey. This ongoing genotyping effort needs to be expanded, as it promises additional benefits for collection management as well as enhanced use.

#### Summary and future needs

The respondents to the survey of the conservers of tea genetic resources represented a significant portion of the known conserved germplasm. Since the collections were established, the respondents have seen a seven-fold increase in their number of accessions, mainly due to local collections. Overall, the ex situ collections were mainly local in nature with very little duplication or redundancies due to germplasm exchange. They mainly conserved local landraces, cultivars, and breeding material from var. sinensis and var. assamica. The majority of collections conserved accessions that were collected by, or acquired from, their own institutes. The respondents concluded that their collections were unique because

Plucking of purple tea field in Pu'er, Yunnan Province.
Image: Paula Bramel/Crop Trust

they held accession with valuable specific traits as well as local landraces and wild species from sites at risk of loss. A number of significant gaps were identified for collection within the next 10 years. These were focused on sites or populations at risk of loss as well as under-represented wild species and *C. sinensis* var. *pubilimba*. There was also a target for acquisition from germplasm exchange to fill institutional gaps in terms of unique diversity. So, the current *ex situ* collections are projected to increase in accession number in the future, when they will hold more diversity that is being lost in the field or forest.

The respondents conserved their accessions in field collections, mainly at one site. There was very limited use of alternative conservation options such as cryopreservation. In terms of operations, protocols had been established for key operations to ensure plant health, normal growth, and longevity in the field. There was a general lack of protocols for distribution and safety duplication. Some key issues, such as the number of plants per accession, the replanting rate, and safety duplication protocols, would benefit from further technical discussions and the establishment of technical guidelines through global action.

The survey identified some key risk for *ex situ* collections of tea genetic resources. Mitigating these risks will require staff succession planning and training on conservation as well as stable, adequate annual funding for routine operations to secure collections for the long term. Effort needs to be made urgently to develop a global strategy to address the need to secure these collections through safety duplication in other field sites or through alternatives such as cryopreservation. Managing risk will require local, national, and global actions but a global conservation system will facilitate the short-term and long-term mitigation.

Overall, the survey of the use of collections found that most distributions of the accessions are within the country. The respondents are actively evaluating and genotyping their collections to enhance use. The main constraint to greater use in the future is the lack of accession level information being shared more widely in a form that better meets users' needs. This limits germplasm exchange and effective use that has resulted in few requests for accessions externally. Furthermore, the lack of clarity or consistency in the terms and agreements for ABS is also a constraint to the use of these collections. Meeting the challenges and opportunities for the tea sector in the future will require more exchange of germplasm and greater genetic gains from its use. Thus, enhancing the use of conserved germplasm needs to be a key focus for the global system.



## SITE VISITS TO KEY TEA GENETIC RESOURCE COLLECTIONS

### China

Visits were made to collections held at the Tea Research Institute, Chinese Academy of Agricultural Sciences (TRICAAS) in Hangzhou and the Tea Research Institute, Yunnan Agricultural Academy of Sciences (TRIYAAS) in Menghai county, Yunnan. A visit was also made to Pu'er tea production areas to visit tea gardens, ancient trees, and tea producers. In all sites, the conservation was field or forest based. Accessions were maintained in pruned and plucked gardens in Hangzhou and Menghai, and also as natural trees in Menghai. The accessions were only replanted when required and mainly just to fill gaps. They used accessions in the germplasm gardens for hybridization through hand pollination. There was no ongoing research on alternative long-term options for conservation such as cryopreservation or seed storage.

In TRICAAS, there is an internal accession level database that is partially shared with CAAS centrally, and a system is being developed to share this publicly from the CAAS website. Genesys could be an option to share data globally in the future, but it would have to be through linkage to the CAAS system. There are agreed descriptors for characterization, as documented in Chen et al (2008). These were originally developed by IPGRI (1997), but in their original version were not very helpful. Now, they are aligned with UPOV distinctness, uniformity, and stability (DUS) testing (Chen et al, 2008) for national

tea variety protection or cultivar registration. There is a significant issue with data sharing for regional or local collections of significance in China. TRICAAS has established technical protocols and standards, at the level of the Ministry of Agriculture and Rural Affairs and above, for tea germplasm descriptors, evaluation, cutting propagation, DUS testing, SSR identification, plantlet quality, etc.

Germplasm distribution is internal in the institute and within China. They have had an exchange with Kenya in recent years as well. They use an institute material transfer agreement to clarify the terms and conditions. Phytosanitary constraints are not an issue within China. Dried leaves for DNA extraction and seeds are the easiest materials to distribute, with rooted plantlets and cuttings being more complex. Tea has been recognized as a national heritage treasure, so there is protection of local cultivars in farmers' fields and local tea germplasm gardens. Farmers do visit each other and take cuttings to try in their own fields. There is resistance to variety protection and licensing fees, even for farmers' own selections.

Germplasm is used directly as selection from seedling accessions or in hybrid combination. There is limited diversity found in crosses of var. *sinensis* x *sinensis*, although it is common to use a resistant *sinensis* source crossed with the best *sinensis* variety to develop a hybrid cloned cultivar for further testing. Hybrids of var. *sinensis* x *assamica* give

higher genetic diversity for selection with some good quality selections and unique traits. Hybrids with wild *Camellia*, initially with closely related species, will give diverse progeny, but selection is for specialty traits, so this is not commonly or routinely done.

There are many collaborations within TRICAAS across disciplines. There are many opportunities to collaborate within the region or locally with the Ministry of Agriculture and Rural Affairs and farmers' cooperatives, especially with the demonstration and evaluation of new cultivars. CAAS also has collaboration across crops, with data being managed centrally, as well as in annual meetings for the National Crop Germplasm Repositories. There is not, however, enough collaboration across tea germplasm gardens in China. There are no central coordination, oversight, monitoring, or reporting arrangements for all tea germplasm across China that would better secure conservation. Each institute has its own agenda or plans that are not shared or aligned. There are some standard protocols or research efforts across collection holders, but it is not so strong across different institutions. Outside of China, there are bilateral or regional groupings of tea researchers. There is no international institutional forum to facilitate research or germplasm collaboration internationally. Thus, ensuring longterm conservation of tea genetic resource is not currently in any international institution's mandate, and while genetic resources are viewed as being of value, they are not a priority currently.

There is complementarity between *ex situ* and *in situ* conservation in China, since there is significant diversity still available in farmers' fields and the forest. This diversity is not currently threatened since there is a high market value for local landraces,

Field genebank for TRICASS in Yunnan Province, China. Image: Paula Bramel/Crop Trust ancient tea plantations, and wild teas. Consumers in China already favor and pay for specialty teas based on local diversity. This can be seen in the core Pu'er tea production system where ancient plants are cultivated and regenerated from seeds that are dropped naturally. Locally, there are seed and seedling nurseries, so the diversity is being used for replants and establishing new fields in local areas. There is also a high market value for Pu'er tea, so as long as this continues, farmers will use and maintain diversity in the core center of diversity. There are risks from oversupply that might reduce prices; marketing of poor quality Pu'er tea that impacts on the branding; or any changes in government policy on planting tea versus other crops. The risk from pests is small, as is that from extreme weather events, since the plants are highly resilient.

In other traditional production areas and local tea types, high genetic diversity is maintained in established fields with farmers. The market favors local seedling fields, so there is very little interest currently to replant with clonal teas. There is widespread use of improved clones, particularly in newly developed fields, as the government is promoting a shift to improved clonal tea cultivars for planting, although some local authorities favor seedling tea. There could be a risk to this diversity in the future if there was a shift in the market to favor increased and lower cost production, or the emergence of a high value specialty market for a new high-quality clonal tea that would result in replants of established fields with clones. The national. regional, and local collections maintain diversity in demonstration or germplasm gardens. So, the current conservation system in China is secure but might not be rational, cost-effective, or sustainable for the longer term, and it is also not used widely, since sharing of germplasm and information is very limited.

The various discussions in China lead to the following conclusions on the future global system. There needs to be greater collaboration on tea genetic resources conservation and use globally and nationally, with a focus on sharing information, sharing expertise, and resolving policy constraints for germplasm exchange. Greater opportunities for collaboration would allow global collective actions on issues such as technical best practice guidelines for more secure collection management; better inventory of collection holders; global genotyping efforts to map diversity, identify gaps, and rationalize collections; responses to emergency threats to key collections or in situ sites; core collections to enhance use; advocacy for sustained support; research on diversity conservation; joint evaluations of germplasm; and more research on options for safety duplication, such as cryopreservation.

#### India

Visits were made to the Tea Research Association (TRA) at the Tocklai Tea Research Institute in Jorhat. Assam, and to the UPASI Tea Research Foundation at the Tea Research Institute in Valparai, Tamil Nadu. At the TRA, the collection conserved includes old accessions; accessions collected from wild patches from the Indo-Burma region; accessions introduced from China, Japan, and Korea; and improved clones from UPASI and the Tea Research Institute of Sri Lanka (TRISL). There are also accessions derived from selections in old tea gardens on estates that were planted prior to 1950, when clone propagation was initiated. When old seedling gardens are to be uprooted and replanted, TRA is notified so they can assess the garden and encourage the estate owners to maintain some areas of the seedling fields for the future. There are also seed gardens established by seed sellers or planters where they have used at least 100 parents and harvested seed from these gardens to get new plants for replanting.

For the tea improvement program, conservation of genetic resources is the first priority. The management of the accession in the field is minimal and does not differ from the breeding program. They have dedicated staff and resources for conservation, but they are undergoing staff changes, which introduces a risk of loss of knowledge on accessions. They have a database for the accessions that includes characterization and evaluation using the Plant Variety Protection – Farmers Rights Act (PPV-FRA) criteria and DUS. The database is only available internally. They also need to digitize passport data that this is currently held in a two-volume book that is the entry journal for all the accessions in the collection.

The land available for tea plantings is limited within the institute, so one key concern for longer term conservation at TRA was the availability of enough land to maintain the number of plants per accession recommended, since the land area in the station is limited. They also need to have a separate, dedicated, and protected site for the germplasm gardens. They have had to move the accessions in the past when the institute has changed sites. This needs to be avoided. They currently get around some of these limitations with their encouragement of estates to maintain portions of old seedling gardens. They have a reliance on one ex situ site and in situ sites that are not monitored. They have no safety duplication. The use of alternative conservation approaches needs to be considered. They concluded that there was a need to keep accessions in at least two sites for security, with the use of molecular markers to check if changes occur



over time. Thus, this could be an *in situ* site as well as *ex situ* conservation in a dedicated site at TRA.

Since they have a collection focused on a key area of original diversity, they have had to assume that they need to maintain a sample in their germplasm garden and ensure diversity will be maintained in situ and can be sampled as needed. They conclude that their ex situ collection is a sample of the diversity from wild stands and old seedling fields as well as special types selected from tea gardens. They recognize there is a risk of loss of var. assamica germplasm from stands conserved by local communities in the tribal areas. They have a plan to do more collections from these areas, but they also recognize a need to ensure ex situ conservation as a compliment to in situ conservation. More effort needs to be made to inform and educate the communities who manage the "wild" stands of original and unique var. assamica, as well as policy makers, to better ensure the long-term conservation. Generally, any risk that will impact on the tea gardens will affect the ex situ collections, since they are only conserved

in the field. Some key risks for the local genetic diversity and the genebank are land conversion to other crops by smallholder farmers or estates, the abandonment of the estate tea gardens, and the significant challenges occurring with changes in the climate. A better understanding of the diversity still held in the old seedling field gardens, the wild stands, and in the genebank will allow more rationalization and a focus on management of the unique diversity for the long term.

The greatest use of the diversity in their collection has been for direct selection of new cultivars. In the use of the collection or in the development of new varieties, the key parameter is quality, and it takes 8–10 years to determine the quality. The planter will decide on new cultivars based upon quality assessments. There is also a focus on drought tolerance, using water use efficiency as the key parameter. TRA also have a project to use molecular markers to identify candidate genes related to drought tolerance and quality. They need to make greater use of molecular markers for genebank management to determine if there is redundancy among the accessions. One suggestion for action by the global system is to create a set of accessions from all collection holders that can be shared widely for use to increase overall diversity in breeding pools and to foster greater collaboration for conservation and use.

UPASI has been designated as the 41st National Active Germplasm Site of the National Bureau of Plant Genetic Resources (NBPGR) for tea genetic resources. They work collaboratively with TRA to conserve accessions. Technical guidelines were developed by two experts for the conservation site that set the optimal plot size, design, and field management to maintain genetic integrity within the minimum population size for each accession. For example, UPASI maintains 25 individuals in each accession in two replications in the same field. Five of these plants are maintained naturally. They have 17 years of funding for routine maintenance from NBPGR. They have completed the establishment of 50-60 accessions in the conservation gardens in seven years following the technical guidelines. The collection is governed by the PPV-FRA of 2003. Funds were allocated to the research station to reestablish the genebank on one site in 2011; the collection had been started in 1962 but at several sites. They now hold 600 accessions in total in their own collection where they do regular plucking and production of tea for income.

The use of these collections in the breeding programs has been limited, although it is recognized as a resource where investments are made in characterization and evaluation for traits of interest.

For the breeding programs, early priority was given only to yield improvement, but with the acute shortage of labor in the tea production fields and the adoption of mechanization, there has been a shift to breeding clones for machine harvestability with reasonable yield and acceptable quality. They promote the establishment of both clones and seedling teas in gardens to maintain resilience in the fields. Other aspects of their current research and training programs have shifted to mechanization in tea production. There is also increased interest in green tea by consumers, so developing clones for green tea production is a new focus that will require diversity conserved currently as well as new diversity from local fields. Another recent interest is in biological control and host plant resistance for major pests and diseases, due to increasing restrictions on pesticide use in tea production and the increasing value of organic production.

UPASI have an interest in the conservation and use of some of the oldest tea cultivars originally brought to the region, including those established by Chinese who were involved in the original planting of tea estates in the Nilgiri area. They brought them from China, and the original plantings still exist, referred to as "Chinese creeper teas". These have been brought to UPASI by farmers, who are recognized as owners of this germplasm; if the accessions are used, the intellectual property rights belong to the farmers. There is also a need to consider the loss of the old seedling fields, but currently these are only valued as sources of unique or outstanding traits. Longterm conservation is not being considered for the genetic diversity in the old seedling fields. Since the Forest Act prohibits new land being made available for tea, land availability for expansion of the collection in the research station will be difficult for any expansion, as well as the considerable cost for maintenance. UPASI see value in using markers to rationalize the collection to maintain maximum genetic diversity within the current land allocation. Currently, priority is not being given to allocation of adequate funds for the maintenance of the collection. They have some of their accessions in cryopreservation and see value in seed conservation as well.

Germplasm exchange is not an issue within India. TRA and NBPGR are engaged in an ongoing tea genome sequencing project together. Even farmers' varieties can be freely exchanged, and collections can be made from anywhere in India, but international exchange is not allowed at present. Participation in a global system will be difficult under current conditions.

### Sri Lanka

A visit was made to the Tea Research Institute of Sri Lanka (TRISL) in Talawakelle. Germplasm conservation is seen as a priority in the TRISL corporate plan, where it is a project along with prebreeding. They also use the collection in their own breeding program where individual plants are used as parents for biclonal or polyclonal hybridization. The main focus for their effort is the Cambod type with its intermediate leaf shape, height, and number of trunks, although this is closer to var. assamica. There are a number of taxonomic controversies around this type that need to be resolved. They have limited introductions of this type from outside Sri Lanka in the collection and have an interest in increasing this. Use of the collection is going to increase in the future, as there is pressure to reduce the use of pesticides in tea production, meaning the use of host plant resistance as part of integrated pest management will need to increase.

TRISL have derived specialty teas from old growth gardens and developed estate cultivars through intense identification and release of individual selections as cultivars. They realize they have only used a small proportion of the seedling diversity

in these old estate fields. They are now evaluating 150-year-old seedling nurseries for shot hole borer and blister blight resistance. They have developed integrated approaches for both pests, but resistance is still a goal for the prebreeding program. They also have a target to acquire more accessions of wild relatives to screen for shot hole borer resistance.

Quality is still an important trait, but now relating to regionally specific and unique aspects, given the increased market for quality teas. Here, too, they realize they have used a limited number of parents and diversity in the past cultivars, so the focus is on increasing the diversity they are using in breeding programs. They have identified gaps for germplasm exchange outside Sri Lanka, for example, more green tea diversity from China and drought or heat tolerance from Kenya. There is a need to increase exchange of germplasm; however, with many government policies and regulations, exchange has not completely stopped but is very restricted.

They still have an interest in increasing conservation of the old growth fields in the areas of high diversity, such as Uva region, an area of high-quality tea with a unique growing environment. The issue for the long term is whether to conserve *ex situ* or





in situ. They have concluded that many of the unique characteristics are environmentally specific, so they have identified diversity hotspots. TRISL has substations that can be used for ex situ conservation in a more relevant environment. Most of the large-scale plantations need permission to replant, so if in a hotspot, they cannot uproot. They have to negotiate for an alternative option such as a land swap. They are putting a priority on protection of production with advice to maintain a ratio of seedling to clonal tea in fields as a policy.

TRISL are conducting ongoing research on the use of tissue culture in tea. They have concluded that it is suitable for micropropagation but not a viable business for application now. Their main focus has been on its use in the breeding program, since they have found that embryo culture can speed up the breeding process by up to six years and that somatic embryogenesis can be used to increase initial multiplication rates for testing of new cultivars. They conclude that an artificial seed production system is needed for cryopreservation of tea for long-term conservation and for exchange of artificial seed.

For TRISL, one of the key concerns for long-term conservation is the lack of a fully dedicated genebank curator with proper training and focus on conservation as their primary job.

From discussion at TRISL, it was concluded that a global conservation system for tea genetic resources needs to consider:

- Safety duplication and exchange that could be addressed with cryopreservation and artificial seeds.
- A global core collection that would allow for safety duplication of the core and breeding products.

- Sharing of accession level data and information on a central platform to secure data as well as enhance use.
- A policy for exchange and conservation, especially for international exchange.
- ➤ A common platform for resolving taxonomic classification difference as well as descriptors so that a common set is used to enhance the globalized accession level database for sharing information.
- A global genotyping platform to describe and map diversity locally and globally for conservation and use.
- Establishing links for collection and conservation with countries that have no significant tea production but hold unique wild or cultivated genetic diversity in lesser known areas, like Myanmar or Thailand.

### Kenya

Visits were made to the Tea Research Institute, Kenya Agricultural & Livestock Research Organization (previously known as the Tea Research Foundation of Kenya) and to Unilever Tea Kenya Ltd., both in Kericho. The Tea Research Institute has a focus on everything related to tea and the environment, such as production, processing, quality, and adaptation to climate change. The institution has gone through significant changes that have included a shift from funding by a producer levy to a government allocation to Kenya Research Fund and annual replenishment. Management has shifted from the Tea Marketing Board to the Tea Research Foundation Advisory Board. They have their own farm with commercial tea processing, but use smaller cottage processing for novel tea.

The clonal nurseries and evaluation trials as well as the biclonal or polyclonal seed nurseries that are established are kept for long-term commercial use. New clonal planting and evaluation trials are established each year, but never uprooted, so they are finding it more difficult to expand the field areas. There is an advantage to this approach since the old clones and trials can still be used for data collection as well as for harvesting cuttings to meet requests from smallholder farmers and estates. They also have a DUS garden where the germplasm accessions are managed as natural trees. This is an area where they do characterization using DUS for new cultivars as well as the germplasm accessions. Otherwise, everything is managed and plucked for commercial tea production.

In the Kericho production area, the Tea Research Institute does not have any significant pest problem that causes major damage in the tea fields, but will select for resistance as needed. Frost can be a periodic problem. Drought can occur with some scorching but not all cultivars are affected. Hail storms are a risk and can result in no plucking for three months. Rainfall and cloud cover are increasing, with a resulting decrease in soil temperature that is reducing growth. There is an increasing risk from landslides in the Mount Kenya tea production area. They also have a focus on a climate smart strategy for tea production with World Bank funds for climate change adaptation. The other big issue for the future is the increasing cost of production for black cut-tear-curl (CTC) tea, especially for smallholder farmers, with subdivision of land occurring in each generation. The farms are getting too small to be sustainable as tea gardens, so they are being converted into other uses or crops. Tea is a very important contributor to the Kenyan economy, so the government has committed to stop subdivision to prevent a reduction in land used for tea.

There is an increasing focus on specialty tea market development, but it is not easy for Kenya to compete with the current traditional producers of white and green tea. One significant focus for breeding for new teas is purple tea. This is a hybrid of C. irradensis (C. taliensis) with C. sinensis that was an introduction from Tocklai, India a long time ago. They have selected within the cross to get high levels of anthocyanin. They are also interested in developing green tea for Kenya and have been able to get some germplasm from Japan. They also have an interest in acquiring new germplasm of Cambod type tea and new var. sinensis accessions from China with greater drought and cold tolerance. They have established partnerships with other countries to acquire germplasm even today. They also exchange germplasm with the private sector where they have an agreement on a research and development fee or licensing fee. They have material transfer agreements with other countries, but certain material they cannot send. They have exchanges with Malawi and other African countries.

Generally the interest by farmers in new cultivars is very low, so the uptake of their new cultivars is low. Thus, there is a limited use of diversity in new cultivars except for specialty uses. They get advice and clones from the Tea Research Institute which they buy and propagate themselves, or they get them from other private nurseries. Very few farmers have seedling fields. Farmers have nurseries of clonal tea cultivars, but this is not encouraged. It is still illegal to sell seed or seedlings. The Tea Research Institute does have an interest in

collecting germplasm from the old seedling fields in the private plantations before they are uprooted.

At Unilever Tea Kenya, they were committed to conserving the genetic diversity in the original seedling fields that had been established by Brook Bond planters in 1924 from original seed sources from India. They are keeping small patches of these fields when the rest is uprooted and replanted to clones. In the 1950s, they started to collect seed and clone bushes from the gardens to get new cultivars. They also established biclonal and polyclonal gardens that are still being maintained. In the 1990s, they realized that to get greater gains they needed to better utilize germplasm through characterization. They needed to bring in diversity with a clear target on traits. In 1991, they started to actually breed tea, so they were able to get germplasm from the old gardens, and they also got germplasm from Japan and the Tea Research Foundation of Central Africa in Malawi. They also started to use grafting and selected for root stocks as well. They evaluated the response to harsh conditions and tested compatibility to get good



root development and high yield. They still collect from seedling gardens by encouraging the pluckers to identify bushes that have good traits, such as drought tolerance. These are then cloned and put into the germplasm gardens. They have a number of germplasm gardens for the conservation of accessions.

They have found that parental selection is important for hybridization to get long-term progress but heritability for the trait was also important. They currently do all hybridizations with hand pollination. Parental or germplasm selection is driven by what the market wants. It started with a focus on yield but now quality is also important. They have a focus on drought tolerance as they face changes in the climate. They do not target pest resistance yet, although they have thrips, red mite, tea mosquito bug, tea weevil, and aphid, but currently they only assess the effects of these pests on economic loss. They look to the Tea Research Institute to select for host plant resistance. Pests are not a current constraint, but they will be in the future, so they need to consider them for new commercial cultivars, although some already have high levels of resistance. They have a focus on climate resilience with frost, cold, and drought tolerance. They can see clear differences in drought tolerance, but they have accessions and cultivars from Kenya with good drought tolerance.

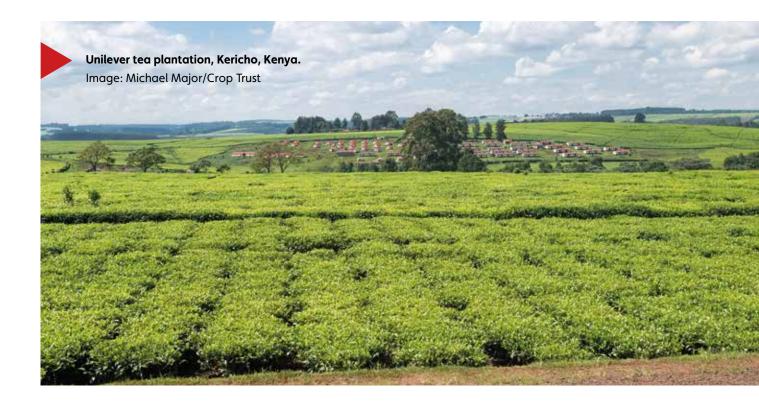
In terms of quality, they have imported Yabukita type cultivars from Japan as seed. Plants were selected that were used for breeding, and they also established a germplasm garden; their objective was to get varieties to manufacture green tea in Kenya for the Japanese market. They also have



var. sinensis accessions that were selected to be used as root stocks but not used much. They have germplasm they got from Malawi and some interspecific crosses with pollen from the Tea Research Institute. They have an interest in acquiring more new germplasm, but this is not easy except from other Unilever locations. They can see the value of a global system to secure conservation as well as to enhance use through greater accession level information sharing, greater germplasm exchange, and more joint evaluations.

### Summary of insights from visits to key genebanks for the future of conservation and use

Visits were made to seven institutes that conserved tea genetic resources. All the institutes managed the accessions in their germplasm collection in the field at one or more sites. There was limited or no safety duplication. There were established technical guidelines for germplasm descriptors, evaluation, cutting propagation, DUS testing, SSR identification, plantlet quality, etc. in China, and for the germplasm collections in India. In most cases, the accessions were plucked, and the tea used for processing by the institute. There was some long-term maintenance of the accessions as natural growing trees. The accessions were being characterized and evaluated, but there was very limited sharing of the accession level information in a searchable database outside the germplasm staff or the institute. Very little of the passport data was digitized. In general, the observation from the field visits confirmed the findings of the survey in terms of the status of secure conservation and use for the collections for the long term. There were a number of opportunities identified for more global collaboration on issues such as an approach to safety duplication in the field or using cryopreservation; common guidelines for costeffective routine operations and descriptors for characterizing accessions; and the development of a common platform to link databases and share accession level information. There are some key conservation research needs as well in terms of cryopreservation and safe exchange of germplasm by using artificial seeds. The development of standard operating procedures (SOPs) based upon collectively agreed best practices for processes, such as acquisition, characterization, maintenance, germplasm health, distribution, and safety back-up, would be a key activity for global collaboration. The implementation of these SOPs across genebanks will guide collection managers in their management decisions, enhance the security of conservation of collections, and facilitate capacity development.



Most of the institutions had links to, and interest in, conservation of diversity in old seedling fields, in wild stands, or in the forest. This local diversity had been used in the past for selection, but it was not very extensive, so there was value for its continual conservation. In some countries, this local diversity has a market and local value that currently secures its conservation. There were specific concerns about diversity held in countries where unique local landraces or wild species are still grown but tea production is not of importance for the country. In some of the countries, this diversity in the old plantations was at risk of loss with uprooting of the old seedling fields for planting new clonal cultivars or in conversion of the land to new crops. In those cases, the institute was involved with the estates to secure parcels of these seedling fields for conservation. There were also efforts to collect this diversity, but most of the institutes visited had limits to the land available for their germplasm gardens. Thus, developing genomic tools to assess diversity still held in the fields and rationalizing it for conservation is a critical need they all share for the future.

In all cases, the germplasm was conserved as a priority for the institutes' breeding programs and it was shared with others within the country. There were only a few examples of international exchange, but all the institutes visited had an interest in acquiring specific new germplasm from outside their country. Within the countries, most of the institutes also had an interest in utilizing genotyping to better characterize the accessions in their collection and

the material still held locally in fields and forests. The development and use of a globally agreed genotyping platform was a key action proposed for the global system. This would allow for global level mapping of diversity; identification of key gaps to fill for collection; rationalization of ex situ collections as well as in situ sites; and the opportunity to build this as part of a global platform for sharing accession level information for conservation and use.

In summary, the visits to the sites of seven key collection holders confirmed the conclusion of the background review and the survey of conservers and users. The current conservation system for tea genetic resources is nationally focused with the primary aims of conserving local diversity and providing some key introductions to institutes' breeding programs. There is an appreciation of the value of conservation, and it is a priority for the institutes and nationally. There are no formal links between these nationally based institutes globally, and no mechanism for international collaboration. This has resulted in very little knowledge of the diversity conserved and its use outside each institute and country. This is not the secure, rational, cost-effective, or sustainable system needed to ensure long-term conservation or use. This is very unfortunate when the risks facing tea production systems and forests are considered. Thus, there was a consensus among all the institutes visited that there is value in a more global conservation and use system. A significant impact of the global system would be to provide increased access to and use of tea genetic resources for the long term.





# A GLOBAL SYSTEM FOR THE CONSERVATION AND USE OF TEA GENETIC RESOURCES

The current conservation system for tea genetic resources is nationally based, with *ex situ* and *in situ* conservation of local landraces and wild relatives as well as *ex situ* conservation for products of breeding programs such as released cultivars or special cultivars/clones with specific traits. This local, unique germplasm is mainly used in breeding programs for direct selection or by local farmers for tea production. It is not currently securely conserved for the long term.

### Who conserves tea genetic resources?

Tea genetic resources are being conserved ex situ and in situ in a number of tea-producing countries. Conservation of tea genetic resources is being done by designated national repositories, public and private tea research institutes, regional and local governments, botanical gardens, universities, farmers' associations, tea estates and plantations, individual farmers, and national, regional, or local protected sites (nature reserves or special germplasm protection areas). These conservers hold local landraces, improved seed or clonal cultivars, breeding material, and accession of closely related wild relatives. The value of much of the diversity conserved is unknown, and few of these conservers have formal links with each other.

## What needs to be conserved for the long term?

The primary focus for conservation should be the center of diversity in South and Southwest China, as well as Northeast India and in the northern border areas of Myanmar, Thailand, and Vietnam adjacent to China. This would secure unique genetic diversity from all the botanical varieties of *C. sinensis* as well as the wild related species.

The secondary focus for conservation should be the diversity currently found in old seedling gardens in many areas of the world. The genetic diversity from these recombinant populations is a product of the movement and hybridization that occurred when tea was planted from various sources of seed during the early establishment of tea production in China, Japan, Korea, Vietnam, Indonesia, India, Sri Lanka, Malawi, Kenya, and Madagascar. These seed derived populations have adapted to local conditions and can be sources of more specific adaptation. There has been direct selection of cultivars from these local populations in the past. There is general recognition that these old seed gardens are still important sources of genetic diversity for future crop improvement and for evolutionary studies.

While there is value in the conservation of old and newly developed cultivars, this is not of the highest priority for long-term conservation. These cultivars might be of shorter-term value for users. They are seen to have limited genetic diversity but could have very important traits that are more readily available for use by breeders. Some of these cultivars or clones have specific chemical components, distinguishing leaf color, or unique physiological characteristics that need to be conserved. Many of the current collections conserve a significant number of the cultivars developed or released from their institute. There has been greater sharing of these accessions globally, so the limited number of these are likely to have some duplication across countries and institutes.

## How will long-term conservation be secured?

The global nature of production and consumption of tea means that there is global interdependence for genetic resources. Currently, conservation of tea genetic resources is the responsibility of nationally or locally based public and private entities who mainly conserve unique local landraces and released cultivars. There is limited conservation of the wild related species. There is limited knowledge of the accessions conserved and limited exchange, especially internationally.

Tea fields near UPASI Tea Research Foundation at the Tea Research Institute in Valparai, Tamil Nadu. Image: Paula Bramel/Crop Trust

In some of the key countries, national policy has favored protecting these resources and recognizing the rights of farmers to benefit from their use. This is an important aspect of securing these global resources but there is also a need for a careful consideration of an ABS regime that enhances the use of tea genetic resources to generate a benefit to all producing countries, farmers, private industry, and consumers. This is very important for the future of the tea sector with its needs to meet the opportunities of expanding markets and the increased challenges to tea production systems. There are a number of options for ABS terms that could be considered and applied for germplasm exchange, but resolving this constraint would require a more national and global recognition that the conservation of this very important genetic resource for the future does depend upon the value added through sustained use.

The conservation of tea genetic resources is *ex situ* and *in situ* in the field, where they are vulnerable to challenges such as climate change, biotic threats, land use changes, increased uprooting/replanting with new clonal cultivars, and other changes in the tea sector. Mitigating these risks will require much greater global collaboration on genetic resource conservation and use to secure collections with greater accession level information sharing, secure monitoring systems, and mechanisms to respond to significant threats to key collections or centers of diversity. There will be a need for more collaborative research on alternative safety duplication approaches such as cryopreservation using artificial seeds.

The goal of a global system for the conservation and use of tea genetic resources is secure, rational, cost-effective, sustainable, long-term conservation and use. Compared to other major crops, tea has lagged behind in the development of coordinated global or regional research and conservation programs. The prerequisite for the global system to function is an enabling environment for cooperation, collaboration, and collective action. This enabling environment has four key elements.

The first element is a platform for global collaboration with leadership that has commitment, outreach, credibility, and recognition within the tea community. The platform should offer opportunities to engage governments, the private sector, conservers, and users in its activities. The leadership of this platform could be an existing international forum. Secondly, there is a need for global sharing of accession level documentation in a publicly accessible, searchable format that meets the needs of conservers and users. Thirdly, national

and institutional policies for ABS are needed, as well as phytosanitary regulations, to facilitate tea germplasm exchange more widely and easily both nationally and internationally. Finally, adequate resources should be available to allow global actions to secure long-term conservation and use.

It is clear that the current conservation system for tea genetic resources lacks the key enabling elements to facilitate the development of a more secure global system. The current "system" basically consists of unconnected national conservation and use activities with a few bilateral international exchanges and collaborations. Thus, key priority investments are required to build the global system:

- As governments, industry, farmers' organizations, and NGOs consider the future needs for tea sector development, securing the global conservation and use of tea genetic resources needs to be considered as a priority. This will require global leadership and advocacy within current international organizations or NGO consortia. One option would be the Crop Trust, FAO Intergovernmental Group on Tea, and Ethical Tea Partnership working together to facilitate a global collaborative platform and promote greater global actions.
- 2. Resources need to be made available to support key global actions and collaboration to address the priority needs. National institutions funded with national resources have a clear priority to focus on conservation and use within countries. The current, inadequately funded, national collection holders cannot be expected to also accommodate the resources needed for global actions or partnerships. The national resources should complement global resources to allow for national needs to be addressed through global collaborations with outreach for tea sector development locally as well as globally.
- 3. Greater global dialog and collaboration among governments, collection holders and users will be facilitated by a global meeting among conservers and key users to share knowledge on accessions, conservation standards and technical guidelines, genotyping, characterization, conservation research, etc. It will be used as an opportunity to communicate the needs of conservation for tea genetic resources within the tea sector and develop collaboration on projects to address key needs for the global system. With committed global leadership and advocacy, a global meeting on tea genetic resources should be held as soon as possible to enable further discussion on securing



long-term conservation and use as well as greater global collaboration on priority needs.

- 4. There is a need to address the priority focal areas for global actions that have been i dentified in the development of this global strategy. These are:
  - Accession level documentation sharing, including internationally agreed standard phenotypic and genotypic characterization and evaluation data, on a global platform such as Genesys, that will allow for global searching of linked or compiled databases with a standard interface that will also enable a user to select accessions based upon collective knowledge.
  - Internationally agreed standards and technical guidelines for secure conservation, safety duplication (including the use of cryopreservation), germplasm exchange, and other key issues.
  - A standard genotyping platform agreed upon and used for a global genotyping effort to better focus long-term conservation and use on unique diversity, address gap filling, and facilitate rationalization.
  - ► Enhanced use of the diversity in conserved accessions through greater collaboration in research and breeding globally that will be facilitated with a clearer policy for international exchange of accessions to benefit the tea sector overall.

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# ANNEX I: LIST OF PARTICIPANTS IN THE SURVEY

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