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An assessment of genetic relationships in cultivated tea clones and native wild tea in Taiwan using RAPD and ISSR markers

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Abstract. Tea (*Camellia sinensis* [L.] O. Kuntze) is an important beverage crop in Taiwan. Most of the cultivated clones were introduced from China and India though some native wild teas are distributed in the mountains of central and southern Taiwan. In this study, 37 tea samples were evaluated using RAPD and ISSR markers. The samples comprised 21 clones of China, 3 clones of Assam, 7 hybrid clones between China and Assam tea, and 6 individual samples of native Taiwanese wild tea. A total of 53 and 56 polymorphic RAPD and ISSR markers respectively, were scored. The results of cluster analysis based on RAPDs revealed that three major groups could be recognized, i.e., cultivars of China tea and the cultivars developed in Taiwan from hybridization and selection; Assam tea; native Taiwanese wild tea. The native Taiwanese wild teas were, however, most distant in the clustering tree. In the ISSR dendrogram, Taiwanese native wild teas clustered closely with Assam tea then with China tea and the Taiwanese hybrid cultivars. The population gene diversity of the native wild tea was found to be the highest among the three populations studied. Analysis of molecular variance (AMOVA) revealed that the variance component within groups was larger than that among groups. The correlation coefficient between similarity matrices based on RAPD and ISSR was 0.811. A Mantel test revealed that the correlation was highly significant ($p<0.001$), indicating good congruence between the results of these two molecular markers.

Keywords: AMOVA; *Camellia sinensis*; Genetic variation; ISSR; RAPD; Tea.

Introduction

Tea (*Camellia sinensis* [L.] O. Kuntze) is an important beverage crop in Taiwan, having been cultivated on the island for more than two hundred years. The tea trade dates back to 1865. The export of tea from Taiwan has, however, been decreasing gradually since 1980 due to competition from other tea-exporting countries (Zuan and Shi, 1995). The production of high quality tea with regional characteristics has nevertheless remained a highly profitable business. The breeding of the new high quality varieties and the conservation of tea germplasm resources continue to be important for the sustainable cultivation of tea in Taiwan.

Tea is an out-crossing species, and selected elite genotypes are propagated vegetatively and released as clonal varieties. Clonal identification has traditionally been based on morphological descriptors such as plant shape, leaf shape, young leaf type, and fruit shape. However, as in many out-crossing crops, tea is highly heterozygous with most of its morphological, physiological and biochemical descriptors showing continuous variation and high plasticity. Similarly, most morphological traits are influenced by environmental factors, plant age and phenology. Furthermore, since objectivity is crucial to accurate morphological typing, the above factors render the use of such descriptors in plant identification and discernment of genetic relationships difficult. Molecular markers such as RAPD and ISSR can, however, be used for these purposes. The advantages of molecular markers are that they are least affected by environmental factors and are almost unlimited in number. They also offer a possibility to observe the genome directly, and thus eliminate the shortcomings inherent in a phenotype observation.

The first tea cultivar cultivated in Taiwan was introduced from Fukien (China), between 1796 and 1820. More cultivars were introduced from China thereafter. From 1926, some of the new cultivars were introduced from India. The cultivars introduced from China belong to China tea (var. *sinensis* [L] O. Kuntze) while those introduced from India belong to Assam tea (var. *assamica* [Mast.] Kitam.). The majority of tea clones cultivated in Taiwan are China tea, reflecting the order of introduction and the preference for domestic consumption. Besides the introduced clones, new clones have been developed by hybridization and selection. The native wild tea (var. *assamica* [Mast.] Kitam. f. *formosensis* Kitam.) grows in the central and southern mountains of Taiwan. Based on leaf characteristics and tree type, they have been taxonomically classified as a form of var. *assamica*. The leaves and young leaf buds of the native wild tea can also be used in the production of tea (Ho and Wang, 1984). Although they are not commonly exploited, the native wild teas provide an important reservoir of genes which can be introgressed into the current generation of clones. Their genetic relationship with cul-

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tivated clones, therefore, deserves investigation. Random Amplified Polymorphic DNA (RAPD) markers, developed by Williams et al. (1990) have been employed widely in cultivar identification and characterisation (Hu and Quiros, 1991; Halward et al., 1992; Hsiao and Rieseberg, 1994; Sharma et al., 1995; Lai and Hsiao, 1997; Mandolino et al., 1999). In tea, this molecular marker system has been used to determine genetic diversity and differentiation within and between cultivated tea and related *Camellia* species (Wachira et al., 1995; Wachira et al., 1997). Intersimple Sequence Repeat Polymorphism (ISSR) markers developed by Zietkiewicz et al. (1994) can also be employed for the same purpose (Wolff et al., 1995; Tsumura et al., 1996; Fang and Roose, 1997; Chen et al., 1998).

Materials and Methods

Materials

Leaf samples were collected from 37 tea cultivars and from native Taiwanese wild tea listed in Table 1. The samples included 21 clones of China tea, 3 clones of Assam tea, 7 China/Assam hybrid clones developed in Taiwan, and 6 individual samples of Taiwanese native wild tea. The samples were collected from theYuchih Substation of the Taiwan Tea Experiment Station.

Table 1. Samples studied and the codes used in the present study.

Code	Clone or accession name
China tea 1	Gungtuyy
China tea 2	Hwanggung
China tea 3	Chinshingungtuyy
China tea 4	Wuuyi
China tea 5	Hongshinwuuyi
China tea 6	Tieeguanin
China tea 7	Sheauyehtieeguanin
China tea 8	Yingtzetzaojoong
China tea 9	Tutuyykengpeimaohu
China tea 10	Heimaohu
China tea 11	Dananuanpeimaohu
China tea 12	Peimaohu
China tea 13	Kimen 1
China tea 14	Kimen 2
China tea 15	Gaolu
China tea 16	Shiangyuan
China tea 17	Dahpang
China tea 18	Chingshindahpang
China tea 19	Dahyehoolong
China tea 20	Hangshinoolong
China tea 21	Chingshinoolong
Hybrid tea 1	TTE No. 11
Hybrid tea 2	TTES No. 12
Hybrid tea 3	TTES No. 13
Hybrid tea 4	Taicha 14
Hybrid tea 5	Taicha 15
Hybrid tea 6	Taicha 16
Hybrid tea 7	Taicha 17
Native wild tea 1	Shueijing (native wild tea)
Native wild tea 2	Laitou (native wild tea)
Native wild tea 3	Fenghuang (native wild tea)
Native wild tea 4	Senshan (native wild tea)
Native wild tea 5	Leye (native wild tea)
Native wild tea 6	Minghai (native wild tea)
Assam tea 1	Assam Indigenous
Assam tea 2	Manipuri
Assam tea 3	Jaripuri

DNA Extraction and Amplification

The DNA was extracted from the leaf samples according to the method of Kobayashi et al. (1998). RAPD amplification generally followed the method of Williams et al. (1990) with some minor modifications of thermal cycles as follows: 94°C for 2 min; 44 cycles of 94°C for 30 s, 36°C for 30 s, 72°C for 2 min; 72°C for 5 min; 4°C end. One hundred and forty decanucleotide primers from kits A, B, C, E, M, Q, and V of Operon Technology Inc. (USA) were screened for polymorphism. The PCR amplifications were carried out on a PTC-100 Thermal Cycler Controller (MJ Research Inc.). ISSR amplification generally followed the method of Tsumura et al. (1996) with a minor modification of the thermal cycles as follows: 94°C for 3 min; 45 cycles of 94°C for 40 s, 50°C for 45 s, 72°C for 100 s; 72°C for 5 min; 4°C end. The DNA polymerase from HT Biotechnology (UK) was used in the optimized RAPD and ISSR polymerase chain reactions. A negative control with no DNA was included in each PCR run. The amplification was repeated at least twice. The amplification products along with a Bio100 DNA ladder (PROtech technology, Inc.) were size fractionated in 1.5% agarose and electrophoresed in 0.5X TBE buffer at 200 V for 2 h and stained with ethidium bromide. The banding patterns were documented on Polaroid 667 films.

Statistical Analysis

Only intensely stained, unambiguous, and reproducible polymorphic bands were scored for presence (1) and absence (0). The binary data was used to calculate similarity matrix among samples using Dice's (1945) algorithm. The similarity matrix was used in an unweighted pair group method using arithmetic averages (UPGMA) cluster analysis. The Pearson's correlation coefficient between the similarity matrices based on RAPD and ISSR data was calculated and the significance of the correlation tested by the Mantel test (Sokal and Rohlf, 1995). The samples, excluding hybrid tea clones developed in Taiwan, were separated into the three groups of China, Assam, and native wild tea. A matrix of inter-phenotypic distances was constructed and analyzed with WINAMOVA 1.55 (Analysis of Molecular variance) according to the formula of Excoffier et al. (1992), $D=N(1-(N_{11}/N))$, where N is the total number of polymorphic bands and N_{11} the number of bands shared by two samples. Genetic variation was then partitioned within and among groups and significance values assigned to variance components based on the random permutation (10,000 times) of individuals assuming no genetic structure. Nei's gene diversity (H_s; Nei, 1973) for each group was derived using the computer program, POPGENE 3.2 (Yeh et al., 1999) assuming Herdy-Weinberg equilibrium.

Results

RAPD

Out of the primers screened, 12 (Table 2) produced intensely stained, reproducible bands. An example of the polymorphisms detected among some test samples by primer OPV6 is shown in Figure 1. A total of 53 polymorphic bands (Table 2) were scored from the amplifications using these primers. The average number of polymorphic bands per primer was 4.4. All of the tea clones could be distinguished from one another based on these polymorphic bands except "Heimaohu" and "Dananuanpeimaohu", which had identical profiles. Similarities among the test samples calculated with Dice's formula (Dice, 1945) indicated that the highest similarity (1.000) was between "Heimaohu" and "Dananuanpeimaohu" while the lowest (0.238) was between "Shiangyuan" and "Laitou". The similarity matrix obtained (not presented) was used in an UPGMA cluster analysis. The cophenetic correlation coefficient of this cluster analysis was 0.870, indicating good agreement between the similarity matrix and cophenetic matrix although a certain degree of distortion is usually inevitable in the clustering. The dendogram (Figure 2) showed that three groups could be recognized at a similarity index of 0.5. The first group consisted of all cultivars of China tea. The second group consisted of three cultivars of Assam tea while the third group consisted of samples of native wild tea. The dendrogram also indicated that the group of native wild tea was the most isolated group. Seven clones developed in Taiwan and selected from a hybridization program between China and Assam tea were clustered in the China tea group. The clustering was consistent with morphological similarity except in the case of cultivar "TTE No. 11", which clustered with the China cultivars though it is morphologically more similar to Assam tea. Within the group of native wild tea, two subgroups could be recognized at the similarity index of 0.6. One subgroup consisted of Shueijing and Laitou, both of which have red leaf buds. The other subgroup was composed of native wild tea without the red color.

The Nei's gene diversity measure for each group is listed in Table 3. The native Taiwanese wild tea group had the largest within group diversity (H=0.280) while the Assam tea group had the least diversity (H_s=0.074). The diversity within the China tea group was H_s=0.197. The results of AMOVA (Table 4) showed that the variance components among groups and among individual samples within groups were 5.099 (46.69%) and 5.822 (53.31%), respectively.

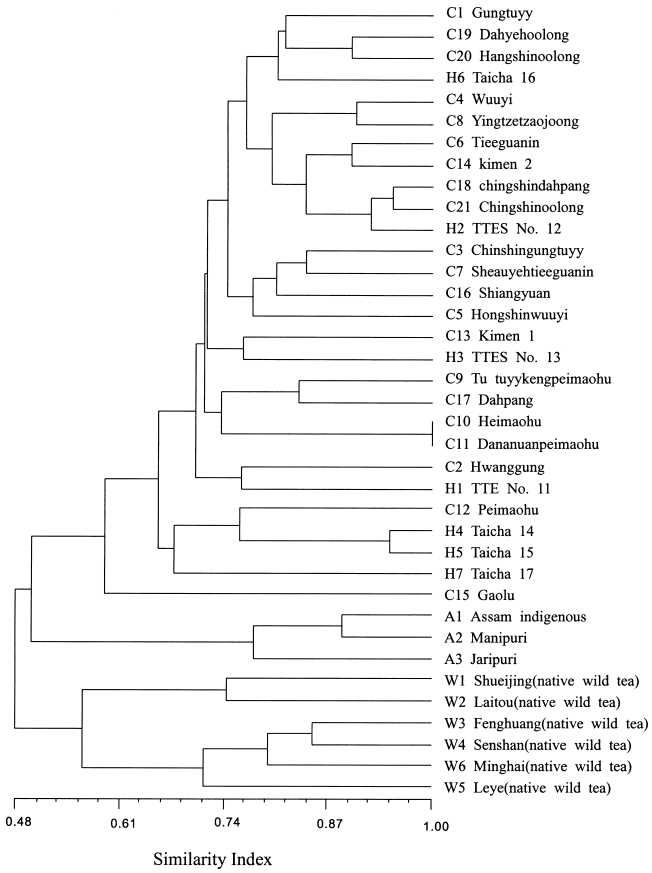

Figure 2. Dendrogram based on polymorphic RAPD bands.

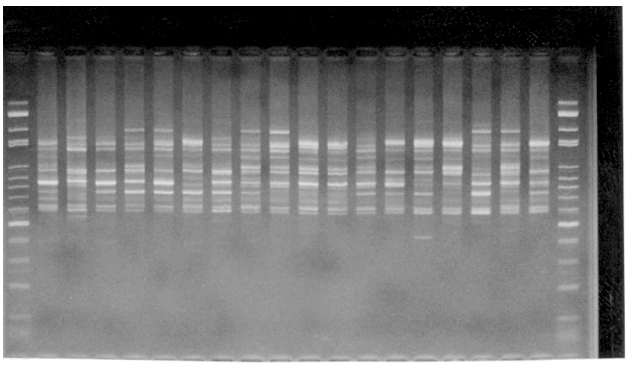

Figure 1. An example of the polymorphisms detected among some test samples using RAPD primer OPV6. (left to right: lane 1, 100 bp marker; lane 2 - lane 19, samples C1-C18; lane 20, 100 bp marker).

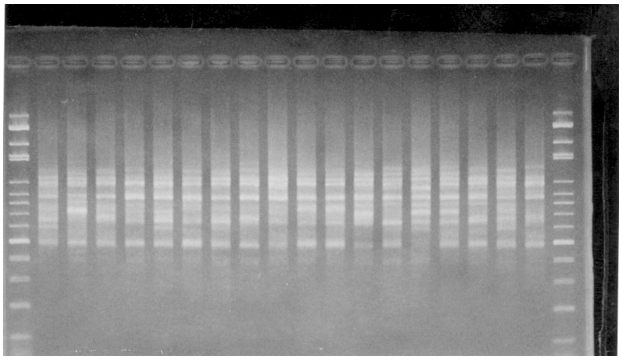

Figure 3. An example of the polymorphisms detected among some test samples using ISSR primer 814.1. (left to right: lane 1, 100 bp marker; lane 2 - lane 19, samples C1-C18; lane 20, 100 bp marker).

Table 2. Nucleotide sequences of primers used and the fragments recorded.

Primer code	Sequence (5'-3')	Fragments recorded
OPB10	CTGCTGGGAC	0.55 kb, 0.75 kb, 1.2 kb, 1.6 kb, 1.8 kb
OPQ13	GGAGTGGACA	0.65 kb, 0.71 kb, 0.8 kb, 0.95 kb, 1.2 kb
OPM2	ACAACGCCTC	0.85 kb, 0.95 kb, 1.2 kb, 1.45 kb, 1.8 kb
OPV2	AGTCACTCCC	0.45 kb, 0.71 kb, 0.95 kb, 1.55 kb, 2.1 kb
OPQ5	CCGCGTCTTG	0.38 kb, 0.45 kb, 0.5 kb, 0.65 kb, 0.68 kb
OPE12	TTATCGCCCC	0.31 kb, 0.45 kb, 0.55kb, 0.88 kb
OPV6	ACGCCCAGGT	0.52 kb, 0.65 kb,0.71 kb, 0.95 kb,1.5 kb
OPA8	GTGACGTAGG	0.72kb, 0.73 kb, 1.2kb
OPA9	GGGTAACGCC	0.49 kb, 0.95 kb, 1.1 kb, 1.2 kb, 2.8 kb
OPA12	TCGGCGATAG	0.8 kb, 0.92 kb, 1.2 kb, 1.7 kb
OPA4	AATCGGGCTG	0.43 kb, 0.65 kb,1.1 kb
OPV12	ACCCCCCACT	0.72kb, 0.84 kb, 1.2kb, 1.3 kb
814.1	$(CT)_8TG$	0.51 kb, 0.55kb, 0.64 kb, 0.65kb, 0.81 kb, 0.82kb, 0.94 kb, 1.15kb, 1.2 kb, 1.4kb, 1.41 kb
UBC830	$(GA)_8C$	0.52 kb, 0.54kb, 0.55 kb, 0.65kb, 0.7 kb, 0.84kb, 0.85 kb, 0.96kb, 0.98 kb, 1.05kb, 1.1 kb
UBC825	$(AC)_8YT$	0.55 kb, 0.66kb, 0.82 kb, 0.84kb, 0.86 kb, 0.93kb, 0.96 kb, 1.05kb, 1.2 kb
UBC840	$(AC)_8YG$	0.51 kb, 0.63kb, 0.66 kb, 0.84kb, 0.95 kb, 1.1kb, 1.35 kb, 1.4kb, 1.6 kb
UBC842	$(CT)_8RG$	0.5 kb, 0.72kb, 0.74 kb, 0.95kb, 0.96 kb, 1.2kb, 1.22 kb, 1.26kb, 1.28kb
17901	$(GT)_6YR$	0.71 kb,0.72 kb, 0.83 kb,0.84 kb, 1.2 kb,1.3 kb, 1.35 kb

ISSR

Out of the twenty 3'-anchored ISSR primers screened, six (Table 2) produced non-ambiguous and reproducible bands. An example of the polymorphisms detected among some test samples by primer 814.1 is shown in Figure 3. A total of 56 polymorphic bands were scored in the test samples from the primers. The average number of polymorphic bands per primer was 9.3. All the tea clones could be distinguished from one another based on these polymorphic bands.

Similarities among samples calculated with Dice's formula (data not presented) indicated that the highest similarity (0.923), was between "Heimaohu" and "Dananuanpeimaohu" while the lowest similarity (0.270) was between Minghai of native wild tea and "Gaolu". The similarity matrix (not presented) was used in an UPGMA cluster analysis. The cophenetic correlation coefficient of this cluster analysis was 0.843. The dendogram observed (Figure 4) showed that two groups could be recognized at the similarity index of 0.5. The first group consisted of all China and hybrid cultivars. As was demonstrated with RAPDs, cultivar "TTE No. 11", which is morphologically similar to Assam tea also clustered with China tea. The second group consisted of samples of native wild tea and cultivars of Assam tea. Within this group, two subgroups could be recognized at the similarity index of 0.55. One subgroup consisted of the red leafed native wild teas Shueijing and Laitou. The second subgroup consisted of Assam tea cultivars and some non-red leafed native wild teas. The cultivars of Assam tea were separated from samples of native wild tea within the second subgroup.

The Nei's gene diversity measure for each group is listed in Table 3. The native wild tea group had the largest within group diversity (H_s=0.253) while the Assam tea had the least diversity (H_s=0.114). The diversity within the China tea group was H_s=0.181. AMOVA (Table 4) revealed that the variance components among groups and among individual samples within groups were 5.021 (44.11%) and 6.362 (55.89%), respectively.

Table 3. Gene diversity (H_s) within China, Assam and native wild tea.

	China tea	Assam tea	Native wild tea
RAPD	0.197	0.074	0.280
ISSR	0.181	0.114	0.253

RAPD and ISSR Combined

When RAPD and ISSR data were combined, the results of cluster analysis (Figure 5) showed two major groupings similar to the result of ISSR.

Table 4. Result of analysis of molecular variance (AMOVA).

	RAPD						ISSR					
Source of variation	d.f.	SSD	MSD	Variance component	% Total	P*	d.f.	SSD	MSD	Variance component	% Total	P*
Among groups	2	82.0	41.0	5.099	46.69	<0.001	2	82.0	41.0	5.021	44.11	<0.001
Among individuals wthin group	27	156.6	5.8	5.822	53.31	<0.001	27	172.8	6.4	6.362	55.89	<0.001

*After 10,000 random permutations.

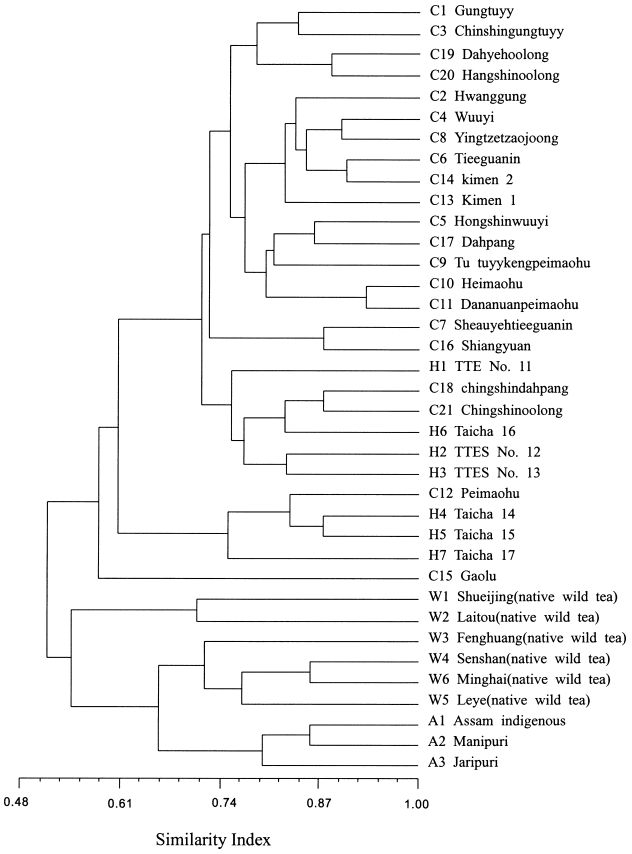

Figure 4. Dendrogram based on polymorphic ISSR bands.

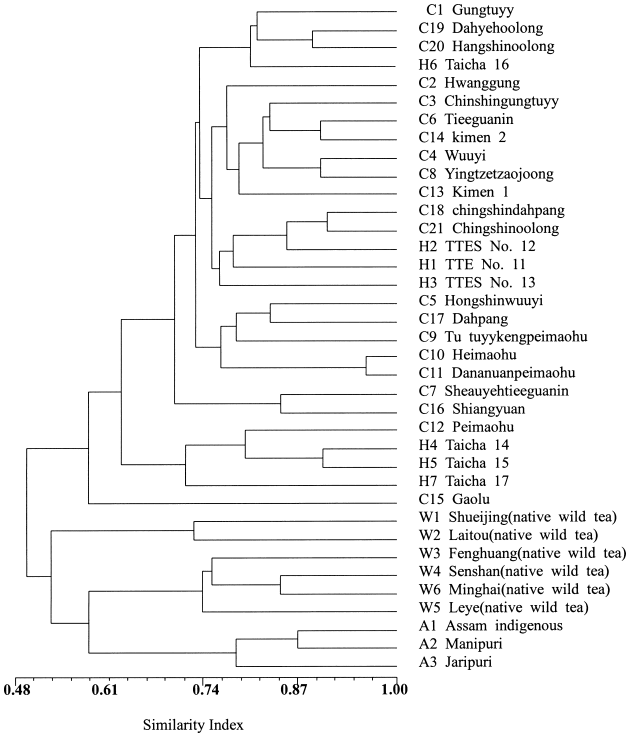

Figure 5. Dendrogram based on polymorphic RAPD and ISSR bands.

Discussion

Three main groups could be recognized from the result of cluster analysis based on RAPD data. Samples of Assam tea formed a group while those of native wild tea formed another group. The remaining samples clustered in a group that consisted of samples of China tea and the hybrid tea clones developed in Taiwan. The hybrid tea clones are generally closer to China tea morphologically with the exception of clone "TTE No. 11", which is closer to Assam tea. The major groupings in the dendrogram based on RAPD markers are thus, with the exception of "TTE No. 11", consistent with morphological differentiation. This result seems to indicate that "TTE No. 11" is closer to China tea in overall genetic relationship. Wachira et al. (1995) studied the relationship among tea clones using RAPD markers and found that, among the clones studied, some clones clustered in the Assam tea group despite their seemingly China characteristics. These clones were thought to be putative hybrids of China and Assam varieties which had retained morphological traits of the former. Genetic differentiation based on ISSR polymorphism was similar to that obtained using RAPD markers. When RAPD and ISSR data were pooled together, the results of cluster analysis showed two major groups, similar to the result obtained using ISSR markers alone. These results support the taxonomic treatment in which the native wild tea was treated as an Assam variety.

Clones "TTES No. 12" and "TTES No. 13" are difficult to distinguish morphologically. However, these clones are different in parentage, method of cultivation, and tea quality. They can be distinguished based on their RAPD profiles, indicating that there is genetic difference between them. These two cultivars could also be separated based on ISSR markers. The result indicated that both RAPD and ISSR have high resolution in the genotype identification of tea.

Clones "Peimaohu", "Dananuanpeimaohu", and "Tutuyykengpeimaohu" were introduced from Fukien, China (Zuan and Shi, 1995). They had been suspected to be the same clone, despite their minor morphological differences. They could, however, be separated based on their RAPD and ISSR profiles. The similarities among these cultivars ranged from 0.68 to 0.74 for RAPD and from 0.58 to 0.76 for ISSR.

Clones "Taicha 14", "Taicha 15", "Taicha 16", and "Taicha 17" were developed from "Peimaohu". Both RAPD and ISSR dendrograms showed that "Taicha 14", "Taicha 15", and "Taicha 17" clustered with "Peimaohu", reflecting pedigree relationship. Although "Taicha 16" did not cluster with "Peimaohu", it had a high RAPD similarity (0.80) and high ISSR similarity (0.77) with "Peimaohu". The result confirmed the potential of RAPD and ISSR in reflecting a pedigree relationship.

Clones "Shiangyuan" and "Gaolu" are similar in morphology. They are triploids and thus flower rarely. "Gaolu" was introduced from Japan while "Shiangyuan"

was introduced from Fukien, China. The RAPD similarity (0.58) and ISSR similarity (0.60) between them indicated a large genetic difference in spite of morphological similarity. "Shiangyuan" has the highest RAPD similarity (0.81) and ISSR similarity (0.86) with "Sheauyehtieequanin". They were both introduced from Fukien, China and have similar leaf morphology.

Taiwan native wild tea is close to Assam tea morphologically, and it has been taxonomically treated as an Assam variety. The cluster analysis based on RAPD markers revealed that it is the most isolated group. The variety *assamica* is distributed from southern China to northern India. In the present study, three clones introduced from India were analyzed, representing only a small portion of the total variation of the variety *assamica*. The result of the RAPD study suggests that the native wild tea is native to Taiwan and not the result of an escape from cultivation because there is considerable genetic difference between it and the cultivated teas. The germplasm of native wild tea might be useful in plant breeding programs and deserve conservation. The result of the ISSR study suggests that the native Taiwanese wild tea is close to Assam tea genetically and thus supports the current taxonomic treatment.

Table 3 showed that Assam tea had the smallest within group diversity while native wild tea had the largest for both RAPD and ISSR markers. Three clones of Assam tea were included in the present study. These clones represent only a small portion of the genetic variation of Assam tea. This might account for the small within group diversity of Assam tea observed. Six samples of native wild tea were studied, and the diversity within native wild tea was larger than China tea, which consisted of 21 cultivars. The result indicated that the native wild tea is highly variable and the germplasms of native wild tea might be useful in breeding programs.

Wachira et al. (1995) studied the genetic diversity and relationship of 38 tea clones of China, Assam, and Cambod tea (*C. assamica* ssp. *lasiocalyx* Planchon ex Watt) using RAPD markers and showed that 30% of the total diversity resided among populations while 70% resided within populations based on Shannon's diversity index. The result of cluster analysis was generally consistent with the taxonomic treatment. Wachira et al. (1997) studied the genetic relationship of 20 species of the genus *Camellia* using RAPD and organelle-specific PCR. The result was generally consistent with the classification based on morphology and the relationship revealed from comparative phytochemical data. Paul et al. (1997) studied the diversity and genetic differentiation of India and Kenyan tea using AFLP markers. Shannon's index of diversity was used to partition the total variation, and they found that most of the diversity was detected within populations, with 79% of the variation being within and 21% being between populations of India and Kenyan tea. A dendrogram constructed on the basis of band sharing separated the tea samples into China, Assam and Cambod types. The genetic diversity and relationships of tea clones available in Taiwan, together with samples of native wild tea, were investigated in the present study using RAPD and ISSR markers. The results may serve as reference information for tea breeding and germplasm conservation purposes. In the present study, native wild tea was included as a group while Cambod tea was not studied. The AMOVA analysis revealed that the percentages of variances attributable to the differences among and within groups were 46.69% and 53.31% for RAPD and 44.11% and 55.89% for ISSR, respectively. These results indicated considerable genetic differentiation among the three groups studied. Wachira et al. (1995) didn't analyze their data using AMOVA.

The Pearson's correlation coefficient between similarity matrices based on RAPD and ISSR was 0.811. The Mantel test revealed that the correlation was highly significant ($P<0.001$). This implied that the results of RAPD and ISSR studies were highly congruent. However, ISSRs seemed to be more discriminative in cultivar identification then RAPDs because cultivars that could not be distinguished by RAPD markers were separable based on ISSR markers. This finding suggests that the evolution rate of ISSRs might be faster then that of RAPDs in the tea samples studied. ISSR markers are, therefore, suitable for use in the study of genetic diversity and determination of genetic relationships of closely related tea cultivars. Other studies also indicated that ISSR markers exhibit higher levels of polymorphism than RAPD markers (Yang et al., 1996; Nagaoka and Ogihara, 1997; Parsons et al., 1997; Esselman et al., 1999). The observed difference between ISSR and RAPD might be explained in terms of functional constraints since some RAPD bands may be associated with functionally important loci (Penner, 1996). The fast evolutionary rate and the hypervariability of ISSR may suggest that ISSR bands represent neutral markers (Esselman et al., 1999).

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Literature Cited

Chen, Y., G. Hausner, E. Kenaschuk, D. Procunier, P. Dribnenki, and G. Penner. 1998. Identification of microspore-derived plants in anther culture of flax (*Lilium usitatissimum* L.) using molecular markers. Plant Cell Rep. **18:** 44-48.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology **26:** 297-302.

Esselman, E.J., L. Jianqiang, D.J. Crawford, J.L. Windus, and A.D. Wolfe. 1999. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (Poaceae): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and intersimple sequence repeat (ISSR) markers.

Mol. Ecol. **8:** 443-451.

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from matrix distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131:** 479-491.

Fang, D.Q. and M.L. Roose. 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. Theor. Appl. Genet. **95:** 408-417.

Halward, T., T. Stakker, E. LaRue, and G. Kochert. 1992. Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.). Plant Mol. Biol. **18:** 315-325.

Ho, S.H. and L.C. Wang. 1984. The collection of native wild tea in Taiwan. Taiwan Tea Res. Bull. **3:** 133-155 (in Chinese).

Hsiao, J.Y. and L.H. Rieseberg. 1994. Population genetic structure of *Yushania niitakayamensis* (Bambusoideae, Poaceae) in Taiwan. Mol. Ecol. **3:** 201-208.

Hu, J. and C.F. Quiros. 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep. **10:** 501-511.

Kobayashi, N., T. Horikoshi, H. Katsuyama, T. Handa, and K. Takayanagi. 1998. A simple and efficient DNA extraction method for plants, especially woody plants. Plant Tissue Cult. Biotech. **4:** 76-80.

Lai, C.C. and J.Y. Hsiao. 1997. Genetic variation of *Phyllostachys pubescens* (Bambusoideae, Poaceae) in Taiwan based on DNA polymorphisms. Bot. Bull. Acad. Sin. **38:** 145-152.

Mandolino, G., A. Carboni, S. Forapani, V. Faeti, and P. Ranalli. 1999. Identification of DNA markers linked to the male sex in dioecious hemp (*Cannabis sativa* L.). Theor. Appl. Genet. **98:** 86-92.

Nagaoka, T. and Y. Ogihara. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. **94:** 597-602.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA **70:** 3321-3323.

Parsons, B.J., H.J. Newbury, M.T. Jackson, and B.V. Ford-Lloyd. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. Mol. Breeding **3:** 115-125.

Paul, S., F. N. Wachira, W. Powell, and R. Waugh. 1997. Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) Kuntze, O) revealed by AFLP markers. Theor. Appl. Genet. **94:** 255-263.

Penner, G.A. 1996. RAPD analysis of plant genomes. *In* P.P. Jauhar (ed.), Methods of Genome Analysis in Plants. CRC Press, Boca Raton, pp. 251-268.

Sharma, S.K., I. K. Dawson, and R. Waugh. 1995. Relationship among cultivated and wild lentils revealed by RAPD analysis. Theor. Appl. Genet. **91:** 647-654.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry. Third Edition. Freeman, New York.

Tsumura, Y., K. Ohba, and S.H. Strauss. 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theor. Appl. Genet. **92:** 40-45.

Wachira, F.N., R. Waugh, C.A. Hackett, and W. Powell. 1995. Detection of genetic diversity in tea (*Camellia sinensis*) using RAPD markers. Genome **38:** 201-210.

Wachira, F.N., W. Powell, and R. Waugh. 1997. An assessment of genetic diversity among *Camellia sinensis* L. (cultivated tea) and its wild relatives based on randomly amplified polymorphic DNA and organelle-specific STS. Heredity **78:** 603-611.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. **18:** 6531-6535.

Wolff, K., E. Zietkiewicz, and H. Hofstra. 1995. Identification of chrysanthemum cultivars and stability of DNA fingerprinting patterns. Theor. Appl. Genet. **91:** 439-447.

Yang, W., A.C. de Oliveira, I. Godwin, K. Scheritz, and J.L. Bennetzen. 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. Crop Sci. **36:** 1669-1676.

Yeh, F.C., R.-C. Yang, T.B. J. Boyle, Z.-H. Ye, and J.X. Mao. 1999. POPGENE 3.2, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Alberta, Canada.

Zietkiewicz, E., A. Rafalski, and D. Labuda. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics **20:** 176-183.

Zuan, Y.M. and Y.S. Shi. 1995. The History of Tea Plantation in Taiwan. Taiwan Tea Agriculture Improvement Institute, Taipei (in Chinese).

應用 RAPD 及 ISSR 研究臺灣栽培與野生茶樹之遺傳歧異度及親緣關係

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茶樹（*Camellia sinensis* L. (O. Kuntz)）是重要的經濟作物。臺灣的茶樹栽培品種主要源自於中國及印度，而分佈在台灣山區的野生茶樹未被廣泛的利用與研究。在本研究中，應用 RAPD 及 ISSR 技術分析臺灣地區引進的品種、雜交育成的品種和臺灣野生茶樹共 37 個樣本，其中包括中國茶 21 個樣本，阿薩姆茶 3 個樣本，中國茶與阿薩姆茶雜交所育出之品種 7 個樣本，及臺灣原產野生茶樹 6 個樣本。結果以 12 個 RAPD 引子及 6 個 ISSR 引子分別得到 53 及 56 個多型性條帶。RAPD 分子標誌除了大南灣白毛猴及黑毛猴不能區分外能區分其他的樣本，而 ISSR 分子標誌能區分所有的樣本。由 RAPD 分子性狀的歸群分析得到三群，第一群包含所有的中國茶栽培品種及雜交育成的品種，第二群包含阿薩姆茶之三個品種，第三群則包含臺灣原產野生茶樹。而在ISSR之歸群圖，原產野生茶樹先與阿薩姆茶連結，然後與中國茶及雜交育成品種所構成之一群連結。各栽培品種大致依照發源地及分類地位而分群。臺灣的野生茶樹的分子性狀偏向於阿薩姆變種，且野生茶樹樣本間的歧異度最大。依歸群結果之分群進行 AMOVA 分析的結果指出群內變方成分大於群間的變方成分，與前人研究相符。比較 RAPD 及 ISSR 相似度矩陣，兩矩陣間相關係數為 0.811，Mantel 檢定之結果顯示此相關極顯著（P<0.001），因此兩種分子標誌所得結果有高的吻合度。以品系的區分能力顯示 ISSR 技術具有較高的靈敏度。

關鍵詞：AMOVA；*Camellia sinensis*；遺傳歧異度；RAPD；ISSR；茶。