



Review on Tea (*Camellia sinensis*) Research Achievements, Challenges and Future Prospective Including Ethiopian Status

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Abstract: Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most popular and lowest cost beverages in the world, and consumed by a wide range of age groups in all levels of society with more than three billion cups daily worldwide. Tea breeding strategy such as source of gene pool, selection of elite mother bushes and assessment of made tea quality reviewed. Tea research achievement in breeding, eco-physiology, and biochemical; production of tea in China, India, Kenya, Sri Lanka and Vietnam was considered. The challenges like mini-manufacture, cost assessment, climate change, time consuming protocols, pest and diseases; cost of assessment including constraints of Ethiopian tea industry was reviewed well. Future prospects of tea research main research emphases of tea genetics and breeding in the future, deepening the functional genome research to reveal special gene resources, enhancing the basic genetic research for breeding, conducting distant hybridization to enhance germplasm innovation, improving gene transformation technique to use exogenous genes, Perfecting molecular marker assisted technique to effectively conduct early-stage appraisal and similar procedures should be integrated in the future tea research in order to cope up with the currently existing environmental phenomena.

Keywords: Tea, Research, achievement, Challenges, Prospective, Ethiopia

1. INTRODUCTION

Tea [*Camellia sinensis* (L.) O. Kuntze], is one of the most popular and lowest cost beverages in the world, and consumed by a wide range of age groups in all levels of society with more than three billion cups daily worldwide (Hick, 2009). Majority of the tea producing countries are located in the continent of Asia where China, India, Sri Lanka are the major producers. African tea growing countries are located mostly around the tropical regions where Kenya, Malawi, Rwanda, Tanzania, Uganda are major producers. Apart from these regions, some quantities of tea are also being produced in South America (Argentina, Brazil and others), the Near East (Iran and Turkey) and the CIS (Russia and Georgia). Amongst tea producing countries, the principal producers are China, India, Sri Lanka, Kenya and Indonesia. In terms of area under tea plantation, on an average during the last two decade (1991 – 2010), China lead (45%) the world followed by India (21%), Sri Lanka (7%), Kenya (5%), Vietnam (3%) (Basu *et al*, 2012).

The Tea Research Institute (TRI) is the only comprehensive institute dedicated to tea research at the national level in China. The institute holds the second largest tea preservation resource in the world and currently conserves 3,013 different tea germplasm resources, encompassing the most genetic diversity of any tea resource in the world. In total, researchers at TRI have contributed 225 scientific and technological advances to the field 106 of these achievements have been recognized with awards, including eight national and 39 ministerial prizes. The institute has bred eight nationally certified tea varieties, has been granted 45 patents, and has established one international standard, 25 national standards, and 51 industrial standards for tea production (Sean, 2013). The first tea planted in Africa was in 1850 in the Durban Botanical Garden on the eastern shore of South Africa near the port of Durban which was China type. In order to improve yield and quality of the crop in Southern Africa the Tea Research Foundation of Central Africa (TRFCA) was established in Malawi in 1933, initially as a tea research station under the department of agriculture before gaining autonomy in 1966. The TRFCA has an active plant breeding and selection program aimed at solving tea production problems such as drought, extreme temperatures, pests and diseases, low yield and low quality, under local

growing conditions in order to increase productivity and maximize total value and income from the crop (Apostolides *et al.*, 2006).

2. BODY OF THE TEXT

2.1. The Achievements of Tea Genetic Improvement and Breeding

2.1.1. Breeding of the Tea Plant (*Camellia sinensis*) in China

China has abundant tea genetic resources capable of providing diverse parent materials for tea breeding. Different methods, which included ex-situ, in-situ and in vitro, were employed to prevent the loss of tea germplasm and ensure the preservation of genetic diversity. The tea germplasms have been evaluated and appraised based on morphology, physiology, agronomy, cytology and molecular biology, through which the elite and unique tea accessions were screened and further utilized in tea breeding. An efficient tea breeding system, in which controlled hybridization and individual selection are the main breeding approaches, combined with molecular marker assisted selection and micropropagation techniques, has now been established and gradually developed in China. Up to date, a total of 123 cultivars have been registered as national tea cultivars. More than 150 cultivars have been accredited for release in given provinces and 4 clones have been covered by Plant Varieties Protection (PVP) in China (Ming and Liang, 2012). By the end of 2005, China had 97 national registered cultivars, among them 17 are jats, 80 are clones, 30 are landraces and 67 are improved clones (Table 1). They are bred by 23 different institutions including the national and local tea research institutes, agricultural universities, local agricultural departments, tea experimental stations, etc. There were also about 130 provincial registered cultivars, among them 16 are jats, 114 are clones, 29 are landraces and 101 are improved clones, respectively (Yu, 2005; Liang *et al.*, 2007).

Table1: Brief introduction to the Chinese national registered cultivars

no	Cultivars	Registered year	Breeding method	no.	Cultivars	Registered year	Breeding method
1	Fuding Dabaicha	1985	Landrace	50	Yunkang 10	1987	Field clone
2	Fuding Dahoacha	1985	Landrace	51	Yunkang 14	1987	Field clone
3	Fu'an Dabaicha	1985	Landrace	52	Juhuachun	1987	OP
4	Meizhan	1985	Landrace	53	Guihong 3	1994	Field clone
5	Zhenghe Dabaicha	1985	Landrace	54	Guihong 4	1994	Field clone
6	Maoxie	1985	Landrace	55	Yangshuling 783	1994	Field clone
7	Tieguanyin	1985	Landrace	56	Wannong 95	1994	Field clone
8	Huangdan	1985	Landrace	57	Xicha 5	1994	Field clone
9	Fujian Shuixian	1985	Landrace	58	Xicha 11	1994	Field clone
10	Benshan	1985	Landrace	59	Hanlv	1994	Field clone
11	Daye Wulong	1985	Landrace	60	Longjing Changye	1994	Field clone
12	Mengku Dayecha*	1985	Landrace	61	Zhenong 113	1994	OP
13	Fengqing Dayecha*	1985	Landrace	62	Qingfeng	1994	Field clone
14	Menghai Dayecha*	1985	Landrace	63	Xingyang 10	1994	Field clone
15	Lechang Baimaicha*	1985	Landrace	64	Baxiancha	1994	Field clone
16	Hainan Daye*	1985	Landrace	65	Qianmei 601	1994	HP
17	Fenghuang Shuixian*	1985	Landrace	66	Qianmei 701	1994	HP
18	Damianbai	1985	Landrace	67	Gaoyaqi	1994	Field clone
19	Shangmeizhou Zhong	1985	Landrace	68	Zhuyeqi 12	1994	Field clone
20	Ningzhouzhong*	1985	Landrace	69	Baihaozao	1994	Field clone
21	Huangshang Zhong*	1985	Landrace	70	Jiebohuang 13	1994	Field clone
22	Qimenzhong*	1985	Landrace	71	Shuyong 703	1994	HP
23	Jiukengzhong*	1985	Landrace	72	Shuyong 808	1994	HP
24	Yuntaishanzhong*	1985	Landrace	73	Shuyong 307	1994	HP
25	Meitan Taicha*	1985	Landrace	74	Shuyong 401	1994	HP
26	LingyunBaimaocha	1985	Landrace	75	Shuyong 3	1994	HP

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27	Ziyangzhong*	1985	Landrace	76	Shuyong 906	1994	HP
28	Zaobaijie*	1985	Landrace	77	Yihongzao	1998	Field clone
29	Yichang Dayecha*	1985	Landrace	78	Fuzao 2	2002	Field clone
30	Yixingzhong*	1985	Landrace	79	LingtouDancong	2002	Field clone
31	Qianmei 419	1987	OP	80	Xiuhong	2002	Field clone
32	Qianmei 502	1987	HP	81	Wulinghong	2002	Field clone
33	Fuyun 6	1987	OP	82	Yunda Danlv	2002	Field clone
34	Fuyun 7	1987	OP	83	Gancha 2	2002	OP
35	Fuyun 10	1987	OP	84	Shuyong 808	2002	HP
36	Zhuyeqi	1987	Field clone	85	Shuchazao	2002	Field clone
37	Longjing 43	1987	Field clone	86	Wannong 111	2002	Mutation
38	Anhui 1	1987	Field clone	87	Zaobaijian 5	2002	Field clone
39	Anhui 3	1987	Field clone	88	Nanjiang 2	2002	Field clone
40	Anhui 7	1987	Field clone	89	Zhenong 21	2002	Field clone
41	Yingshuang	1987	OP	90	E'cha 1	2002	HP
42	Cuifeng	1987	OP	91	Zhongcha 102	2002	Field clone
43	Jingfeng	1987	OP	92	Mingke 2	2002	HP
44	Biyun	1987	OP	93	Yuemingxiang	2002	Field clone
45	Zhenong12	1987	OP	94	Mingke 1	2002	HP
46	Shuyong 1	1987	HP	95	Huangqi	2002	OP
47	Yinghong 1	1987	Field clone	96	Guilv 1	2003	Field clone
48	Shuyong 2	1987	HP	97	Mingshan Baihao	2005	Field clone
49	Ningzhou 2	1987	Field clone				

Source: *China Tea Varieties Compilation Committee, 2001; MOA Bulletin No. 191, 2002*

* *Jat cultivars: (propagated by seeds), the others are clones Breeding methods:*

Landrace: traditional cultivars, including jat cultivars and clones;

Field clone: individual selected clones from seedlings/jats;

OP: Clones selected from open-pollinated progenies;

HP: Clones selected from hand-pollinated progenies

2.1.2. Breeding of the Tea Plant (*Camellia sinensis*) in India

The indigenous Assam tea plant was discovered by Robert Bruce in 1823. The better cup quality produced by the Assam tea plant popularized it as an important planting material in the tea industries of the country. Considering the need for improved planting materials for the tea industry, Tocklai Experimental Station (TES) Tea Research Association, initiated a tea breeding programme in 1930, under which germplasms were collected based on trait specific phenotypic characteristics. The Tea Research Foundation, United Planters' Association of Southern India (UPASI), initiated a similar program in 1963 and collected germplasms were preserved in their gene bank. The technique of vegetative propagation standardized in 1955, provided scope for developing improved clonal cultivars as well as biclonal seed cultivars through hybridization. From the selected plants from old seed jats and progenies of biclonal hybrids, 153 locally adapted and 31 universal clones were developed for the tea industry. Marker development for draught resistance and blister blight disease is under progress using cDNA-AFLP techniques. The micropropagation technique has also been standardized for quick multiplication of these biotechnologically modified plantlets (Chen *et al.*, 2012).

2.1.3. Tea Improvement in Kenya

Although tea was introduced into Kenya at the turn of the 20th century, concerted efforts at tea improvement could not take root until the early 1960s after the country gained self-rule from Britain. Owing to the heterogeneity of pioneer seedling populations that was accompanied by management constraints, early research efforts resulted in the development of whole single cuttings as propagation materials which, coupled with clonal selection, led to the release and commercialization of high yielding and quality clones. To date, the Tea Research Foundation has released a total of 50 high yielding and good quality tea clones for commercial utilization, not just in Kenya alone but also in the entire East African region. Furthermore, tea improvement activities integrating molecular markers

and participatory clonal selection involving farmers and consumers are expected taste-track the development and adoption of novel varieties within a relatively short period (Samson *et al.*, 2012).

2.1.4. Breeding of tea plant (*Camellia sinensis*) in Sri Lanka

Tea germplasm accessions conserved in field gene banks in Sri Lanka have not been adequately characterized in order to maximize its utilization in tea breeding programs. The unique morphological identity of each group was further confirmed using the available information related to their origin, ancestral relationships and locations of selections of the accessions. Moreover, it was able to identify key descriptors contributing to the phenotypic variation of the tea germplasm in Sri Lanka. This is the first extensive study reported so far on tea germplasm characterization using morphological descriptors, both locally and internationally. Furthermore, the exceptional morphological descriptors identified in this study would facilitate cultivar identification, which has become one of most demanding needs among tea growers after the introduction of new improved cultivars by the Tea Research Institute of Sri Lanka (Piyasundara *et al.*, 2009).

2.1.5. Breeding of tea plant (*Camellia sinensis*) in Vietnam

Some new tea cultivars of high yield and good quality, suitable for production of green, black and Oolong teas such as LDP 1, LDP 2, PH 8, PH 9, have been developed. Tea breeding, however, needs to be further strengthened, and to this end the following activities need to be promoted: collecting and conservation of tea genetic resources, hybridization of native and exotic cultivars of *C. sinensis* and *C. sinensis* var. *assamica*, and use of mutation for speeding up the breeding cycle (Chen *et al.*, 2012).

2.2. Breeding strategy

2.2.1. Source of variation (gene pool)

Tea production in the Southern Africa region is largely limited by climatic factors, particularly low and unevenly distributed rainfall and large fluctuation in temperatures, resulting in three distinct seasons. The thrust has therefore been to look for vegetatively propagated cultivars that are drought tolerant, have a low base temperature and are high yielding and that would thrive under these harsh conditions and make high quality teas in the main growing season. Initially the established seedling fields provided the gene pool from which elite mother bushes for VP (Vegetatively Propagated) cultivars were selected. This started in the mid 1950s and selection was based on drought tolerance and rate of recovery from prune. Some of the field selections were made at Swazi (now Nsuwadzi) research station in Malawi, e.g. SFS150 and SFS204 have become very popular cultivars in the region, each now covering over 100 hectares. In recognition of the limited natural variation for specific attributes expected of seedling tea population, a deliberate hybridization programme to create variable F₂ progeny populations for specific attributes from which to select elite mother bushes for bulking up vegetatively, started in 1960 at the TRFCA (Ellis and Nyirenda, 1995).

2.2.2. Selection of elite mother bushes

Selection of elite mother bushes for quality attributes is based on fast fermentation, growth vigor, shoot size and tolerance to major pests and diseases. The chloroform test is very inexpensive (about USD 1/ genotype) and is used to classify plants as "slow", "medium" or "fast" fermenters (Sanderson, 1963). Using this test, the "slow" fermenters are eliminated from the selection programme in year 4. This occurs in the nursery plants, thus eliminating them from expensive field trials (Table 2). Selection of these attributes requires establishment of rapid and reliable selection criteria and methods (Apostolides *et al.*, 2006).

Table2: Breeding and selection programme for new cultivars at the Tea Research Foundation in Central Africa (Malawi)

Place	Year	Activity	Number of genotypes	Number of plants per genotype
Seed Garden	0	Controlled crosses of flowers in seed garden	5000	5000
Nursery	1	Germinate viable seeds in pots	3750	3750
Nursery	2,3	Select seedlings on the basis of plant vigor; Take single cuttings for vegetative propagation	2500	2500

Nursery	4	Select seedlings on the basis of rooting ability and nursery performance. Eliminate slow fermenters (chloroform test) take 30-50 cuttings from selected seedlings	350	350
Field	5	Plant cultivars in 2 x 8 = 16 bush observation plots in field. Mini-manufacture for organoleptic assessment by expert tea taster	150	2400
Field	6,7,8	Select cultivars on field performance and organoleptic quality	20	2400
Field	9	Establish cultivars in replicated field trials of 5 x 6 = 30 bushes per plot replicated five times, on several research stations	20	3000 per station
Field	10	Early release of cultivars to estates	5	as required
Field	11,12,13,14	Evaluation of cultivars on all traits for yield and quality	5	10,000 to 100,000
Industry	15	Possible release of 1-2 new cultivars to industry	0-3	as required

Source: *Apostolides et al., 2006*

2.2.2.1. Collection and conservation

The investigation of tea germplasms was initiated in China in the 1930s. However, large-scale and well-planned investigation and collection began in the 1980s. In 1990, the China National Germplasm Tea Repositories (CNGTR), including Hangzhou Tea Repository in the Tea Research Institute Chinese Academy of Agricultural Sciences (TRICAAS) and Menghai Tea Repository Branch in the TRI Yunnan Academy of Agricultural Sciences (TRIYAAS) were established as national level permanent ex-situ conservation repositories (Chen *et al.*, 2004). About 2,665 germplasm accessions, including wild tea plants, landraces, improved clones, strains, genetic materials and others, from the tea growing provinces of China and several other countries around the world had been collected and preserved in the CNGTR by the end of 2003 (Chen *et al.*, 2004). Meanwhile, repositories of different scale were established in the TRIs of other tea producing provinces mainly to conserve their local landraces (Liang *et al.*, 2007).

2.2.2.2. Early-stage appraisal technique for breeding

Cup tea quality, yield and resistance are main tea breeding objectives. The desirable attributes for high crop yield and perhaps quality may not be likely to occur in more than one out of every 40,000 or more bushes (Wright, 1956; Hajra, 2001). It will take 22–25 years to successfully breed a new clone using traditional methods (Takeda, 2000). Thus, in order to shorten the breeding time and improve the breeding efficiency lots of research projects on early-stage appraisal technique for breeding have been carried out and achieved significantly. In early 1970s, research mainly focused on the simple relationships between the morphological characteristics and yield, cup tea quality and resistance. Since the late 1990s, DNA molecular markers have been used in early-stage appraisal (Chen *et al.*, 2006c). The early stage appraisal of tea breeding is now advancing from morphological, physiological and chemical level to more precise DNA level (Liang *et al.*, 2007).

2.2.2.3. Genetic introgression to enhance germplasm innovation

Germplasm innovation by distant hybridization has important role of broadening the genetic base. Ackerman (1973) attempted a large number of interspecific crosses involving 20 *Camellia* species. Tea could be easily crossed with 10 different species. In Japan, the distant hybridization of tea plant and 26 species in the genus *Camellia* were conducted. An interspecific hybrid between tea plant (*C. sinensis*) and flower camellia (*C. japonica*) and named Chatsubaki was obtained. It proved highly resistant to tea anthracnose, gray blight and cold damage in winter and furthermore had low caffeine content (Takeda *et al.*, 1987). It has become one of the three promising parental materials for tea breeding in Japan. A clone TV 24 in Assam, India was produced by crossing between F1 hybrids (*C. irrawadiensis* X *C. assamica*) and TV1, an Assam-China hybrid (Bezbaruah, 1987).

2.2.2.4. Hybridization breeding

Hybridization is one of the main methods of obtaining genetic variation, and it is an important method of breeding new varieties. Totally 11 clones, corresponding to 73.3 percent of all clones bred using

hybridization method, were bred from the controlled hybridization of Yunnan Dayecha (including Fengqing Dayecha) as female parent or male parent (data not shown). The percentage of clones bred using hybridization method increased significantly, from 9.1 percent for the first time (1987) to 29.1 percent for the second (1994) and 22.2 percent for the third (2002) time, respectively. Distant hybridization is a powerful method for broadening the genetic base of new varieties. Thanks to sterility or extremely weak fecundity, currently it could not be a routinely used method in the breeding of tea plant. Young embryo rescuing tissue culture strategy is now being developed to improve the success ratio of distant hybridization in the TRICAAS (Liang *et al.*, 2007).

2.2.2.5. Mutation breeding

The combined effects of c-ray and chemical mutagens on biological damage to tea plant were systematically analyzed (Yang and Lin, 1992). The suitable dose rate and physicochemical combined mutation technique and criteria of main Chinese tea cultivars and a math model between radiation dose and effect of dose rate were proposed. One excellent new strain, which is very early sprouting in the spring, high cup tea quality, resistant to disease and suitable for fine green tea, has been selected from the offsprings of Longjing 43 cuttings under Co60c-ray radiation (Yang *et al.*, 2003a). The TRI Hunan and Anhui Agricultural University had bred and registered a provincial and national clone using tea seedlings or seeds as radiation objects and Co60c-ray as radiation source in 1997 and 2002, respectively (Liang *et al.*, 2007).

2.2.2.6. Molecular marker assisted selection

Presently, several molecular markers, such as RFLP, RAPD, CAPS, AFLP, ALPs, SSR, ISSR, have been developed and widely applied in the tea plant. Remarkable advances have been achieved for the analysis of genetic diversity and relationship, discrimination of varieties and cultivars, studies on molecular phylogenetics, detection of genetic stability and fidelity and, genome mapping (Chen *et al.*, 2006c). It is possible to genetically manipulate and control the tea plant. This will bring tremendous effects to tea genetic improvement and breeding in the foreseeable future (Chen *et al.*, 2006c; Liang *et al.*, 2007).

2.2.2.7. Micropropagation

One of the difficulties in early breeding and extension stages of tea plant is reproduction. It is time consuming to propagate enough plantlets for strain comparative test, provincial and national adaptability tests. Moreover, it will take long time to propagate the new released clone for large scale extension. Where very few cuttings were available, it might take 8–10 years to multiply 4,000–5,000 plantlets using the conventional single node cutting method. However, only about 2 years might be sufficient to obtain the same quantity of plantlets using micropropagation methods (Cheng and Li, 1998).

2.2.2.8. Polyploidy and its application in tea breeding

In tea, gigantism in various morphological characters has been found to be associated with tetraploids (Amma, 1974; Chaudhuri and Bezbaruah, 1985). Apart from increasing the biomass of crops (Allard, 1960), induced polyploids could be used as an additional source of genetic variation to improve the overall performance of existing diploids, or to enhance particular characters such as the shoot size trait (Wachira and Kiplangat, 1991), while retaining most of the characteristics of the diploid progenitor. In addition, they could also be used as a source of breeding material for the production of secondary polyploidy cultivars by crossing with diploids (Singh, 1980). Although the value of polyploids in overcoming barriers to inter-specific gene introgression has been recognized in plant species (Allard, 1960), this has not been fully exploited in perennial crops (Wachira and Ng'etich, 1999). On the other hand, non-conventional methods, such as polyploidy breeding, may induce more vigour and some degree of resistance to biotic and abiotic stresses in existing tea cultivars, without causing changes in the desired parts of the genome (Simura and Inabe, 1952). There is a positive correlation between size of the leaf and the yield in tea (Satyanarayan and Sharma, 1982). Further, in asexually-propagated perennial crops such as tea, where the vegetative organs are of economic value, polyploidy breeding can be used effectively in their genetic improvement (Gunasekara and Ranatunga, 2003).

Table3: Identified polyploid clones, their chromosome number and ancestry

Clone number	Ploidy-Chromosome No.	Original Seed Source
*TRFK 311/287	4n = 60	TRFK 6/8 x TRFK 31/11 - hand-pollinated from TRIEA Uganda
*TRFK 31/30	4n = 60	Seed from Ambangulu Estate, Tanzania
*TRFK 52/1	3n=45	“Between Seed from Kanywankoko Estate, Uganda
*TRFK 72/1	3n=45	Open-Pollinated Seed from Mimosa Estate, Malawi
*Dimbolil 3	3n=45	Seedlings from James finlay (K) Ltd. Dimbolil Estate
*TRFK 77/2	3n=45	Open-Pollinated Seed from koiwa estate
*TRFK 383/1	3n=45	Open-pollinated BB35 X BB5, Koiwa Estate
*TRFK 331/2	3n=45	Seed from Chemosit Estate barie, Unilever tea
*TRFK 378/1	3n=45	Open-pollinated BB35 X BB7, Koiwa Estate
*TRFK 412/1	3n=45	Open-pollinated BB21 X BB5, Koiwa Estate
*TRFK 371/1	3n=45	Open-pollinated seed of AHP S15/10 from Chepgoiben Estate
*TRFK 400/1	3n=45	eed from Chepgoiben Estate barie
*TRFK 389/1	3n=45	Open-pollinated BB35 X BB152, Koiwa Estate
*TRFK 392/1	3n=45	Open-pollinated BB7 X BB35, Koiwa, Estate
*TRFK 394/1	3n=45	Open-pollinated BB2 X BB35, Koiwa, Estate
*TRFK 395/1	3n=45	Open-pollinated BB5 X BB35, Koiwa, Estate
*TRFK 54/49	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 386/1	3n=45	Seed from Sotik Tea Co
TRFK 381/1	3n=45	Seed from BB5 X BB2
TRFK 84/1	3n=45	Mixed seed from Congo, Toro and Entebbe, Uganda
TRFK 84/2	3n=45	Mixed seed from Congo, Toro and Entebbe,Uganda
TRFK 85/1	3n=45	Seed collected from clones from Kakonde Estate
TRFK 382/2	3n=45	Seeds from BB5 X BB35
TRFK 382/1	3n=45	Seeds from BB5 X BB35
TRFK 386/2	3n=45	Seed from Sotik Tea Co
TRFK 76/3	3n=45	Seed from Ramjat, Lugeri Estate, Malawi
TRFK 76/1	3n=45	Seed from Ramjat, Lugeri Estate, Malawi
TRFK 76/2	3n=45	Seed from Ramjat, Lugeri Estate, Malawi
TRFK 75/1	3n=45	Commercial seed from Lugeri Estate, Malawi
TRFK 31/36	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 31/38	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 31/39	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 31/40	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 31/41	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 18/7	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 18/27	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 18/26	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 18/28	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 54/50	3n=45	Seed from Ambangulu Estate, Tanzania
TRFK 550/1	3n=45	Open-pollinated seed from polyclonal mixture, Timbilil Estate, Kericho

Source: Wachira and Kiplangat (1991); Kamunya, 2010 NB: in tea, $n = x = 15$.

2.2.2.9. Genetic transformation in tea

Foreign genes have been introduced in several woody crops including the rubber tree by using the *Agrobacterium tumefaciens* Ti plasmid (Horsch *et al.*, 1985; Venkatachalam *et al.*, 2006). Although the transgenic technology has immense potential for the genetic improvement of tea (Mondal *et al.*, 2004; Lopez *et al.*, 2004), the technique was hardly tried prior to 2000. This may have been caused by initial challenges in developing a viable protocol for gene transfer as well as efficient protocols for the *in vitro* regeneration of tea. Mondal *et al.* (2001) were able to optimize transformation conditions and production of transgenic tea via *Agrobacterium tumefaciens*.

2.3. Tea industry in Ethiopia

Tea is a new crop to Ethiopia which introduced in the early 19th century. The main government objectives to be self sufficient domestic consumption and save the foreign exchange spent for importing tea, to supply for export market by increasing the production and quality of tea through

time and, to create employment opportunity for the citizens (MCTD, 2001). In Ethiopia, tea is produced by three estates (private tea plantation) viz. Wush-Wush and Gумero (Ethio Agri-Ceft P.L.C), and Chewaka (East Africa P.L.C) in south west of the country. Moreover, out-growers have produced and supplied green tea leaves to the three estates. Currently, the three estates hold a total area of 2660 ha under tea production and out-growers hold 437 ha for tea production. In the last five years (2011/12 to 2015/16), the three estates supplied a total of 4570.78 tons export and 34,126.1 tons of tea for domestic market. Tea industry in Ethiopia has provided income and employment opportunities to 575 out-growers, 7139 temporary per annum and 1157 permanent workers at tea estates (EIAR, 2017).

Ethiopian high land tea is thus considered to be one of the best East African teas that could fetch premium prices in the world market (MCTD, 1991); the comfortable growth of tea in all coffee production areas is a good opportunity which permits the use of tea as an alternative crop in coffee diversification program in the country. Besides, it offers the coffee farmers the possibility to generate continuous income from the tea yield which is obtainable at every 10-15 days. The availability of plenty labor force in the country, as the production of tea requires an intensive human labor (EIAR, 2017).

2.4. Challenges of tea research

2.4.1. Mini-manufacture

This is limited by the age of the bushes when adequate leaf can be harvested and number of cultivars that can be mini-manufactured and tasted by expert tea tasters per season. There are also high electricity costs involved. Being a final confirmatory quality assessment, only short-listed potentially high quality selections should undergo this test. This can only be achieved when rapid, inexpensive screening methods are in place to eliminate poor quality genotypes in the early stages of selection (Maina *et al.*, 2015).

2.4.2. Time consuming protocols

Most of the current selection methods can only start once the bushes have attained a particular stage. This is responsible for the long selection cycle making the programme expensive. The early methods of catechin analysis had to be abandoned because they were expensive and time consuming.

2.4.3. Cost of assessment

The current biochemical methods are expensive due to the need for special equipment and expertise. The limited funding to most research organizations makes it impossible to analyze all samples going through the breeding and selection process. For example, the fresh leaf catechins can be measured in a single two and a bud shoot, but cost about USD 101 per sample. The flavonoid costs only about USD 8 per sample but can only be done after mini-manufacture that costs USD 50 per sample, plus 50g fresh shoots costing another USD 10, for a total cost of USD 68. The catechin test is quite attractive due to the possibility of doing this test early (single bush, year 4) in the selection process (Maina *et al.*, 2015).

2.4.4. Climate change

The impacts of climate change on tea production are the following: drying of the soils causing reduced water content in the tea, decreasing yields and negative impacts on quality; appearance of new pests and diseases; changes in the suitability of existing tea growing areas (In extreme cases, as temperatures become too high for tea cultivation, new suitable land areas need to be found; reduced biodiversity and ecosystem function as the result of habitat conversion, high-energy consumption (logged timber); sun scorch damage decreasing yields and lowering tea quality; reduced resilience of tea crops; uncertainty with application of fertilizers, high application of pesticides in some countries; increase in extreme weather events such as droughts, hail storms, floods, frosts, extreme rainfall and landslides (MCCTS, 2014; Maina *et al.*, 2015).

2.4.5. Pests and diseases

The pests observed in farmers' fields were red spider mites, scales, thrips, aphids and crevice mites. Control measures suggested were cultural measures such as good nutrition. Over half of the farmers

(54%) indicated that *Armillaria* root rot is a problem in tea production. The control measure of this disease was reported to be uprooting the affected plants and venturing into different enterprises. Disease control measures suggested were; for *Armillaria* root rot; uprooting of the tea, for hypoxylon wood root; proper pruning, and for stem canker; use of chemicals. Other studies have also indicated these diseases affect tea (Otieno, 1997; Maina *et al.*, 2015).

2.4.6. Major constraints of Ethiopian tea industry

The problem of low yield, which is mainly accounted to lack of technology recommendations suiting to Ethiopian condition, the use of adopted research technologies from other countries; particularly, that of Kenya's has been in use till presently. Tea Yield/ha in Ethiopia has been showing an increasing tendency from its early stage below 1300 kg/ha is still very low as compared to other tea producing countries. As per the Food and Agricultural Report (FAO, 2001), in Assam tea, there was a world high production yield of 2,586 kg/ha in Bolivia and as high as 6,700 Kg/ha has also been reported in Sri Lanka. Limitation of tea genetic resource base; thus less than 15 tea clones from introductions made so far. This has hindered attempts that could have been made to broaden and improve the existing narrow genetic base through different breeding approaches. The cultivation of tea in Ethiopia has been practiced by very few private large scales under limited locations and the most proportion (about 99.95%) of potentially suitable tea lands is left unexploited till presently

2.5. Future strategy or prospects of tea research

2.5.1. Main research emphases of tea genetics and breeding in the future

Significant advances in tea breeding have been achieved and the breeding system has also progressed. The ultimate purpose of tea germplasms collection, conservation, appraisal and evaluation is to use their desirable genes. About 60% of the tea germplasms preserved in the CNGTR is jats (Chen *et al.*, 2004). If the genetic background, superior characteristics and genes of the most important key hybridization parents are well studied, tea breeding in China will have a breakthrough and the change from germplasms predominance to new clone predominance will be realized with the assistance of new breeding techniques and methods (Chen *et al.*, 2004). Our newly developed medium density cDNA microarray of tea plant may provide a possible tool for high-throughput germplasms appraisal on the gene level (Zhao *et al.*, 2006; Liang *et al.*, 2007).

2.5.2. Deepening the functional genome research to reveal special gene resources

The isolation of functional genes from tea plant was initiated in 1992 and advanced slowly owing to the distinctness of tea plant from other taxa (Zhao *et al.*, 2003). Totally 1,684 ESTs and the partial sequences of more than 300 functional genes, including some important secondary metabolism and stress/defense related genes have been generated (Chen *et al.*, 2005c). Consequently, it is necessary to increase input and promptly deepen the functional genome research, in order to reveal specific genes of tea plant, to better understand the mechanism of genetic variation on the whole genome level for tea breeding (Liang *et al.*, 2007).

2.5.3. Enhancing basic genetic research for breeding

The genetic characteristics of the tea plant are still poorly understood and it causes significant negative effects to tea breeding. Thereby, the techniques and approaches of modern molecular biology should be employed to enhance the genetic understanding of important agronomic traits, quality, metabolism and resistance. It will help to guide tea breeding, to enhance the precision and to improve the efficiency (Liang *et al.*, 2007).

2.5.4. Conducting distant hybridization to enhance germplasm innovation

Germplasm innovation has the function of pre-breeding and has the important role of broadening the genetic base and improving the effectiveness of breeding. Along with the discovery of excellent germplasms, innovation should be enhanced in order to provide breakthrough germplasms for breeding. The method of innovation is diverse; a main important one is hybridization, including distant hybridization. In Japan, the distant hybridizations of tea plant and 26 species in the genus *Camellia* were conducted. An interspecific hybrid between tea plant (*C. sinensis*) and flower camellia (*C. japonica*) named Chatsubaki was obtained. It proved highly resistant to tea anthracnose, gray blight and cold damage in winter and, furthermore had low caffeine content (Takeda *et al.*, 1987).

2.5.5. Improving gene transformation technique to use exogenous genes

Using gene engineering techniques, desirable exogenous genes from other organisms are able to be introduced into tea plants, new varieties with new characteristics are able to be created and desirable traits of two clones are able to be combined together. There is a successful case of transgenic tea plant in India (Mondal *et al.*, 2001). Meanwhile, there are several research projects in China, using both particle bombardment and *Agrobacterium* mediated or combined transformation methods (Luo and Liang, 2000; Zhao *et al.*, 2001; Wu *et al.*, 2003, 2005). Furthermore, RNA mediated post-transcriptional gene silencing technique might be used to make low or nil caffeine tea clones. The key genes of caffeine synthesis pathway such as caffeine synthase (Kato *et al.*, 2000) and S-adenosyl methionine synthase (Feng and Liang, 2001) genes could be sequence specifically degraded by double stranded RNA (dsRNA) using antisense messenger RNA technique. The bottleneck is that the transformation receptor system is still problematic. So, great attentions should be paid in the gene transformation technique to improve it, and make transgene an effective routine approach for germplasm innovation (Liang *et al.*, 2007).

2.5.6. Molecular marker assisted technique to effectively conduct early-stage appraisal

There are many early-stage appraisal approaches available in tea breeding which include morphological, physiological, chemical and molecular methods. Informative DNA markers are used to construct high density genetic linkage map, to locate the quantitative trait locus (QTL) of important agronomic traits, quality and resistance, showing bright prospects in tea breeding via marker assisted selection. The first linkage map for tea plant was constructed with RAPD markers by Tanaka (1996), and the markers related with theanine content, date of bud sprouting, resistance to anthracnose and tolerance to cold were detected (Tanaka,1996). Another linkage map from the female parent, SFS150, was established with RAPD and AFLP markers (Hackett *et al.*, 2000).

3. SUMMARY AND CONCLUSION

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most popular and lowest cost beverages in the world, and consumed by a wide range of age groups in all levels of society with more than three billion cups daily worldwide. The TRI holds the second largest tea preservation resource in the world and currently conserves 3,013 different tea germplasm resources, encompassing the most genetic diversity of any tea resource in the world. In total, researchers at TRI have contributed 225 scientific and technological advances to the field 106 of these achievements have been recognized with awards, including eight national and 39 ministerial prizes. The TRFCA has an active plant breeding and selection programme aimed at solving tea production problems such as drought, extreme temperatures, pests and diseases, low yield and low quality, under local growing conditions in order to increase productivity and maximize total value and income from the crop. Early sprouting in the spring, high cup quality, high yield, high resistant to biotic and a biotic stresses and, some special characteristics (such as low caffeine, high Catechins, etc.) are the main tea breeding objectives in the present stage and foreseeable future. Many improved, high-yielding tea cultivars possess one or a few undesirable traits such as susceptibility to pests and diseases, which preclude their extensive use in commercial planting. The problem of low yield, which is mainly, accounted to lack of technology recommendations that suiting to Ethiopian condition.

In conclusion to develop promising cultivars, breeding strategies, which combine high yield, good cup quality and resistance to biotic and a biotic stresses, are required to obtain high productivity and to reduce the cost of production. The challenges like mini-manufacture, cost assessment, climate change, time consuming protocols, pest and diseases, labour, soil erosion, machine picking were affected mainly tea industry including Ethiopian. In the future the tea research must emphasize on tea genetics and breeding, deepening the functional genome research to reveal special gene resources, enhancing the basic genetic research for breeding, conducting distant hybridization to enhance germplasm innovation, improving gene transformation technique to use exogenous genes, Perfecting molecular marker assisted technique to effectively conduct early-stage appraisal and similar procedures should be integrated in the future tea research in order to cope up with the currently existing environmental phenomena.

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