

# Studies of genetic diversity and the establish of the molecular data base of Taiwan tea cultivars

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## ABSTRACT

In this study, different tea varieties preserved in Taitung Tea Research and Extension Station (TTES) were used as materials. Tea leaves were manufactured to green tea and 10 kinds of tea catechins were determined by HPLC, and to study the genes Diversity based on these chemical markers. Principal component analysis and UPGMA clustering analysis based on the contents of tea catechins have been used in the study of correlations between manufacturing suitabilities and agronomic traits. Molecular markers, including the nucleotide sequences of pITS2, trnL intron, trnL-trnF IGS were also used in determining the genetic diversity of teas preserved in Tea Research and Extension Station of tea in Taiwan. Our results have shown that the breed tea varieties are generally have higher total catechin content. TTES No.4 has the highest methylation catechins of 3.3 tea (g/100g d.w.), with the greatest potential used in anti-allergy products, for all the tea species tested. Catechins contents in wild *Camellia* speciesis generally low. Even though these indigenous tea species are much more resist to the environment stress. Unfortunately, more or less negative agronomic traits will be carriered. Total catechins (TC) content is correlated to the contents of caffeine, EC, ECG, EGC, EGCG and four methylated catechin content. Caffeine 's content is correlated to the content of EGCG, ECG. Principal component analysis (PCA) results have shown that Taiwan wild camellia Tea fell the third and fourth quadrant. Taiwan wild camellia Tea is best for making black tea, and most of the tea cultivars located in third and fourth quadrant are suitable for manufacturing black tea too. For those tea cultivars suitable for manufacturing green tea are scattering in all four quadrantswith overlapping some cultivars suitable for manufacturing Fermented tea. PCA results also indicated that the content and distribution of catechins are correlated with the tea varieties and the manufacturing suitablility and are valuable data for future tea breeding reference. The higher content of caffeine and EGCG are related to the capacities ofinsect resistance of tea, such as anti-mite, anti-leaf beetle, anti-thrips and leaf beetle resistance. Cluster analysis for tea varieties based on UPGMA method using MVSP software has separated two wild *Camellia* Tea (TD85 and TD100) can be species with other into an independent cluster, showing their genetic pecifity. Single nucleotide polymorphisms were observed based on three multiple sequence alignments of pITS2 sequence of 112 tea varieties, 104 trnL intron sequences, and 98 trnL-trnF IGS sequencespITS2 has the highest nucleotide sequence polymorphism with sequence similarity between 0.379 ~ 0.994, and up to 149 variable sites. Two cpDNA sequences are relatively conserved in these two area, with sequence similarity of 0.948~1.000 for trnL intron and sequence similarity of 0.979 to 1.000 for trnL-trnF IGS. The sequence variation of pITS2 can be used in establishing the molecular fingerprinting database for Taiwan tea plants. Phylogenetic trees constructed based on nucleotide sequence of pITS2 using Neighbor Joining Method, Minimum Evolution methods and Mmaximum parsimony methods can clustered wild teas in Taiwan into two independent clustersalong with other five groups. Our results in genetic diversity is important in future tea breeding selection program.

Keywords : tea、catechins、molecular markers、germplasm resource、genetic diversity

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## REFERENCES

- 王建波、張文駒、陳家寬。1999。核rDNA的ITS序列在被子植物系統與進化研究中的應用。植物分類學報。37(4):407-416。
- 史樞、陳永盛、楊宗國、石振原、廖增祿。1972。臺灣野生茶之調查。臺灣農業 8 ( 4 ) :199-201。
- 李臺強、張清寬。2003。臺灣茶樹種原圖誌-建廠100週年紀念特刊。第1-202頁。行政院農委會茶葉改良場編印。臺北，臺灣。
- 甘子能。1983。近二十年來茶葉化學的研究發展。食品工業15 ( 10 ) :23-27。
- 甘子能。1985。製茶原理的生化觀。食品工業17 ( 7 ) :25-37。
- 池宗憲、林芊玲、何南輝。2002。臺灣茶街。宇河文化出版有限公司。臺北，臺灣。
- 吳振鐸。1973。從茶湯之化學成分談臺灣茶葉品質之改進問題。臺灣農業季刊。9 ( 1 ) :194-198。
- 呂海鵬、譚俊峰、林智。2006。茶樹種質資源EGCG3"Me含量及其變化規律研究。茶葉科學26 ( 4 ) :310-314。
- 汪毅。2006。茶葉中甲基化EGCG的調查研究:34。西南大學碩士論文。重慶，中國。
- 阮逸明、陳英玲。1998。茶葉中兒茶素類萃取及純化之研究。臺灣茶葉研究彙報17:1-8。
- 阮逸明。1996。臺灣省茶業改良場場誌。第130-164頁。臺灣省茶葉改場編印。桃園，臺灣。
- 阮逸明。1997。臺灣之茶文化及其科學。臺灣茶葉研究彙報16:79-85。
- 林木連、蔡右任、張清寬等著。2003。臺灣的茶葉。第23-25頁。行政院農委會茶業改良場。臺北，臺灣。
- 林亞平、胡智益、蔡右任、林福順。2010。成茶品種快速分子鑑定技術之研究及應用。作物、環境與生物資訊7:37-51。
- 林福順。2006。中草藥基原之DNA鑑定。『中醫藥基因體研究及其核心技術訓練』暨『系統生物學虛擬實驗室研討會』，2006年10月20-21日，臺北市。
- 林世昱。2002。應用逢機增殖DNA片段檢測茶樹品種的親緣關係。國立臺灣大學園藝學研究所碩士論文。臺北，臺灣。
- 今惠淑、梁月榮、陳建良。2001。中、韓兩國主要茶樹品種基因組DNA多態性比較研究。茶葉科學，21: 103-107。
- 張捷。2010。臺灣地區高甲基化兒茶素茶樹樹種篩選及其種質資源歧異度研究。大葉大學生物產業科技學研究所碩士論文。彰化，臺灣。
- 胡益智、蔡右任、林順福。2007。利用DNA分子標誌鑑別臺灣茶樹品種極評估種原之遺傳歧異度。中國農學通報，23:380-385。
- 胡智益。2004。臺灣茶樹種原葉部性狀及DNA序列變異之探討。國立臺灣大學園藝學研究所碩士論文。臺北，臺灣。
- 徐英祥編譯。1995。臺灣之茶樹品種-臺灣日據時期茶業文獻編譯。第1-25頁。臺灣省茶業改良場編印。桃園，臺灣。
- 徐英祥、阮逸明。1993。臺灣茶樹育種回顧。臺灣茶業研究彙報12:1-18。
- 張如華、阮逸明、蔡永生。1995。茶葉主要化學成分於製茶過程中之變化及其對品質之影響。農特產品加工研討會專刊:120-148。
- 陳右人。1998。茶樹品種與育種介紹。茶葉技術推廣手冊茶作篇。第7-14頁。臺灣省茶業改良場編印。桃園，臺灣。
- 陳右人。2005。臺灣茶之種源與育種。臺灣學者論文。第11屆。臺北，臺灣。
- 陳志輝。2004。分子標記技術在茶樹研究中的應用。福建茶業試驗方法，3:22-24。
- 劉本英、成浩。2007。分子指紋圖譜技術及其在茶樹品種資源中的應用。茶葉 33卷4期:198-202。
- 蔡俊明、張清寬、陳右人、陳國任、蔡右任、邱垂豐、林金池、范宏杰。2004。2004年度命名茶樹新品種臺茶19 號及臺茶20 號試驗報告。臺灣茶業研究彙報 23:57-78。
- 蔡憲宗、蔡依真、廖文如、張清寬、王裕文。2003。利用AFLP及RAPD分子標誌分析臺灣茶樹品種(系)遺傳歧異度。臺灣茶業研究彙報，22:17-32。
- 蔡憲宗、蔡依真、廖文如、張清寬、王裕文。2004。臺灣地區青心烏龍品種外表型及AFLP標記變異之研究。臺灣茶業研究彙報，23:21-30。
- 蔡依真。2003。臺灣茶樹原遺傳歧異度研究。國立臺灣大學農藝研究所碩士論文。臺北，臺灣。
- 蘇夢淮。2006。臺灣山茶之分類研究。國立臺灣大學生態學與演化生物學研究所碩士論文。臺北，臺灣。
- 賴正南編譯。1990。茶業技術推廣手冊製茶技術。行政院農業委員會茶葉改良場編印。桃園，臺灣。
- 賴昭安。1998。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。國立中興大學植物學系碩士論文。
- Astrid Nehlig, Daval Jean-Luc, Gerard Debry. 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Research Reviews 17 ( 2 ) :139-170.
- Alvarez, I., Wendel, J. F. 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. 29:417-434.
- Ariyarathna, C. and Gunasekare, K. 2006. Genetic base of tea (*Camellia sinensis* L.) cultivars in Sri Lanka as revealed by pedigree analysis. J. Appl. Genet., 48:125-128.
- Cao, G.. Sofic, E. Prior, R. 1996. Antioxidant capacity of tea and common vegetables. J Agric Food Chemistry 44:3426 – 3431.
- Carmen Cabrera, Reyes Artacho, Rafael Giménez. 2006. Beneficial Effects of Green Tea—A Review. Journal of the American College of Nutrition. 25 ( 2 ) :79 – 99.

- 40.Chiu Feng-Lan. Lin Jen-Kun. 2005. HPLC Analysis of Naturally Occurring Methylated Catechins,3 " - and 4 " -Methyl-epigallocatechin Gallate, in Various Fresh Tea Leaves and Commercial Teas and Their Potent Inhibitory Effects on Inducible Nitric Oxide Synthase in Macrophages. *J. Agric. Food Chemistry* 53:7035-7042. 41.Chiou, S. J. Yen, J. H. Fang, C. L. Chen, H. L. Lin, T. Y. 2007. Authentication of medicinal herbs using PCR-amplified ITS2 with specific primers. *Planta Med* 73:1421-1426. 42.Chen, S. Yao, H. Han, J. Liu, C. Song, J. Shi, L. Zhu, Y. Ma, X. Gao, T. Pang, X. Luo, K. Li, Y. Li, X. Jia, X. Lin, Y. Leon, C. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS One*. 5:e8613. 43.Chung, F.L. Schwartz, J. Herzog, C.R. Yang, Y. M. 2003. Tea and cancer prevention: Studies in animals and humans. *J Nutr* 133:3268 – 3274. 44.Fujimura Yoshinori. Umeda Daisuke. Yamada Koji. Tachibana Hirofumi. 2008. The impact of the 67 kDa laminin receptor on both cell-surface binding and anti-allergic action of tea catechins. *Archives of Biochemistry and Biophysics* Volume 476 ( 2 ) :133-138. 45.Fukuda, T. Yokoyama, J. Ohashi, H. 2001. Phylogeny and biogeography of the genus *Lycium* (Solanaceae): inferences from chloroplast DNA sequences. *Mol. Phylogenetic Evol.* 19:246 – 258. 46.Gerats, A. M. Martin, C. 1992. Flavanoid synthesis in *Petunia hybrida*: genetics and molecular biology of flower colour. *Phenolic Metabolism in Plants* 167 – 175. 47.Gupta,S., K. Hastak., N. Ahmad, J.S. Lewin, and H. Mukhtar. 2001. Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. *Proc. Natl. Acad. Sci. USA* 98:10350-10355. 48.Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41:95-98. 49.Hirasawa M., Takada K. 2004. Multiple effects of green tea catechin on the antifungal activity of antimycotics against *Candida albicans*. *J Antimicrob Chemother* 53:225 – 229. 50.Hodgson, J.M. Devine, A. Pudsey, I.B. Chan, S.Y. Beilin,L.J. Prince, R. L. 2003. Tea intake is inversely related to blood pressure in older women. *J Nutr* 133:2883 – 2886. 51.Kaundun,S.S., A. Zhyvoloup, and Y.G. Park. 2000. Evaluation of genetic diversity among elite tea (*Camellia sinensis* var. *sinensis*) accessions using RAPD markers. *Euphytica* 115:7-16. 52.Kim, J. H. Kang, B. H. Jeong, J. M. 2003. Antioxidant antimutagenic and chemopreventive activities of a phyto-extract mixture derived from various vegetables fruits and oriental herbs. *Food Sci Biotechnol* 12:631 – 638. 53.Kojoma, M. Kurihara, K. Yamada, K. Sekita, S. Satake, M. Iida, O. 2002. Genetic identification of cinnamon (*Cinnamomum* spp.) based on the *trnLtrnF* chloroplast DNA. *Planta Med.* 68:94 – 96. 54.Lambert, J. D. Yang, C.S. 2003. Mechanisms of cancer prevention by tea constituents. *J Nutr* 133:3262 – 3267. 55.Lau, D. T. Shaw, P. C. Wang, J. But, P. P. 2001. Authentication of medicinal *Dendrobium* species by the internal transcribed spacer of ribosomal DNA. *Planta Med* 67:456-460. 56.Lee, S. C. Lee, C. H. Lin, M. Y. Ho., K. Y. 2010. Genetic identification of *Cinnamomum* species based on partial internal transcribed spacer 2 of ribosomal DNA. *J. Food and Drug Anal.* 18:225-231. 57.Lee Shin-Chieh. Yan Rui-Hong. Cheng Hun-Yuan. Wu Sang-Shung. Liu Shu-Ying. 2009. Screen and Genetic Assessment of Tea Germplasms with Elevated Methylated Catechin, (-)-Epigallocatechin-3-O-(3-Omethyl)gallate. *Food Chemistry*. 57 ( 19 ) :8906 – 8912 58.Magoma, G. N. Wachira, F. N. Obanda, M. Imbuga, M. Agong, S. G. 2000. The use of catechins as biochemical markers in diversity studies of tea ( *Camellia sinensis* ). *Genetic Resources and Crop Evolution* 47:107-114. 59.Millin, D. J. and Rustidge, D. W. 1967. Tea manufacture. *Process Biochem.* 6:9-13. 60.Mittal,A. Pate, M. S. Wylie, R. C. 2004. EGCG down regulates telomerase in human breast carcinoma MCF-7 cells, leading to suppression of cell viability and induction of apoptosis. *Int J Oncol* 24:703 – 710. 61.Negishi, H . Xu, J. W. Ikeda, K. Njelekela, M. Nara, Y. Yamory, Y. 2004. Black and green tea polyphenols attenuate blood pressure increases in stroke-prone spontaneously hypertensive rats. *J Nutr* 134:38 – 42. 62.Ni, S., Yao, M., Chen, L., Zhao, L., Wang, X. 2008. Germplasm and breeding research of tea lant based on DNA maker approaches. *Front. Agric. China*, 2:200-207. 63.Saravanan, M. Maria John, K. M. Raj Kumar, R. Pius, P.K. Sasikumar, R. 2004. Genetic diversity of UPASI tea clones ( *Camellia sinensis* ( L. ) O.Kuntze ) on the basis of total catechins and their fractions. *Phytochemistry* 66:561 – 565. 64.Saini, A. Reddy, S. K. Jawali, N. 2008. Intra-individual and intra-species heterogeneity in nuclear rDNA ITS region of *Vigna* species from subgenus *Ceratotropis*. *Genet Rres (Camb)*. 90:299-316. 65.Sano Mitsuaki. Suzuki Masazumi. Miyase Toshio. Yoshino Kyoji. Mari Maeda-Yamamoto. 1999. Novel Antiallergic Catechin Derivatives Isolated from Oolong Tea. *J. Agric. Food Chemistry* 47:1906-1910. 66.Takabayashi, F. Harada, N. Yamada, M. Murohisa, B. Oguni, I. 2004. Inhibitory effect of green tea catechins in combination with sucralfate on *Helicobacter pylori* infection in Mongolian gerbils. *J Gastroenterol* 39:61 – 63. 67.Taberlet P. Gielly, L. Pautou, G. Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol Biol*. 17:1105-1109. 68.Tamura, K. Dudley, J. Nei, M. Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24:1596-1599. 69.Tsai, L. C. Yu, Y. C. Hsieh, H. M. Wang, J. C. Linacre, A. Lee, J. C. 2006. Species identification using sequences of the *trnL* intron and the *trnL-trnF* IGS of chloroplast genome among popular plants in Taiwan. *Forensic Sci Int.* 164:193-200. 70.Vijayan, K. and Tsou, C.-H. 2008. Technical report on the molecular phylogeny of *Camellia* with nrITS: the need for high quality DNA and PCR amplification with Pfu-DNA polymerase. *Bot. Stud.*, 49:177-188. 71.White, T. J.; Bruns, T.; Lee, S.; Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In “ PCR protocols: a guide to methods and applications. ” , 315-322 72.Wight, W., 1959. Nomenclature and classification of the tea plant. *Nature* 183:1726 – 1728. 73.Yang, Y. C. Lu, F. H. Wu, J.S. Wu, C. H. Chang, C. J. 2003. The protective effect of habitual tea consumption on hypertension. *Arch Intern Med* 164:1534 – 1540. 74.Yao, H. Song, J. Liu, C. Luo, K. Han, J. Li, Y. Pang, X. Xu, H. Zhu, Y. Xiao, P. Chen, S. 2010. Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS One*. 5:e13102. 75.Yip, P. Y. Chau, C. F. Mak, C. Y. Kwan, H. S. 2007. DNA methods for identification of Chinese medicinal materials. *Chin. Med.* 2:9. (doi:10.1186/1749-8546-2-9) 76.Yi, T. Miller, A. J. Wen, J. 2004. Phylogenetic and biogeographic diversification of *Rhus* (Anacardiaceae) in the Northern Hemisphere. *Mol Phylogenetic Evol.* 33:861 – 879. 77.Zhang, Y.-B., Shaw, P.-C., Sue, C.-W., Wang, Z.-T. Tong, Y. 2007. Molecular authentication of Chinese herbal materials. *J. Food and Drug Anal.*, 15:1-9.