

Development of HPLC Fingerprinting Techniques and ITS DNA Authentication for Taiwan Tea

顏睿宏、李世傑

E-mail: 9701082@mail.dyu.edu.tw

ABSTRACT

50 Taiwan teas were used for catechin content analysis and their ITS sequences determination. There are twenty Tai-tea cultural cultivars (TA01~TA20), fourteen Partial-Fermented tea (PF01~PF14), twelve Mountain tea (TM01~TM12) and five commercial available teas (TC01~TC05) . The order catechins contents in Taiwan tea is Epigallocatechin gallate (EGCG) > Epigallocatechin (EGC) > Epicatechin (EC) > Gallocatechin (GC) > Epicatechin gallate (ECG) > Catechin (C) . The cultivars rich in ester-catechin are TA03, TA07, TA08, TA12, TA14, TA17, TA18, TM03 and TM05, there are excellent clones in developing health food products. EGCG3 " Me content is determined to be higher than nine tea cultivars 1% dry leaf weight. The length of ITS region is range from 594 bp to 647 bp in our studied. The inter-specific variations in the ITS region was very high with 0.571~1 of divergence among the test samples. In conclusion, the HPLC fingerprinting and ITS authentication techniques for teas in Taiwan area were established.

Keywords : catechins, Epigallocatechin gallate, fingerprint, ITS, HPLC.

Table of Contents

封面內頁 簽名頁 授權書	iii 中文摘要
iv 英文摘要	vi 誌謝
viii 圖目錄	xii 目錄
茶樹之地理位置分布 1 1.1.3 茶葉在分類學上的研究 1 1.1.4 台灣茶樹品種的簡史 2 1.2 茶葉的應用 2 1.2.1 茶葉的化學成份 3 1.2.2 茶葉之生理活性 3 1.2.3 茶葉抗過敏活性成分EGCG3 " Me 4 1.3 植物在分子基原鑑定上的應用 5 1.3.1 RAPD在植物分子標記的研究進展 5 1.3.2 AFLP在植物分子標記的研究進展 8 1.3.3 ITS在植物分子標記的研究進展 10 1.4 台灣茶葉未來的展望 11 2. 材料與方法 13 2.1 實驗材料 13 2.1.1 實驗茶葉 13 2.1.2 實驗藥品 13 2.1.3 實驗試劑組 14 2.1.4 酵素 14 2.1.5 儀器設備 15 2.2 研究方法 15 2.2.1 兒茶素測定 15 2.2.2 DNA萃取 16 2.2.3 DNA的定量與稀釋 rDNA核酸引子進行聚合?鏈鎖反應 18 2.2.5 膠體電泳回收純化 18 2.2.6 質體DNA之構築 20 2.2.9	
質體DNA定序	19 2.2.7 質體DNA以熱休克 (heat shock) 之轉形 19 2.2.8 質體DNA之萃取 21 2.3 茶葉ITS-rDNA序列分析 21 2.3.1 序列分析 21 2.3.2 親緣關係圖的建立 21 2.4
統計分析 23 3. 結果與討論	24 3.1 台灣茶優良品系與高酯型兒茶素茶種源之篩選 24 3.1.2 沸水萃取時間比較 24 3.1.3 茶葉中兒茶素與咖啡因含量檢測 25 3.1.4 台灣雜交茶種兒茶素含量差異 26 3.1.5 台灣部份發酵茶種和紅茶兒茶素含量差異 28 3.1.6 台灣野生茶種和商業化茶種兒茶素含量差異 29 3.1.7 EGCG3 " Me 兒茶素定量與定性 30 3.1.8 台灣綠茶EGCG3 " Me 的篩選 30 3.1.9 50個茶葉品種之主成份聚類分析 (PCA) 31 3.2 探討台灣茶樹品種 (山茶屬) ITS片段之變異性 32 3.2.1 山茶屬ITS片段 32 3.2.2 茶葉總DNA的抽取 33 3.2.3 ITS-PCR結果 34 3.2.4 40種品系ITS定序結果 34 3.2.5 40種品系演化樹探討 35 4. 結論 38 參考文獻 76 附錄 81

REFERENCES

王建波、張文駒、陳家寬1999植物分類學報。37(4): 450~490。 史全良、諸葛強、黃敏仁2001 分子系統學原理及其在林木上的應用 生命科學。13(2):90-93。 伊東明、?敏仁 1997 AFLP分子標記及其在植物育種上的應用生物工程。25(170): 6~11。 汪小全、洪德元 1997植物分類學報。35(5): 465~480。 邱湧忠 2005 綠茶生機。一橋出版社，台北。 胡智益、蔡右任、林順福 2004 利用簡單重複序列 (ISSR) DNA分子標誌評估台灣茶樹種原之遺傳變異，中華農學會報。原征彥 1998 茶兒茶素 (Catechin) 類的生理活性作用 茶葉多元酚在食品/保健食品產業之製造及應用研討會 食品工業發展研究所。新竹，台灣。 陳宗懋 2003 中國茶經 上海文化出版社。 蔡永生、劉士綸、王雪芳、區少梅 2004 台灣茶業研究彙報 23: 115-132。 張同吳 2003 保健植物應用於休閒產業之研究。 休閒作物資源之開發與應用研討會專刊，農改場，151-164。 譚和平、徐利遠、余桂容、杜文平、馬增強 2004 RAPD技術對茶樹品種鑑別的研究。中國測試技術:30 3-6。 Balasaravanan,T., P.K. Pius, R.R. Kumar, N. Muraleedharan, and A.K.Shasany. (2003) Genetic diversity among south India tea germplasm

(*Camellia sinensis*, *C. assamica* and *C. assamica* spp. *lasiocalyx*) using AFLP markers. *Plant Sci.* 165:365-372. Cao,Y. and R. Cao. (1999) Angiogenesis inhibited by drinking tea. *Nature* 398: 381-382 Facts and Comparisons, (2002) The review of natural products : the most complete source of natural product information. 189:20-21 Chiu F.L., Lin J.K. (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 Chem.* 53 ; 7035-7042. 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. Hour,T.C., Y.C. Liang, I.S. Chu and J.K. Lin. (1999) Inhibition of eleven mutagens by various tea extracts, (-)epigallocatechin-3-gallate, gallic acid and caffeine. *Food Chem. Toxicol.* 37:569-579. Kremer, A. and Petit, R.j. (1993) Gene diversity in natural pulations of oak Species .*Ann.Sci.*50:186-20. KYOJI YOSHINO, KENJIRO OGAWA, TOSHIO MIYASE,(2007) Inhibitory effects of the C-2 epimeric isomers of tea catechins on mouse type IV allergy. *J Agric. Food Chem.* 52:4660-4663.23. 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. Li Y.G., Dewald C.L., Sims P.L.(1999) Genetic relationships with in tripsacum and detected by RAPD variation .*Ann.Bot.*84:695-704. Lai,J.A., Yang, W.C., and Hsiao,J.Y. (2001) An assessment of genetic relationship in cultivated tea clones and native wild tea in Taiwan using RAPD and ISSR markers. *Bot. Bull. Acad. Sin.* 42:93-100. Liao, S., Kao, YH, Hiipakka, RA (2001) Green tea: biochemical and biological basis for health benefits. *Vitam.Horm.* 62:1-94. Lu,Y.P., Y.R. Lou, J.G. Xie, Q.Y. Peng, J. Liao, C.S. Yang, and M.T. Huang.(2002) Topical applications of caffeine or (-)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. *Proc. Natl. Acad. Sci. USA* 99:12455-12460. Lotito, S. B. and Fraga, C. G. 1998. (+)-Catechin prevents human plasma xidation Free Radic. Biol. Med. 24(3):435-441. Jankun, J., Selman, S. H., and Swiercz, R. 1997. Why drinking green tea could prevent cancer. *Nature*, 387(5):561. Mangiapane, H., Thomson, J., Salter, A., Brown, S., Bell, D.,and White, D. A. 1992. The inhibition of low-density lipoprotein by (+) catechin, a naturally occurring flavonoid. *Biochem Pharmacol* 43: 445-450. Miura, S., Watanabe, J., Tomita, T., Sano, M., and Tomita, I.1994. Inhibitory effects of tea polyphenols (flavan-3-oldervatives) on Cu2+ mediated oxidative modification of low-density lipoprotein. *Biol Pharm. Bull.*17:1567-72. Mondal,T.K.(2002) Assessment of genetic diversity of tea (*Camellia sinensis* (L.) O. Kuntz) by inter-simple sequence repeat polymerase chain reaction. *Euphytica* 128:307-315. Maeda-Yamamoto, M.(1998) Effects of tea infusions of varieties or different manufacturing types on inhibition of mouse mast cell activation. *Biosci. Biotechnol.Biochem*,62:2277-2279. Maeda-Yamamoto, M.(2001) The change of Epigallocatechin-3-o-(3-o-methyl) gallate content in tea of different varieties, tea seasons of crop and processing method. *J Agric. Food Chem.*,1:64-68. Mitsuaki Sano,Massazumi Suzuki,Toshio Miyase,,(1999) Novel anti-allergic catechin derives isolated form oolong tea.*J. Agric. Food. Chem.*,47:1906-1910. Nakagawa,K., M. Ninomiya, T. Okubo, N. Aoi, L.R. Juneja, M. Kim, K. Yamanak, and T. Miyazawa. (1999) Tea catechin supplementation increases antioxidant capacity and prevents phospholipid hydorperoxidation in plasma of humans. *J. Agric. Food Chem.* 47:3967-3973. Oiki S,Kawahara TK., (2001) Random amplified polymorphic DNA (RAPD) variation among poplations of the insularendemic plant *Campanulamicrodonta* (*Campanulaceae*).*Ann.Bot.*, 87:661-667. Petit,S.,and phisms R.J. (1993) Geographic strture of chloroplast DNA polymorphisms in European oaks .*I'heor. Appl. Genet.* 1993,87:122-128. Risesberg,L.H. (1996) Homology among RAPD fragments in interspecific comparisons.*Mol.Ecol*,5:99-105. Shaw, P. C. (2002) Authentication of Chinese Medicinal Materials by DNA Technology. World Scientific Publishing. Singapore, 220:123-124. Sharma, S.K.(1996) AFLP analysis of the diversity and phylogeny of 1ens and its comparison with RAPD analysis .*Theo Appl Genet*, 93:751-758. Swofford, D. L. (2001) Phylogenetic Analysis Using Parsimony, PAUP 4.08. Sinauer, Sunderland, MA. Saitou, U. and Nei, M. (1987) The neighbor-joining methods: A new method for reconstructing phylogenetics tree. *Mol. Evol.* 4:25-26 Visser, T., Ferwarda, F., Wit, H., Veenman N.V and Zonen E., (1969) Outline of perennial crop breeding in the tropics. 22:459 – 493. White T. J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* 38:315-322. Wachira,F., J. Tanaka, and Y. Takeda. (2001) Genetic variation and differentiation in tea (*Camellia sinensis*) germplasm revealed by RAPD and AFLP variation. *J. Horti. Sci. Biotech.* 76:557-563. Wight, W., (1959) Nomenclature and classification of the tea plant. *Nature* 183: 1726 – 1728. Welsh, J.and Mmclell, M. (1990) Fingerprinting genomes using PCR with arbitrary primers .*Nucleic.Acid.Res.*1818:7213-7218. Widmer A.and Baltisberger M. (1999) Molecular evidence for allopolyploid speciation and asingle origin of the narrow endemic draba ladina (Brassicaceae). 86: 1282-1289. Willams, P. L. and Fitch, W. M. (1989) Finding the minimalchang in a given tree. In “ The hieracy of life. ” P. Fernholm eds. Elsevier Science Publishers B. 453-469. Yang, T. T. and Koo, M. W. 1997. Hypcholesterolemic effect of Chinese tea. *Pharma. Res.* 35(6):505-1