

## EVALUATION ON POPULATIONS GENETIC DIVERSITY IN OOLONG TEA GERMPLASMS BY AFLP FINGERPRINTING

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### Summary

Genetic diversity in 45 Oolong germplasms collected from three provinces: Fujian, Guangdong and Taiwan was evaluated by AFLP fingerprinting based on silver staining technique using 5 primer combinations. The 5 primer combinations generated 208 polymorphic bands. Largest genetic distance between germplasm was 0.481, and smallest 0.124. Estimated genetic diversity within the 45 germplasm was up to 0.311. Genetic diversity within Wuyi Mountain population was highest, while genetic diversity within Anxi population was higher than Guangdong and Taiwan populations and lowest within Taiwan population. A dendrogram generated by NT-SYS software indicated that the 45 Oolong tea germplasms could be divided as Wuyi type and Chaoan type. Genetic similarity determined by Popgene 32 software was highest between Anxi and Wuyi populations which was up to 0.9505, and was lowest between Taiwan and Chaoan populations which was up to 0.77. It could be inferred that Fujian Oolong tea germplasm originated from Wuyi Mountains.

**Keywords:** *Camellia sinensis*, Oolong tea germplasm; Genetic diversity; AFLPs

### Introduction

Oolong tea is a semi-fermented type of tea, give us a floral and sweet aroma and a pleasant taste. Its consumption is increasing around the world. Oolong tea germplasm is a valuable part of the overall tea genetic resources. At present, breeding and conservation programs to the Oolong tea breeding were still based on the conventional morphological and agronomic descriptors. These are dependent on environmental and developmental factors. With a view to reveal the genotype differences within Oolong tea germplasms other than phenotypes for Oolong tea breeding, and its classification. AFLP (Amplified Fragment Length Polymorphism) marker were used to analyze 45 Oolong tea from 4 main Oolong tea producing areas; Wuyi Mountains, Anxi county, Chaoan county and Taiwan.

### Materials and Methods

**Plant material:** 45 Oolong tea germplasm from Fujian, Guangdong and Taiwan was used in the study.

**DNA Isolation:** DNA was extracted from half mature tea leaves using the CTAB method as described by Hongya et al., (1999).

**AFLP analysis:** The amplified fragment length polymorphism (AFLP) protocol as described by Vos et al. (1995) was used to detect the diversity. AFLP banding patterns were revealed by silver staining according the methods of Bassam et al (1991).

**Data analysis:** Each band was treated as a separate putative locus, and scored as present (1) or absent (0) in each accession. Estimate for genetic distance was based on Nei and Li's (1979). Cluster analysis was based on UPGMA by NTSYS pc 2.02 software. Genetic diversity was detected by Shannon Informative Index based on Nei (1973) and Shannon (1949), using the PopGene32 (Yeh F.C.) software under Hardy-Weinberg equilibrium.

### Results and Discussion

5 out of 64 primer combinations screened produced clear polymorphic bands, and were selected for analysis. Out of total 226 clear bands scored, 208 were polymorphic (Fig.1). The average number of polymorphic bands per primer combination was 45.

Average polymorphic ratio was 92.03%. Nei's dissimilarity coefficient matrix showed the closest correlation between Daye-wurong and Lunzhi-wurong, and was farthest between the Lvya-fushou and Xingrenxiang-dancong. The dendrogram revealed that 45 Oolong tea cultivars could clearly be distinguished into two groups: Guangdong, and the Fujian and Taiwan types (Fig. 2). Index of Diversity assessed was 0.31 among the 45 accessions. Genetic diversity within Wuyi Mountain population was highest, while genetic diversity within Anxi population was higher than Guangdong and Taiwan.

Studies on genetic similarity among populations revealed maximum genetic similarity (0.9505) between Wuyi and Anxi populations, and minimum genetic similarity (0.77) between Taiwan and Chaoan populations. The Dendrogram of the 4 populations generated by Popgene32 (Fig.3) revealed that 3 populations, Wuyi, Anxi and Taiwan population, were closely related, and could be considered as one type, while Chaoan population belongs to another type. This concurred with the results from PCA analysis (Fig 4). It could therefore be inferred that Fujian Oolong tea germplasm originated from Wuyi Mountains which is in agreement with the evolution route of Oolong tea processing techniques. On the other hand, AFLP fingerprinting is a powerful tool to distinguish the accessions, which were very similar morphologically.

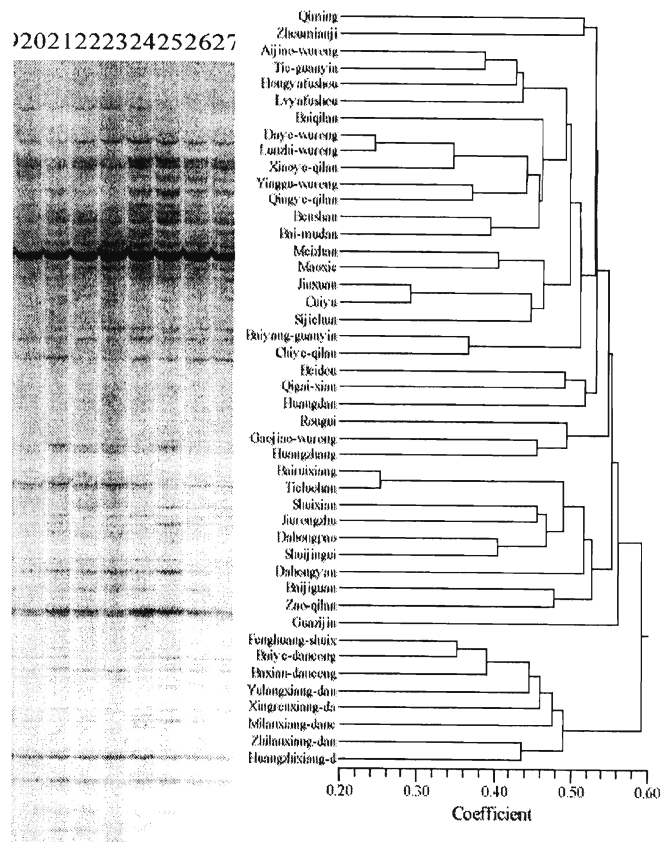


Fig. 1

Fig.2

Fig.1 Part of AFLP fingerprint patterns using primer combination E-ACG/M-CTC

Fig. 2 Dendrogram of Oolong tea germplasm based on AFLP data generated by UPGMA methods (NT-SYS)



Fig.3 Dendrogram of the 4 populations generated by Popgene32.

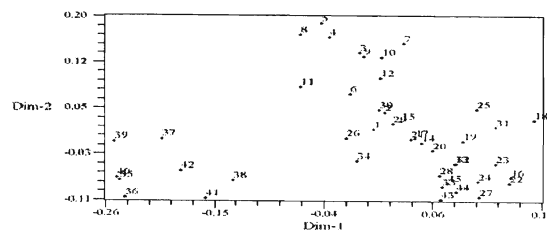


Fig. 4. PCA plot showing the origin and diversity of Oolong tea germplasm.