

Extended Abstract

International Symposium of Sabaragamuwa University of Sri Lanka (ICSUSL) - 2017

Genetic relationships based on SSR polymorphism in wild and ancient cultivated tea trees
(*Camellia sinensis* L.) from China and India.

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1. Abstract

Tea is the most consumed non-alcoholic beverage in the world. China and India are the biggest tea producing countries and accounted for 58% of the global tea production in 2016. These two countries are also vital as domestication centres of tea. Ancient tea germplasm with over 1500 years old tea trees represents the most valuable tea germplasm resource in the world. Related wild species also represent vital gene pools for further genetic improvement. Yet, comprehensive studies on the population structure, genetic relationships among wild and ancient tree tea types lacking. Therefore, in the current study, we used 211 accessions including 78 wild tea plants representing 17 species (12 close wild relatives and 5 distantly related wild species) genotyped with 23 SSRs to investigate the genetic diversity, relationships and wild species contributed to the domestication of cultivated tea. SSR allelic polymorphisms revealed highest expected heterozygosity/genetic diversity ($He=0.812$) and private allele percentages (74.3%) in wild teas. Lowest expected heterozygosity ($He=0.686$) and second highest private allele percentage (13.3%) was reported in Chinese Assam tea (CAS).

Conservation of related wild species and private allele rich CSA is of great importance. PCoA analysis revealed 4 distinct non-overlapping groups. Similarly NJ tree and Structure analysis also reported similar results. This indicates that cultivated tea possibly represent three independent domestication events in China and India. Yet, multiple analysis revealed that Chinese Assam type (CSA) and Indian Assam type tea (CSAIN) were more genetically similar than China type tea (CT). Close wild relative *Camellia taliensis* was found to be possibly contributed to the domestication of CSA in Yunnan, China. Chinese Assam tea was found to be not cultivating in India. Additionally, Indian tea germplasm reported being having a limited gene pool. Therefore, we recommend incorporating wild teas and CSA into the tea breeding programmes in India.

Keywords: Cultivated tea; Domestication; Genetic relationships; Molecular analysis;

2. Introduction and research problem/issue

Tea is the most consumed beverage after water. Tea plant thought to have been firstly domesticated in China around 4500 years ago. China and India are the biggest tea exporters in the world and almost all the tea cultivars were grown in the world directly or indirectly introduced from these two countries.

Classification of tea plant long subjected to debate due to its high plasticity of morphological traits. According to the currently accepted classification system, cultivated tea treated as two varieties, i.e. *C. sinensis* var. *sinensis* (China type tea) and *C. sinensis* var. *assamica* (Masters) Chang (Assam type tea). Recently research revealed that Assam type tea in China is genetically different to that of India and this newly identified tea type from Yunnan, China was referred to as

“Chinese Assam type tea”. While Assam tea from India was noted as

“Indian Assam type tea”. There are ancient cultivated tea populations (100-1500 years old) both in China and India which have not been systematically studied. These ancient populations are key for domestication history studies and important as vital germplasm resources. There are over 120 species in the Genus *Camellia* and 12 of the close wild relatives used to prepare beverage “tea” in China. Genetic relationship of these 12 wild relatives and cultivated tea plant not fully resolved too. In the current study we used 211 tea accessions and employed 23 SSR markers 1) to understand the genetic diversity, genetic relationships of wild and cultivated tea plants; 2) to identify the wild species contributed for the domestication of the tea plant. These findings will be very useful for future tea breeding and systematic germplasm conservation initiatives.

3. Research Methodology

A total of 211 accessions from China and India were included in the present study, and DNA were extracted using modified CTAB method. DNA were quantified using Nanodrop 1000 and subjected to PCR using fluorescently labelled 23 SSR primers. Capillary electrophoresis was performed using ABI 3730 Genome Sequencer. Output profiles from the sequences were manually checked and Gene marker V2.2.0 software was used to record the allele sizes. SSR data was used to calculate genetic diversity indices; Observed heterozygosity (H_o), Expected heterozygosity (H_e), total allele number (A), private alleles (P_A) and Fixation index (F) using the GenA1Ex v.6.5b4 computer package. Principal coordinate analysis was also obtained with the same package. A population structure analysis was performed in STRUCTURE, using

the admixture model and evaluated for 1 to 10 genetic clusters (K) with 20 permutations for each K value. STRUCTURE was run with 100,000 generations of burn-in followed by

100,000 Markov Chain Monte Carlo (MCMC) iterations. The optimal number of genetic clusters, K was obtained using the method of Evonno in STRUCTURE HARVESTER.

We constructed a Neighbour Joining tree (NJ tree) using Nei's genetic distances using MSA v.4.05 with 1,000 multiple runs followed by with 1,000 bootstrap replications Phylip v.3.67. The NJ tree was viewed using FigTree v.1.4.

4. Results

According to the results of the genetic diversity analysis, highest expected heterozygosity/diversity ($H_e=0.812$) was reported for the wild tea accessions (WT) followed by the ancient accessions of Indian Assam type tea (CSAIN). On the other hand, Chinese Assam type tea (CSA) reported the lowest diversity values ($H_e=0.686$). It's natural that wild populations/species exhibiting high genetic diversity than cultivated crop types. However, extensive hybridization of the CSAIN with the China type tea (CSS) in an early 18th century would have reported these higher diversity indices. 74.3% of the private alleles only present in WT followed by 13.3% in CSA. These private alleles may represent unique traits, therefore, conservation and systematic utilisation of WT species/populations and CSA are of great importance. On the other hand, CSAIN reported the lowest private allele percentages. Ancient accessions of CSAIN possible selected from limited elite tea trees by

British in the 1830s. It is vital to incorporate wild species and private allele rich CSA into the tea breeding programmes in India.

Principal coordinate analysis (PCoA) showed four distinct groups with limited overlap.

Population structure analysis revealed similar results too. At $K=4$ of the structure analysis, 17 wild species formed into two clusters yet, the two Assam type teas (CSA and CSAIN) remained intact. Genetic divergence of CSA and CSAIN possibly lower than that of wild species and CSS. Wild species *C. taliensis* showed introgression with CSA. At $K=5$, all three cultivated tea types (CSS, CSA and CSAIN) showed distinct clustering pattern while genetically similar wild species *C. taliensis* and *C. tachangensis* clustered together into one group too. The fifth group represented all other wild species.

Neighbour Joining tree showed similar results to structure and PCoA analysis. Populations/accessions of CSS, WT, CSA and CSAIN showed distinct clustering pattern. Among cultivated tea types, CSS was found to be more genetically divergent compared to CSA and CSAIN. Clustering of some accessions of *C. taliensis* with CSA and also vice versa showed possible introgression and contamination of wild tea gene pools with

cultivated tea. Structure and NJ tree results further confirmed the three independent domestications for cultivated tea. Structure and NJ tree also showed *C. taliensis* possibly contributed to the domestication of CSA in Yunnan province, China.

5. Conclusions, implications and significance

This research shed light into the genetic diversity relationships and domestication of ancient tea trees and their close wild relatives in China and India. Wild tea species *C. taliensis* possibly contributed to the domestication of Chinese Assam tea. Wild tea populations/species and CSA identified as sources of unique private alleles. Incorporation of CSA and WT into the breeding programme in India is recommended.

Results generated is vital for planning the future conservation strategies.

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