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以基因序列多型性作為臺灣檜木之分子標誌

Nucleotide Polymorphisms in Specific Genes as

Molecular Markers for Taiwan Cypress

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## 摘要

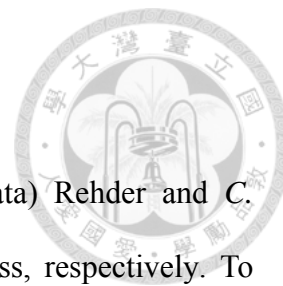


臺灣檜木包含臺灣扁柏 (*Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder) 與紅檜 (*C. formosensis* Matsum.) 兩種。本論文為了輔助鑑別臺灣檜木物種，透過基因序列分析，找出序列之多型性，單一核苷酸多型性 (single nucleotide polymorphism; SNP)、片段插入及缺失 (insertion-deletion; indel)，開發為分子標誌。選殖臺灣檜木 caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) 基因、alpha-pinene synthase (*APS*) 基因、cadinene synthase (*CAS*) 基因並進行序列分析，三個基因分別具有 5、10、10 個顯子。臺灣扁柏及紅檜之 *CCoAOMT* 基因於 Intron 1、2 及 3 具有 Indel；*CAS* 基因於 Intron 1 及 5 存在 Indel；而在 *APS* 基因中僅有 SNP 差異。依據 *CCoAOMT* 基因序列設計引子進行聚合酶連鎖反應 (polymerase chain reaction; PCR)，除了共同條帶，可合成紅檜特有片段約 1.4 kb。針對 *CAS* 基因 Intron 1 之 Indel 設計的引子，可得兩條紅檜特有 PCR 產物分別為 750 及 800 bp；另得到紅檜及臺灣扁柏共同條帶為 650 bp。若經毛細管電泳分析，臺灣扁柏樣品尚出現另一相近條帶，但紅檜則沒有。將 650 bp 序列分析後，臺灣扁柏樣品合成之產物比紅檜多 2 bp 片段插入序列，可用以鑑別臺灣扁柏。另根據 *CAS* 基因 Intron 5 之 Indel 設計的引子，於紅檜及臺灣扁柏均可合成約 700 bp 片段，序列分析結果指出，11 bp 片段缺失之序列為紅檜特有序列。以上三組引子可應用於臺灣扁柏及紅檜分子標誌鑑定用。

關鍵詞：物種鑑定、單一核苷酸多型性、片段插入及缺失、

杜松烯生合成基因

## Abstract



*Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder and *C. formosensis* Matsum. are known as Taiwan yellow and red cypress, respectively. To develop molecular markers to identify cypress, polymorphic gene sequences such as single nucleotide polymorphism (SNP) and insertion-deletion (Indel) were used as molecular markers to identify the species of timber and wood products. Caffeoyl CoA *O*-methyltransferase (*CCoAOMT*), alpha-pinene synthase (*APS*) and cadinene synthase (*CAS*) genes were cloned from both Taiwan yellow and red cypress and sequenced. Gene structure analysis revealed that there are 5, 10, 10 exons in *CCoAOMT*, *APS*, *CAS*, respectively. Indels were found in intron 1, 2 and 3 of *CCoAOMT* and intron 1 and 5 of *CAS* but only SNPs were found in *APS*. Specific primers have been designed based on the polymorphic DNA sequences in *CCoAOMT*, 1.4 kb fragment was synthesized only in red cypress with other common fragments. Two molecular markers based on the *CAS* indels sequence in intron 1 and 5 have been developed. For the marker based on intron 1 can identify cypress species through electrophoresis, in red cypress two specific bands about 750 and 800 bp were found together with the common 650 bp fragment in both cypresses. One extra fragment was separated away from the 650 bp common fragment by capillary electrophoresis, using DNA extracted from yellow cypress. After sequencing, the yellow cypress could be distinguished with red cypress by 650 bp PCR product by 2 bp insertion. Using primers designed based on intron 5 of *CAS*, 700 bp fragment was synthesized in both red cypress and yellow cypress but with 11 bp deletion in red cypress.

Key words: species identification, single nucleotide polymorphisms, insertion and deletion, cadinene synthase gene



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## 壹、前言

全世界柏木科扁柏屬 (*Chamaecyparis* Spach) 成員共有五種及一變種，包含日本扁柏(*C. obtusa* Siebold & Zucc.)、日本花柏 (*C. pisifera* (Siebold & Zucc.) Endl.)、北美西部的羅生扁柏 (*C. lawsoniana* (A. Murray bis) Parl.)、北美東部的側葉扁柏 (*C. thyoides* Sargent)、臺灣扁柏 (*C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder) 及紅檜 (*C. formosensis* Matsum.) (陳等，2012)，其中臺灣扁柏與紅檜合稱臺灣檜木 (林和陳，2007)。紅檜樹皮裂溝較淺剝片較薄，常稱為「薄皮仔」；臺灣扁柏之樹皮較厚，裂溝較深，稱之為「厚殼仔」。紅檜偏好生活在涼爽、潮濕的地區，主要分布於海拔 1000-2900 公尺的中高海拔雲霧帶山區，常和臺灣扁柏及其他針葉樹木混生或形成純林，分布範圍較廣泛，常可在全省中海拔山區發現，例如宜蘭羅東、嘉義阿里山、南投溪頭以及新竹觀霧等 (黃等，2007)。

臺灣檜木因形態優美為高價之景觀園藝樹種；木材質地良好、不易腐壞等優良條件，為高級木材之一，具有高度經濟價，屢遭盜伐並非法交易。過去受限於分子技術尚未成熟及物種來源判斷不易，導致面臨非法盜採時常因物種鑑定或來源判定資料不足而束手無策，而個體間不管在形態方面或是遺傳組成方面均擁有獨特表型及基因型 (黃等，2007)。隨著木材 DNA 抽取方法的突破，分子生物技術開始應用於木材及木製品的材種鑑定及來源地識別，對於阻止非法砍伐及貿易具有重要意義 (伏等，2013)。

本論文針對臺灣檜木重要基因進行選殖及定序分析，找出個體間序列之多型性，包括單一核苷酸多型性 (single nucleotide polymorphism; SNP)、片段插入及缺失 (insertion-deletion; INDEL)，建立臺灣檜木之分子標誌，以作為其木材及木製品材種識別之依據，並運用於探討來源地，期望可於未來臺灣檜木盜採案件中，提供相關科學數據以供判斷分析及鑑定。研究材料為羅東林區太平山工作站採集之臺灣檜木木材及葉片，首先針對臺灣檜木不同部位及木材深度分析 DNA 之含量及完整性，作為日後採樣標準，並利用木材 DNA 測試以葉片 DNA 開發之分子標誌應用於木材樣品之可行性。

## 貳、前人研究



### 一、以 DNA 分子標誌分析遺傳歧異度

植物遺傳性狀差異可由外表型或基因型來進行鑑別，但外表型性狀易受生育環境所影響。為了改善外表型分析造成的缺失，可透過檢測 DNA 間的變異，作為遺傳標記。作物之遺傳標記可分為形態標記 (morphological marker)、細胞遺傳標記 (cytological marker)、生化和化學成分標記 (biochemical marker) 及分子標記 (molecular marker) (Kumar, 1999)。DNA 分子標記廣泛存在於基因組 DNA 各區域，可由電泳圖譜區別不同基因型個體，亦可直接比較生物基因體的遺傳變異。以 DNA 為基礎的分子標記，因具有高水平的多態性及不受環境因子影響的特點，為品種遺傳鑑定工具之一 (Spooner et al., 2005)。其中分子標記是指能顯現生物個體或種群間基因組中某種差異特徵的 DNA 片段 (周，2005)。近 20 年來 DNA 分子標記大量發展，由於 DNA 定序技術的成熟且成本大幅降低，成為今日常使用的方法。DNA 分子標記包含隨機增幅多型性 DNA (randomly amplified polymorphic DNA; RAPD)、簡單重複序列 (simple sequence repeat; SSR)、簡單重複序列間片段 (inter-simple sequence repeat; ISSR)、單一核苷酸多型性 (single nucleotide polymorphism; SNP) 等方法。

RAPD 為使用隨機選擇的單一短寡核苷酸引子，進行聚合酶連鎖反應 (polymerase chain reaction; PCR) 所獲得的產物。當基因組中與引子互補的鹼基缺失，使引子無法結合，會產生不同 RAPD 條帶 (Parker et al., 1998)。通常引子 (primer) 為 10 個核苷酸左右，由於引子短在 PCR 反應時與模板 DNA 結合可能不完全互補，複製增殖得到的 DNA 標記在重複試驗中的再現性不佳，故 RAPD 的準確性容易受到影響。

SSR 或稱為微衛星 DNA (microsatellite DNA)、短重複序列 (short tandem repeat; STR) 是一種由 2~6 個鹼基為單位，以重複串聯方式組成的 DNA 序列；利用 PCR 增幅後的產物，在 100 個鹼基內，於 SSR 兩旁設計專一性的核酸引子對 (primer pair)，SSR 的兩側序列常較保守，在同種而不同個體間多相同 (周，2005)。因此 SSR 標記的再現性高，透過電泳分析可將同型合子 (homozygote) 和異型合子 (heterozygote) 之條帶加以區分，使 SSR 呈現共顯性 (co-dominant) 特色 (劉等，1998)。許多遺傳機制如核苷酸不均等的取代或突變、染色體複製期間 DNA 滑動

(replication slippage) 等造成核苷酸片段具有不同的重複次數，形成 SSR 序列長度的差異，可作為遺傳變異的指標 (Jeffreys et al., 1985)。


ISSR 係根據一個單位的 SSR 序列，再於其 5'端或 3'端處增加少許核苷酸，作為引子，擴增兩個 SSR 之間的 DNA 片段 (Zietkiewicz et al., 1994)。引子設計上比 SSR 標記簡單，不需要知道 DNA 序列即可進行。

SNP 為 DNA 序列中的單一鹼基對發生變異，亦即同物種不同個體之基因組的單一位點，因刪除、插入或是取代反應，出現兩種或多種核苷酸的可能性。SNP 在高分辨率遺傳圖譜的建構、族群結構的研究中，為一具有價值之指標 (Esteras et al., 2013)。

隨著分子生物技術的發展，近年來已廣泛使用分子標誌作為親緣間遺傳差異之研究工具 (Aga et al., 2005; Vieira et al., 2010)。溫和許 (2003) 利用 RAPD 技術對桃的 29 個品種進行遺傳相似度分析，群集分析結果 29 個試驗品種共分為七群，其中顯示日本桃品種可能源自大陸華中地區。細胞核之微衛星標記 (nuclear simple sequence repeat; nSSR) 的遺傳，已用於鑑定葡萄品種親緣關係 (Lacombe et al., 2013)。DNA 分子標誌，亦可偵測出蘭花種間及種內序列的變異，在 31 種石斛 (*Dendrobium Sw.*) 中，利用具物種特性的 ISSR 標記可鑑定出其中 9 種；利用 ISSR 標記所產生的條帶，進行集群分析 (Cluster analysis) 後，將 31 種石斛分成六個群集 (Wang et al., 2009)。Garriga 等人 (2013) 於藍莓 *Vaccinium corymbosum L.* 和 *Vaccinium ashei Reade* 中選擇十三個品種，利用六個 ISSR 引子，測得 80% 的多態性基因座，且具有再現性，其中親緣關係最相近的兩品種 (Bluegold 和 Nelson) 間具 26% 的遺傳差異。陳等人 (2013) 針對臺灣粳型水稻品種進行隨機型 SNP 分子標誌資料庫的初步建立，結果共成功開發 123 個具有多型性之 SNP 分子標誌。另外，有些學者已利用分子標誌的策略來研究紅檜之種源、遺傳變異及分子親源關係等問題 (黃等, 2000; Hwang et al., 2001)。

## 二、木本植物之遺傳歧異性分析

地理屏障及氣候變遷為驅動現存生物之演化、形態及遺傳結構的兩大力量 (Hewitt, 2000)。利用葉綠體和粒線體 DNA 分子標誌，分析大範圍華山松 (*Pinus armandii Franch.*) 之遺傳變異，結果皆指出華山松具有高度遺傳多樣性及族群分化




明顯。從秦巴山區、雲貴高原、喜馬拉雅山-橫斷山脈，中國亞熱帶區之白松族群中檢測到的不同遺傳結構，也說明地理因素影響遺傳分化 (Liu et al., 2014)。分析 30 個安石榴株系之 RAPD，可由 6 個引子得到 22 個多型性片段，其中由新加坡引進之品系皆擁有一個特殊片段 (王等，2007)。Mahar 等人 (2012) 以三種 DNA 分子標誌法：RAPD、直接擴增小衛星 DNA (directed amplification of minisatellite DNA; DAMD) 和 ISSR，估算三葉無患子 (*Sapindus trifoliatus* L.) 的族群結構及遺傳變異，可作為鑑別族群內個體之方法，並有助於挑選適當的重要植物個體，進行遺傳資源保護。黃等 (2000) 以 PCR/RFLP 及核苷酸序列分析位於棲蘭山及阿里山之紅檜與臺灣扁柏葉綠體基因組之多型性，經由 PCR 擴增出 *petG-trnP* 間之非譯碼區，DNA 定序後可知紅檜為 480 個鹼基對，臺灣扁柏則為 476 個；*trnV-trnM* 基因間區間序列在紅檜與臺灣扁柏之間有兩個 *HinfI* 酶切位置之差異，在 *petG-trnP* 基因間區間序列也發現臺灣扁柏與紅檜之間有限制片段長度及核苷酸多型性，但並無個體或族群之差異，顯示紅檜與臺灣扁柏於棲蘭山及阿里山兩族群間與族群內葉綠體 DNA 變異極小。黃等 (2007) 以微衛星 DNA 分析取自臺灣中央山脈及玉山山脈不同紅檜族群之個體，結果顯示紅檜不同個體擁有獨特之基因型，可作為個體鑑別之依據。藉由測試 13 個微衛星基因座之等位基因分布情形，推測採集自磐城市到屋久島中 25 個野生日本扁柏族群 (*C. obtusa* Siebold & Zucc.)，其遺傳分化及族群結構；根據連鎖不平衡分析、有效族群大小評估及檢測到瓶頸事件等證據，部份族群因族群分化或過度開發，造成遺傳多樣性下降；其中位於日本中部的中心族群，有效族群較大，邊緣族群像是位於磐城市及屋久島之日本扁柏，其有效族群較小，而位於屋久島之日本扁柏族群相較於其他地域，有其遺傳獨特性 (Matsumoto et al., 2010)。

### 三、以遺傳標誌鑑別木本植物之生育地

運用個體間 DNA 序列之變異或稱為多型性及分子標誌，可進行遺傳歧異性分析，鑑別物種生育地及不同地域之族群，亦可用於木材來源地的驗證 (Moodie et al., 1997)。大部分樹種的遺傳多樣性高，可應用於追溯植株及其製品之來源地，如木材、加工木製品 (Finkeldey et al., 2010)。利用 SSR 標記，找出 145 個不同的等位基因，顯示桃花心木族群間遺傳分化程度強，且遺傳與空間分布有明顯相關性，並





根據多位點之基因型分析，已確定兩組桃花心木木材的來源地 (Degen et al., 2013)。葡萄酒的品質與儲存葡萄酒的橡木桶產地具有一定關係，橡木來源地的鑑別對於葡萄酒質量的鑑定有實際意義。Deguilloux 等人 (2004) 於 10 個不同法國橡木桶葉採集 131 個橡木木材樣品，使用葉綠體 DNA 標記檢測不同樣品的單倍型 (haplotype)，測試橡木之來源地是否與橡木桶業者聲稱的來源地一致。經分析後，大部分來源地與業者聲稱一致，但有些聲稱來自法國的橡木，顯示源自東歐，有錯誤標記種源之情況。Tsumura 等人 (2011) 發展葉綠體 DNA 資料庫以鑑別娑羅屬物種 (*Shorea Roxb. ex C.F.Gaertn. species*)，透過 DNA 序列及解剖數據 (anatomical data) 推測木材樣品可能所屬之物種。

#### 四、臺灣檜木基因之鈎取

林等人 (2008) 獲得紅檜及臺灣扁柏木質素生合成相關基因 caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) mRNA 之全長序列，共計 1103 個鹼基對，其中包含 5' 端和 3' 端非轉譯序列各 79 與 274 個鹼基對，可轉譯蛋白序列 (coding sequence region; CDS) 共計 750 個鹼基對，並可轉譯出具有 249 個胺基酸之蛋白。Chu 等人 (2009) 獲得紅檜單萜類化合物 (monoterpenoid) 之生合成酵素 alpha pinene synthase (*APS*) cDNA 之全長序列，包含 5' 端非轉譯區 53 個鹼基對、3' 端非轉譯區 160 個鹼基對及 CDS 1887 個鹼基對，並可轉譯出 628 個胺基酸。Kuo 等人 (2012) 獲得紅檜倍半萜類化合物 (sesquiterpenoid) 之生合成酵素 cadinene synthase (*CAS*) CDS 1812 個鹼基對序列，可轉譯出 603 個胺基酸。

#### 五、木質素及萜類化合物之生合成途徑


木材組成分約有 15% -35% 為木質素，其主要由三種木質醇類單體 (monolignol) 聚合而成 (Zhong et al., 2000)。木質素生合成酵素之基因序列變異，可能使得其活性或特異性改變，造成木質素含量或組成分的變化 (Poke et al., 2003)。木質素主要由對-香豆醇 (*p*-coumaryl alcohol)、針葉醇 (conifer alcohol) 以及芥子醇 (sinapyl alcohol) 三種木質醇類單體聚合而成。此三種化合物之最大差別在

於苯環上之甲氧基數量與鍵結位置 (林等, 2008a)。木質醇類單體為苯丙醇類代謝途徑 (phenylpropanoid metabolism) 之產物, phenylalanine ammonia-lyase (PAL) 首先進行 phenylalanine 之脫氨反應。接著再進行一系列的羥基化、甲基化以及側鏈羧基與醇縮合反應, 其中 CCoAOMT 及 caffeic acid *O*-methyltransferase (COMT) 進行甲基化, 組成 p-hydroxyphenyl lignin (H lignin)、guaiacyl lignin (G lignin)、syringyl lignin (S lignin) 三種木質素 (林等, 2008b; Harakava, 2005)。裸子植物以 G lignin 為主要構成, 而被子植物為 G+S lignin, 草本植物多 H+G+S lignin (林等, 2008b; Borejan et al., 2003)。參與代謝途徑之酵素其高度保守胺基酸序列中多型性的存在, 可能會改變其酵素之功能, 進而造成不同木質素組成份 (Poke et al., 2003)。

萜類化合物為構成森林中芬多精 (phytoncide) 的主要物質。萜類化合物是由異戊二烯 (isoprene) 為構成單元而組成之化合物, 根據異戊二烯的個數又可再細分為不同種類, 包含具有兩個異戊二烯之單萜類、三個異戊二烯所組成之倍半萜類、四個異戊二烯組成之二萜類 (diterpenoid) 等, 其中單萜類及倍半萜類通常具有高揮發性及特殊香味 (陳等, 2012)。萜類化合物以三個常見的異戊二烯基二磷酸 (prenyl diphosphate) 作為前驅物, 包含 geranyl diphosphate (GPP)、farnesyl diphosphate (FPP) 及 geranylgeranyl diphosphate (GGPP), 經由單萜類、倍半萜類、二萜類等多種萜類化合物合成酵素 (terpene synthase, TPS) 進行催化反應而得 (Chappell, 1995)。

## 六、二級代謝物生成相關基因序列多型性

分析甘蔗屬 (*Saccharum* L. spp.)、芒屬 (*Miscanthus* Andersson spp.)、蔗茅屬 (*Erianthus* Michx. spp.)、巴西白茅 (*Imperata brasiliensis* Trin.)、河八王 (*Narenga porphyrocoma* (Hance) Bor) 間之 PAL、4-coumarate coenzyme A ligase (4-CL) 及 CCoAOMT 同源基因序列, 於隱子 (intron) 進行聚合酶連鎖反應, 發現特異性條帶可將蔗茅屬及巴西白茅區別出來, 接著回收 PAL、4-CL、CCoAOMT 基因於聚合酶連鎖反應中產生之 DNA 條帶進行序列分析, 繪製出親緣關係演化樹狀圖, 河八王與甘蔗屬親緣關係較近, 巴西白茅則與甘蔗屬相近。利用序列分析所找出之 SNP, 設計出酶切擴增多型性序列 (cleaved amplified polymorphism sequences, CAPS) 分子標誌進行分析, 結果與親緣樹狀分析相同。序列存在的多型性, 如 SNP、片段插



入及缺失 (insertion-deletion, Indel) 可用於區分不同屬間物種；另於 *CCoAOMT* 同源基因找到 SSR，其中 *Saccharum L.*、*Erianthus Michx.* SSR 之不同重複次數範圍較廣，可運用於 *Saccharum L.*、*Erianthus Michx.* 屬內不同種間之鑑別 (Park et al., 2011)。藍桉 (*Eucalyptus globulus* Labill.) 不同族群間木質素組合不同，挑選來自 Strzelecki 及 King Island 區域之不同木材密度族群，於 cinnamoyl CoA reductase (*CCR*) 及 cinnamyl alcohol dehydrogenase 2 (*CAD2*) 存在序列多型性，少部分序列多型性的發生，造成轉譯的相對應胺基酸序列發生改變，可能是造成酵素活性不同之原因 (Poke et al., 2003)。分析大桉樹及銀葉桉 *CCR* 及 *CAD2* 基因多型性，利用存在於大桉樹 *CCR* 啟動子 (promoter) 的片段插入及 *CAD2* 第一個隱子的片段缺失，設計出引子進行聚合酶連鎖反應分析，可區分大桉樹及銀葉桉，可將此 INDEL 作為分子標誌 (Paiva et al., 2011)。於大葉相思 (*Acacia auriculiformis* A. Cunn. ex Benth.) 及馬占相思 (*Acacia mangium* Willd.) 族群中挑選 480 棵個體進行基因型分析，找到 *CCR*、*COMT* 基因之 SNP，可用於鑑定數量性狀基因座 (quantitative trait loci; QTLs)，進而應用於相思樹分子標誌輔助育種 (Sukganah et al., 2013)。

## 參、材料方法



### 一、試驗材料


本研究針對羅東林區管理處太平山工作站轄區、易遭盜竊高風險地區之臺灣檜木進行採樣 (圖 1)，包含太平山區、棲蘭、明池、鴛鴦湖、思源啞口等地，取自 10 個不同林地，共約 155 株個體，利用 GPS 記錄每一個體之位置並加以編號。材料編號原則：第一個英文字母表示其所屬之物種，Y 為臺灣扁柏 (yellow cypress)、R 為紅檜 (red cypress)；接著後三碼為採集之地點 (T14、T15、T31、T54、T98 分別為太平山事業區 14、15、31、54、98 林班；D45、D46、D53 分別為大溪事業區 45、46、53 林班；H60 為太平山事業區 60 林班、N44 則為南澳事業區 44 林班)；最後一個數字則表示為不同個體。第一次的採樣個體，採取 3-4 種由表皮到心材不同深度 (圖 2) 之樹幹、根部樣品 (表 1)，用來測試何種深度的木材樣品所抽取之 DNA 量最多；其餘個體取其葉片進行 DNA 抽取 (表 2)，其中編號 RT15/14、YT54/4、YT54/24、YH60/13 個體同時採集葉片及木材作為試驗材料。

### 二、樣品前處理

採集後的樣品立即放入含有保冷劑的保冷袋中或室溫保存約 7 小時，回到實驗室後葉片樣品置於  $-80^{\circ}\text{C}$  凍箱儲存；木材樣品放入  $4^{\circ}\text{C}$  冰箱待刨削。木材及刀片表面以 75% 酒精潤濕的紙巾擦拭，利用刀片將木材刨削成薄片後置  $-80^{\circ}\text{C}$  凍箱儲藏，以待抽取 DNA。

### 三、木材 DNA 抽取

木材 DNA 抽取方法依據 Dellaporta 等 (1983) 所述再進行修改。每個樣品 (不同個體、不同部位) 取約 2-3 g 之刨削後木材薄片，以液態氮研磨加入含 15 mL Extraction buffer (內含 100 mM Tris-HCl, pH8.0, 50 mM EDTA, pH8.0, 500 mM NaCl, 10 mM  $\beta$ -mercaptoethanol) 的 Oakridge tube 中，接著加入 1 mL 20% SDS 並震盪混合，每次 10 秒共 4 次，之後置於  $65^{\circ}\text{C}$  水浴 10 分鐘。加入 5 mL 5M 醋酸鉀 (potassium acetate) 後用手上下搖動均勻，置於冰浴上靜置 20 分鐘以上後於  $4^{\circ}\text{C}$  以 8800 rpm 離心 15 分鐘 (Beckman, JA-13)；將離心好的 DNA 溶液倒入含有 10 mL  $-20^{\circ}\text{C}$  異



丙醇 (isopropanol) 的離心管中，並在管口上放置 Nylon mesh 以過濾雜質，溫和地混合溶液後，於-20°C 中靜置 30 分鐘以上，使 DNA 沉澱。於 4°C 以 8800 rpm 離心 15 分鐘並去除上清液，風乾沉澱物後加入 0.7 mL High-TE (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, pH 8.0)，放置 15 分鐘使 DNA 溶解；將溶液移置微量離心管中於 4°C 以 13000 rpm 離心 10 分鐘，取上清液加入 75  $\mu$ L 3M 醋酸鈉 (sodium acetate, pH 5.2) 及 500  $\mu$ L -20°C isopropanol 混和均勻，於 4°C 以 13000 rpm 離心 10 分鐘沉澱 DNA。去除上清液加入 200  $\mu$ L 70%酒精於 4°C 以 13000 rpm 離心 5 分鐘，倒掉上清液後再加入 100  $\mu$ L 100%酒精於 4°C 以 13000 rpm 離心 5 分鐘，去除上清液並風乾沉澱物。以 TE (10 mM Tris-HCl, pH 8.0, 1mM EDTA, pH 8.0) 溶解 DNA，加入 3  $\mu$ L 10 mg  $\cdot$  mL<sup>-1</sup> RNase A 於 37°C 下反應 30 分鐘後置於-20°C 保存。用分光光度計測定 DNA 於 260 nm 的吸光值以換算 DNA 濃度並利用電泳分析推估 DNA 含量再次確認。

#### 四、葉片 DNA 抽取

以 VIOGENE 公司之 Plant Genomic DNA Extraction Miniprep System (GPG1001) 試劑組抽取葉片 DNA。取 0.1 g 之葉片，在液態氮中研磨至粉末後，置入含有 400  $\mu$ L PX1 Buffer 的微量離心管，加入 20  $\mu$ L 20 mg  $\cdot$  mL<sup>-1</sup> RNase A，震盪混合後置於 65°C 水浴 10 分鐘，接著加入 130  $\mu$ L PX2 Buffer，震盪混合均勻後置於冰上 5 分鐘。將混合液移置 shearing tube 中，離心 2 分鐘，並取上清液，加入 0.5 倍體積之 PX3 Buffer 及 1 倍體積的 100% 酒精，混和均勻後將溶液置入 Plant Genomic DNA Mini Column 中，10,000 rpm 離心 1 分鐘。去除上清液後加入 700  $\mu$ L WS Buffer，於 13,000 rpm 離心 1 分鐘，重複上述步驟 2 次後，再離心 3 分鐘。將 Mini Column 置於新的微量離心管中，加入 200  $\mu$ L 65°C TE，靜置 5 分鐘，使 DNA 溶解後離心 1-2 分鐘，取上清液置於-20°C 保存。用分光光度計測定 DNA 於 260 nm 的吸光值以換算 DNA 濃度並利用電泳分析推估 DNA 含量再次確認。

#### 五、臺灣檜木基因之選殖

##### (一) 引子設計

先於 NCBI 資料庫搜尋臺灣檜木相關基因序列，推測臺灣檜木基因結構，根據



獲得之 cDNA 序列設計引子 (primer) (表 3)。

## (二) 聚合酶連鎖反應 (polymerase chain reaction; PCR)

於鈎取基因試驗，使用 KAPA HiFi HotStart ReadyMix (2X) kit 進行 PCR 反應。取 1  $\mu\text{g}$  臺灣檜木之基因組 DNA 作為模板，加入 12.5  $\mu\text{L}$  2X KAPA HiFiTM HotStart ReadyMix、0.3  $\mu\text{M}$  引子、以無菌水補足反應總體積 25  $\mu\text{L}$ 。使用的引子反應條件為 95 $^{\circ}\text{C}$  前處理 3 分鐘，再經 45 個循環，每個循環流程為：98 $^{\circ}\text{C}$ ，20 秒；以各自引子黏合溫度進行黏合 (表 3)，40 秒；72 $^{\circ}\text{C}$ ，1-2 分鐘，最後以 72 $^{\circ}\text{C}$ ，7 分鐘終止反應。PCR 產物加入追蹤染劑後，在 1-2% 瓊脂凝膠 (agarose gel) 中以 100 伏特電壓跑電泳進行檢測。

## (三) 膠體內 DNA 之回收與接合反應

將欲進行接合之載體 (pBluescript SK-) 以酵素 (EcoRV) 進行酶切，以瓊脂膠體進行電泳分離 DNA 片段，於 UV 365 nm 照射下取出含目標片段之膠塊，秤重後以 UltraClen<sup>TM</sup> 15 DNA Purification kit (MO BIO) 分離出膠體中之 DNA，取適當 insert 與 vector 比例混合，加上 1X ligation buffer (含 50 mM Tris-HCl, 10 mM MgCl<sub>2</sub>, 10 mM DTT, 1 mM ATP, 25 mM  $\cdot\text{mL}^{-1}$  BSA, pH 7.5) 及 0.5  $\mu\text{L}$  4 U  $\cdot\mu\text{L}^{-1}$  T4 DNA ligase，於 16 $^{\circ}\text{C}$  進行 16 小時接合反應。

## (四) 質體 DNA 之轉型

採 Hanahan (1983) 之方法，取適量接合反應溶液加入 200  $\mu\text{L}$  待轉型勝任細胞，於冰上共培養 30 分鐘，再以 42 $^{\circ}\text{C}$  水浴進行熱休克反應 1 分鐘，立即置於冰上 1 小時。加入 1 mL 預熱之 LB 液態培養基，於 37 $^{\circ}\text{C}$ ，100 rpm 培養 1 小時，取適量塗佈於選擇性固態 LB 培養基 (含 50  $\mu\text{g} \cdot \text{mL}^{-1}$  Ampicillin, 5% (v/v) 5-bromo-4-chloro-3-indoyl- $\beta$ -D-galactoside, 0.1 M isopropyl-thio-galactoside)，於 37 $^{\circ}\text{C}$  培養過夜。挑選培養基中合適的菌落，接種於 5 mL LB 液態培養基 (含 50  $\mu\text{g} \cdot \text{mL}^{-1}$  Ampicillin) 之試管中培養 12 小時。

## (五) 質體 DNA 小量製備

接種單一菌落至含抗生素之 LB 培養液，於 37 $^{\circ}\text{C}$ 、100 rpm 旋轉培養 12 小時。

取 1.5 mL 菌液裝於微量離心管中，在室溫下以離心機 13,200 rpm 離心 1 分鐘，去除上清液後，再次加入 1.5 mL 菌液並離心，留下約 100  $\mu$ L 之上清液將菌塊懸浮。加入 400  $\mu$ L STET 溶液 (8% sucrose, 50 mM EDTA, 5% Triton X-100, 50 mM Tris-HCl, pH 8.0) 及 30  $\mu$ L 之 10 mg  $\cdot$  mL<sup>-1</sup> lysozyme (溶於 TEN: 10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 0.15 N NaCl)，混勻後於室溫靜置 10 分鐘，以沸水浴處理 1 分鐘，置於冰上冷卻 1 分鐘，再於 4°C 離心機 13,200 rpm 離心 30 分鐘。以滅過菌之牙籤挑除沉澱後，加入等體積 -20°C 異丙醇，混勻後置於 -80°C 5 分鐘。接著於 4°C 以 13,200 rpm 離心 10 分鐘，去除上清液，沉澱之質體 DNA 室溫風乾後，回溶於 40  $\mu$ L 含有 0.2 mg  $\cdot$  mL<sup>-1</sup> RNase A 的 TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 7.5)，保存於 4°C 備用。

#### (六)、質體 DNA 定序

使用自動核酸定序儀 ABI sequencer 377，進行 DNA 之定序。以取得 *CoCCoAOMT*、*CfCCoAOMT*、*CoAPS*、*CfAPS*、*CoCAS*、*CfCAS* 基因序列。

## 六、分子標誌開發

### (一)、引子設計

根據臺灣檜木相關基因序列分析結果，針對插入或缺失片段 (insertion-deletion; Indel) 及單一核苷酸多型性 (single nucleotide polymorphism; SNP) 位置設計引子 (表 4)。

### (二)、聚合酶連鎖反應 (polymerase chain reaction; PCR)

於分子標誌試驗，使用 KAPA HiFi HotStart ReadyMix (2X) kit 進行 PCR 反應。取 50 ng 臺灣檜木之基因組 DNA 作為模板，加入 12.5  $\mu$ L 2X KAPA HiFiTM HotStart ReadyMix、0.3  $\mu$ M 引子、以無菌水補足反應總體積 25  $\mu$ L。使用的引子反應條件為 95°C 前處理 3 分鐘，再經 45 個循環，每個循環流程為：98°C，20 秒；以各自引子黏合溫度進行黏合 (表 4)，40 秒；72°C，1-2 分鐘，最後以 72°C，5 分鐘終止反應。PCR 產物加入染色溶液後，在 2-3% 瓊脂凝膠 (agarose gel) 中以 100 伏特電壓跑電泳進行檢測。

### (三)、DNA 定量與定序

取 12  $\mu\text{L}$  QX DNA Dilution Buffer 與 3  $\mu\text{L}$  DNA。使用 Qiagen QIAxcel 進行毛细管電泳反應，並以 BioCalculator 3.2 軟體分析電泳圖譜及定量。將 DNA 濃度調整為  $0.1 \text{ ng} \cdot \mu\text{L}^{-1}$ ，使用 BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing kit 進行定序反應；取 10  $\mu\text{L}$  的  $0.1 \text{ ng} \cdot \mu\text{L}^{-1}$  DNA、4  $\mu\text{L}$  1  $\mu\text{M}$  引子、2  $\mu\text{L}$  5X sequencing buffer、4  $\mu\text{L}$  Ready Reaction Premix，總反應體積為 20  $\mu\text{L}$ ，使用 Applied Biosystems<sup>®</sup> GeneAmp<sup>®</sup> PCR System 9700 進行 PCR，反應條件為 96 $^{\circ}\text{C}$  前處理 1 分鐘，再經 25 個循環，每個循環流程為：96 $^{\circ}\text{C}$ ，10 秒；50 $^{\circ}\text{C}$ ，5 秒；60 $^{\circ}\text{C}$ ，4 分鐘。PCR 產物以 BigDye<sup>®</sup> XTerminator<sup>™</sup> Purification Kit 進行純化，加入 90  $\mu\text{L}$  SAM solution 與 20  $\mu\text{L}$  Xterminator，劇烈搖晃 30 分鐘，短暫離心後以 Applied Biosystems 3130 Genetic Analyzer 分析序列。



## 肆、結果



### 一、不同部位及木材深度之臺灣檜木 DNA 抽取量變化

共採集六棵個體，以其為活樹體或死樹體分別定義為生立木及枯立木，試驗材料共 4 棵生立木、2 棵枯立木 (表 1)。於樹幹的部分，距樹皮深約 1-2.5 公分之生立木木材中，DNA 抽取量明顯高於其他深度，枯立木則於第一、二深度抽取出較多的 DNA；不論是枯立木或生立木，皆可從第一及二深度抽取出 DNA (圖 3A)。採集自根部之木材，於枯立木及生立木第一深度皆可抽出 DNA (圖 3B)；整體而言抽取之 DNA 量樹幹高於根部 (圖 3)。

### 二、臺灣檜木基因之選殖

#### (一)、Caffeoyl CoA *O*-methyltransferase 基因結構與序列多型性分析

針對臺灣檜木木質素合成相關基因進行選殖及定序分析，合成 RH60/1、YH60/1、YH60/2 及 YH60/4 之 caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) 基因，經與 NCBI 資料庫中已登錄之臺灣檜木 *CCoAOMT* cDNA 序列 (GenBank DQ295802、DQ305976) 比對，已確定臺灣檜木之 *CCoAOMT* 具有 5 個顯子 (Exon) 及 4 個隱子 (Intron)，且於 Intron 1、2、3 皆具有 Indel，使得紅檜及臺灣扁柏 *CCoAOMT* 基因長度不同 (圖 4)。*CCoAOMT* 轉錄區為 1921 bp，轉譯為蛋白質的 coding region (CDS) 則為 750 個鹼基對，可轉譯出 249 個胺基酸，在 *CCoAOMT* 基因中之 Exon 的部分，除了 Exon 1 於不同物種間核苷酸數量差異較大，其餘皆相似；而在 Intron 部分不同物種核苷酸數量差距大，同種之紅檜與臺灣扁柏差異較小 (圖 4)。胺基酸序列，不同物種間十分保守，本論文選殖得到之臺灣扁柏 *CCoAOMT* 所轉譯出之胺基酸序列，與已登錄之臺灣扁柏序列 (GenBank ABB87185) 具有明顯差異，後者多出 29 個胺基酸；紅檜與已登錄之序列 (GenBank ABB89956) 比對後，僅於 C 端有 1 個胺基酸之差異 (圖 5)。紅檜與臺灣扁柏之 *CCoAOMT* 長約 1.9 kb，序列具有 Indel 及 SNP 的多型性，且 SNP 多為臺灣檜木物種間之差異，僅七處 SNP 為個體間之差異，位於 Intron 2 及 *CCoAOMT* 基因 3 端處 (圖 )；其中 Indel 多位於 Intron 2 (表 5)，而轉錄區的 SNP 多位於 Exon 4 及 Exon 5，且僅一處 SNP 會

造成轉譯之胺基酸改變 (表 6)。



## (二)、Alpha-pinene synthase 基因結構與序列多型性分析

針對臺灣檜木萜類生合成相關基因進行選殖及定序分析，合成 RT31/1、RT31/6、YH60/2 及 YT31/6 之 alpha-pinene synthase (APS) 基因，經與 NCBI 資料庫中已登錄之紅檜 APS cDNA 序列 (GenBank EU099434.1) 比對，確定臺灣檜木之 APS 具有 10 個顯子及 9 個隱子，基因轉錄區 4257 bp，CDS 為 1890 個鹼基對，可轉譯出 629 個胺基酸 (圖 7)。在 APS 結構中，臺灣檜木與同為松科的巨冷杉 (*Abies grandis* Lindl.) 具相同的結構，有別於具有 7 個顯子的菊科黃花蒿 (*Artemisia annua* L.)；而在後四個 Exon 中，不同物種核苷酸數量差異小，但在 APS 總長，物種間核苷酸數量差異大 (圖 7)。本論文所選殖得到之紅檜 APS cDNA 序列，與已登錄之紅檜序列 (GenBank EU099434.1) 具有 39 個 SNP 及分別為 1 bp 與 2bp 的 Indel；cDNA 序列的多型性，造成 17 個胺基酸序列不同及 1 個胺基酸數量差異 (圖 8)。臺灣檜木之 APS 長約 4.2 kb，序列僅具有 SNP 的多型性，為個體間之差異，且多位於 Intron 3；四個已定序的個體 (RT31/1、RT31/6、YH60/2 及 YT31/6) APS 序列皆存在差異。於 Intron 3，RT31/6 與其他三個個體具有 13 個 SNP (圖 9)；而 Exon 的部分，SNP 僅發生於 Exon 6 及 Exon 7，且皆不會造成轉譯之胺基酸改變 (表 7)。

## (三)、Cadinene synthase 基因結構與序列多型性分析

針對臺灣檜木萜類生合成相關基因進行選殖及定序分析，合成 RT31/1、RT31/5、RT31/7、YH60/2、YT31/1 及 YT31/6 cadinene synthase (CAS) 基因，其中 YT31/1 與 YT31/6 序列相同；經與 NCBI 資料庫中已登錄之紅檜 CAS cDNA 序列 (GenBank JN715077.1) 比對，確定臺灣檜木之 CAS 具有 10 個顯子及 9 個隱子，基因轉錄區為 1812 bp，可轉譯出 603 個胺基酸；因具有 Indel，使得紅檜及臺灣扁柏 CAS 全長不同 (圖 10)。陸地棉 (*Gossypium hirsutum* L.) 與向日葵 (*Helianthus annuus* L.) 之 CAS 結構相同，比樹棉 (*Gossypium arboreum* L.) 多出 1 個顯子及 1

個隱子，相較於臺灣檜木則少了 3 個顯子 3 個隱子，結構明顯不同。而在臺灣檜木後四個 Exon 中，與陸地棉及向日葵之核苷酸數量差異小 (圖 10)。臺灣檜木之 CAS 序列，具有 Indel 及 SNP 的多型性，其中 Indel 位於 Intron 1 及 5，大小分別為 2 及 11 bp；SNP 多為兩物種間之差異，少數為個體差異。本論文於採自羅東林區的臺灣檜木中選殖之 CAS cDNA 序列與取自臺大實驗林溪頭營林區之已登錄紅檜 (GenBank JN715077) 的 CAS CDS 序列，具有 9 處 SNP 差異 (圖 11)。於 Intron 中的 SNP 多位於 Intron 1、3 及 4 (表 8)，Exon 部分則多位於 Exon 7，大部分的 SNP 會造成轉譯之胺基酸改變 (表 9)。

### 三、臺灣檜木分子標誌之開發

#### (一)、Caffeoyl CoA *O*-methyltransferase 基因之分子標誌分析

依據 *CCoAOMT* 序列開發之引子 OMT/MF-1 與 OMT/MR-1 (*OMT-M1*) 進行 PCR，於約 150 個葉片及木材樣品中，不同區域採集之紅檜樣品，皆合成出約 1.4 kb 特異性片段及兩或三個小於 500bp 之條帶；臺灣扁柏小於 500 bp 之片段則有三個以上，電泳圖譜於葉片樣品 (圖 12) 及木材樣品 (圖 13) 明顯不同，可用以鑑別紅檜與臺灣扁柏。

#### (二)、Cadinene synthase 基因之分子標誌分析

依據 CAS 第一個隱子之 Indel 序列開發引子 CAS5 及 CAS/MR-2 (*CAS-C5R*) 進行 PCR，產物經電泳分析後，臺灣扁柏及紅檜葉片 DNA 均可合成約 650 bp 條帶，但紅檜另具有兩個特異性片段約 750 bp (b)、800 bp (a) (圖 14)；將此兩片段與 CAS 中 CAS5 到 CAS/MR-2 之序列進行比對分析，大部分序列相同，具有多個 SNP 及 Indel，其中片段 a、b 之間，具有兩個 Indel 差異 (圖 15)。回收 650 bp 片段進行毛細管電泳分析，紅檜僅有一個條帶，臺灣扁柏則為兩條；進一步將 650 bp 片段進行定序分析，於約 150 個葉片個體中，臺灣扁柏皆具有 2 bp 插入片段相較於紅檜皆具有 2 bp 缺失片段，兩物種間存在多個 SNP (圖 16)。在約 150 個葉片樣品中，YT31/11、(Y)D45/2、YH60/14、YN44/8 於 *CAS-C5R* 分子標誌具有特異序列，YT31/11、(Y)D45/2 分別具有 8、9 個 SNP，YH60/14、YN44/8 則僅具 1 個 SNP，可用以鑑別個體 (圖 16)。利用抽取自木材的 DNA 作為 PCR 模板進行測試，

於紅檜合成兩條特異性片段 (圖 17)，同以葉片 DNA 測試之結果。挑選 YT54/4、YT54/24、YH60/2、YH60/3 木材樣品進行序列分析，YT54/24、YH60/2 與臺灣扁柏序列相同；YT54/4、YH60/3 與紅檜序列相同 (圖 18)。但以葉片 DNA 進行定序分析時，YT54/4 及 YT54/24 兩者序列皆與臺灣扁柏相同，具有 2 bp 插入片段。綜合葉片及木材樣品試驗結果，臺灣扁柏於 CAS-C5R 具有兩種序列型態，其中含有 2 bp 片段缺失之序列與紅檜相同 (圖 19)。

針對 CAS 基因第五個隱子之 Indel 序列開發引子 CAS/MF-1 及 CAS/MR-1 (CAS-CM1) 進行 PCR，臺灣扁柏及紅檜接合成出約 700 bp 條帶 (圖 20)。合成之產物定序分析後，於約 150 個葉片個體中，紅檜具有一個 11 bp 缺失片段，可用以鑑別紅檜 (圖 22)。取木材樣品進行開發之分子標誌測試，不論紅檜或臺灣扁柏皆合成約 700 bp 片段 (圖 21)。挑選 YT54/4、YT54/24、YH60/1、YH60/2、YH60/3 木材樣品進行序列分析，Indel 處皆與扁柏序列相同，且具有個體 SNP 之差異 (圖 22)。綜合葉片及木材樣品測試結果，紅檜於 CAS-CM1 具有兩種序列型態，經序列比對大部分紅檜具有 11 bp 的缺失片段，少部分的個體與臺灣扁柏序列相同 (圖 23)；其中紅檜於具有 11 bp 缺失片段之序列中，於序列 130、411 處具有個體內的 SNP，於 130 處核苷酸型別為 C/A，411 處為 T/A (圖 23)。

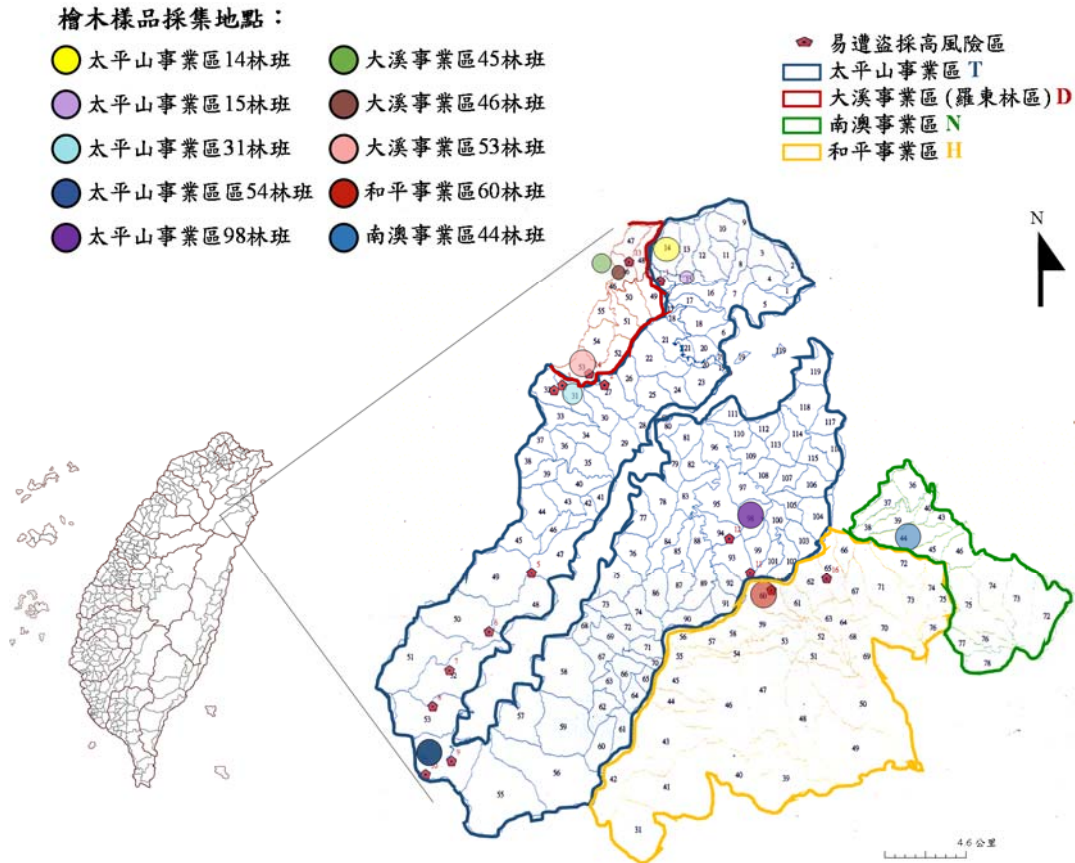


圖 1、臺灣檜木之採集點位於羅東林區管理處太平山工作站轄區

Fig. 1. The distribution of cypress at Yilan county Loudong forestry district office Taipingshan ranger station. High-risk areas mean the site locations which are vulnerable to illegal logging. The map include Taipingshan working circle (T) forest Compartment, Dasi working circle (D) forest compartment-Luotung area, Nanao working circle (N) forest compartment and Ho Ping working circle (H) forest compartment. And the number indicate different area of forest compartment.

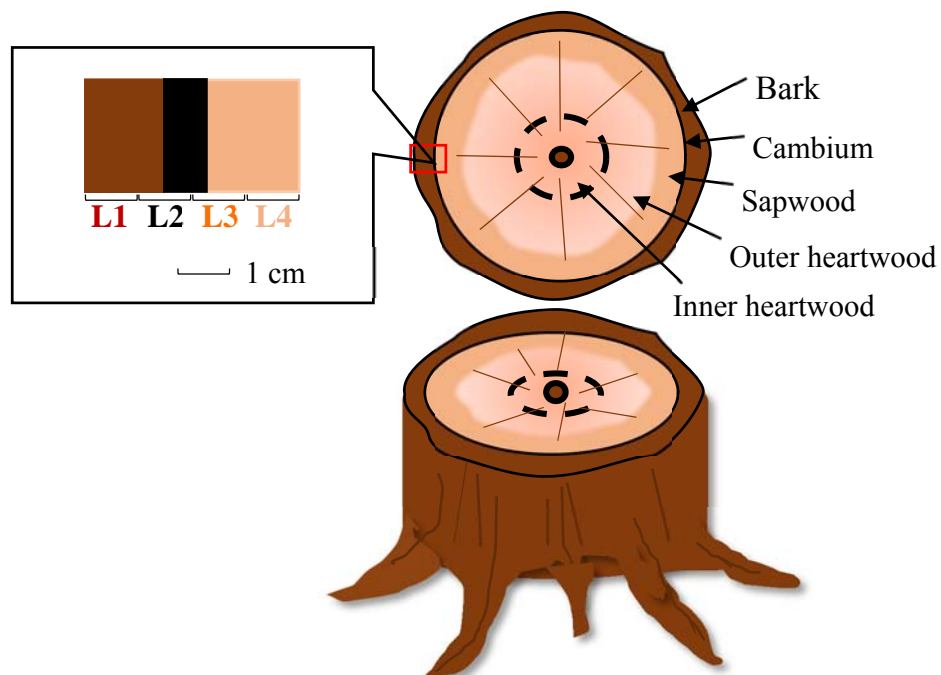


圖 2、木材結構於橫切面之試驗材料示意圖

Fig. 2. Structure of a log indicated on the cross section. The samples were collected from various depths of the log (L1-L4) indicated in the enlarged drawing. The L1 sample was hatched from the outermost of the log whereas the L4 was the innermost part.

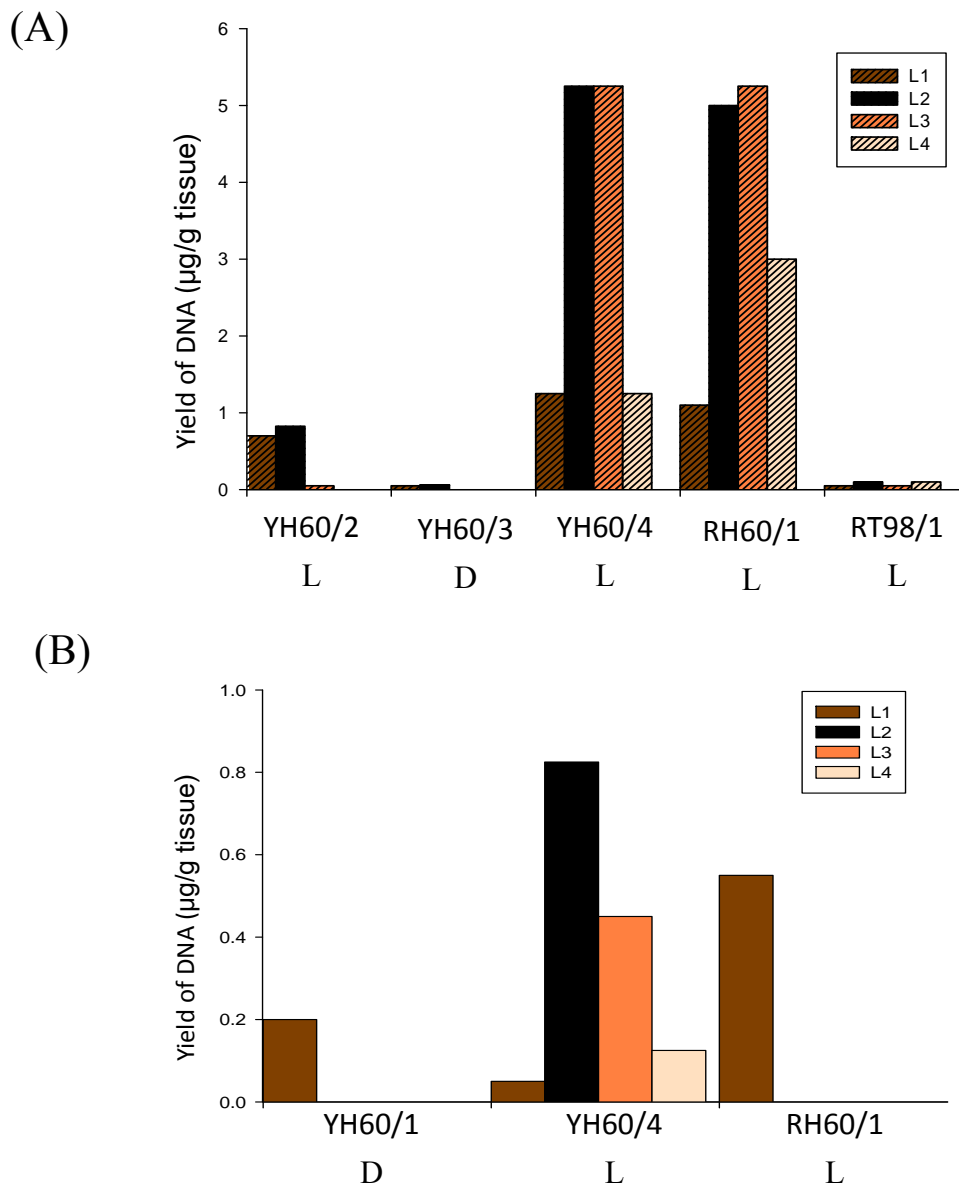
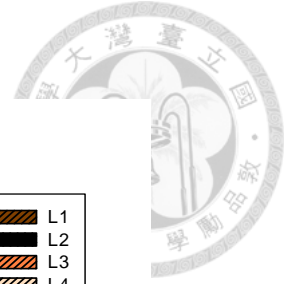


圖 3、不同深度及部位之木材 DNA 萃取量

Fig. 3. Comparisons of the DNA amount extracted from samples collected at different depths of the log. DNA was obtained from trunks (A) or roots (B). The wood samples were collected from living (L) or dead standing (D) tree of *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/1, YH60/2, YH60/3, YH60/4) or *C. formosensis* Matsum. (RH60/1, RT98/1). Wood chunks were collected from various depths of the log (L1-L4). Each layer is one centimeter thick. The L1 sample was hatched from the outermost of the log whereas the L4 was the innermost part.

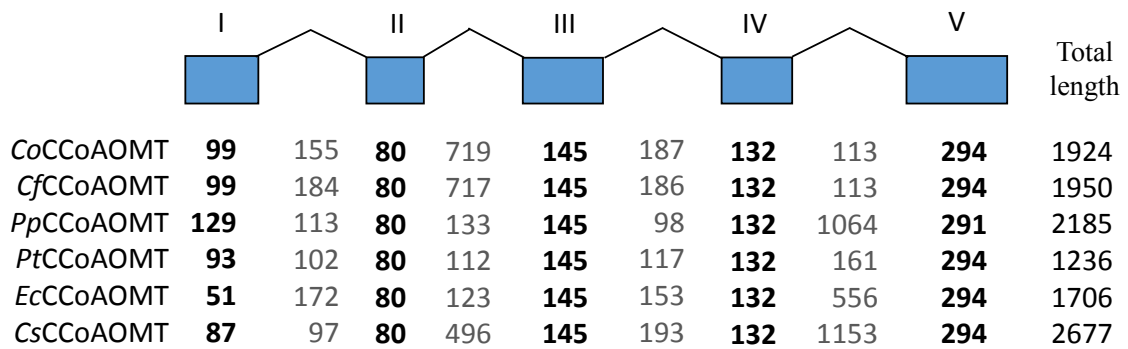


圖 4、不同物種間之咖啡醯輔酶 A 甲基轉移酶基因結構比較

Fig. 4. Schematic diagrams represent structure of genomic DNA of coding region for different caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) genes. Nucleotide sequences from *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (*CoCCoAOMT*, YH60/2), *C. formosensis* Matsum. (*CfCCoAOMT*, RH60/1), *Pinus pinaster* Aiton (*PpCCoAOMT*, CBL95257), *Populus trichocarpa* Torr. & A. Gray ex Hook. (*PtCCoAOMT*, CAA12198), *Eucalyptus camaldulensis* Dehnh. (*EcCCoAOMT*, ADI43381) and *Camellia sinensis* (L.) Kuntze (*CsCCoAOMT*, AFY97680) were analyzed. Exons are indicated by boxes, introns are represented by bended lines between boxes, and the Arabic numerals under the diagrams indicate the lengths (base pair) of the exons and introns. The Roman numerals above the boxes represent the number of exons.





YH60/2 CCoAOMT	(1)	-----MATVEASKDSTQQVSRHQEVGHKSLLOSDALYQYILETSVYPREPBP-----MRELREITAKHPWN
RH60/1 CCoAOMT	(1)	-----MATVEASKDSTQQVSRHQEVGHKSLLOSDALYQYILETSVYPREPBP-----MRELREITAKHPWN
CoCCoAOMT	(1)	-----MATVEATKCDSTQQVSRHQEVGHKSLLOSDALYQYILETSVYPREPESMRELREITQSDALYQYILETSVYPREPESMRELREITAKHPWN
CfCCoAOMT	(1)	-----MATVEATKCDSTQQVSRHQEVGHKSLLOSDALYQYILETSVYPREPBP-----MRELREITAKHPWN
TcCCoAOMT	(1)	-----MATVEATKCDSTQQVSRHQEVGHKSLLOSDALYQYILETSVYPREPES-----MRELREITAKHPWN
PpCCoAOMT	(1)	MASTDVAAAEVKAQTTQAEPEPAKVVRRHQEVGHKSLLOSDALYQYILETSVYPREPBP-----MRELREITAKHPWN
PaCCoAOMT	(1)	MASTDVAAAEVKAQTTQAEPEPAKVVRRHQEVGHKSLLOSDALYQYILETSVYPREPBP-----MRELREITAKHPWN
PtCCoAOMT	(1)	MASTDVAAAEVKAQTTQAEPEPAKVVRRHQEVGHKSLLOSDALYQYILETSVYPREPBP-----MRELREITAKHPWN
CiCCoAOMT	(1)	-----MASTNVQNGADASKDSTKQVSRHQEVGHKSLLOSDALYQYILETSVYPREPES-----MRELREITAKHPWN
EcCCoAOMT	(1)	-----MATAGEESCTQACRHQEVGHKSLLOSDALYQYILETSVYPREPBP-----MRELREITAKHPWN
CsCCoAOMT	(1)	-----MATNGEGEQN--TRHQEVGHKSLLOSDALYQYILETSVYPREPBA-----MRELREITAKHPWN
PtrCCoAOMT	(1)	-----MATNGEGEQSQACRHQEVGHKSLLOSDALYQYILETSVYPREPBC-----MRELREITAKHPWN
PtoCCoAOMT	(1)	-----MATNGEGEQSQACRHQEVGHKSLLOSDALYQYILETSVYPREPBC-----MRELREITAKHPWN
YH60/2 CCoAOMT	(62)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
RH60/1 CCoAOMT	(62)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
CoCCoAOMT	(91)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
CfCCoAOMT	(62)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
TcCCoAOMT	(62)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
PpCCoAOMT	(72)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
PaCCoAOMT	(72)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
PtCCoAOMT	(72)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
CiCCoAOMT	(68)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
EcCCoAOMT	(60)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
CsCCoAOMT	(58)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
PtrCCoAOMT	(60)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
PtoCCoAOMT	(52)	LMTTSADGGQFLHLLKLIINAKNTMEIGVYTGYSLLSFTALALPDDGKILAMDINRENVEYELGLPVIQKAGVAHKIDFREGPALFVLLQMLENKEMHGSFDF
YH60/2 CCoAOMT	(161)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
RH60/1 CCoAOMT	(161)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
CoCCoAOMT	(190)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
CfCCoAOMT	(161)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
TcCCoAOMT	(161)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
PpCCoAOMT	(171)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
PaCCoAOMT	(171)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
PtCCoAOMT	(171)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
CiCCoAOMT	(167)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
EcCCoAOMT	(158)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
CsCCoAOMT	(157)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
PtrCCoAOMT	(159)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI
PtoCCoAOMT	(151)	IFVDAADKDNVINYHRRLLIDLVKIIGGVTCYDNTLWNGSVVAPPDAPMRKYVRYRDFVIELNKALAADPRIEIQMLPVEDGHTLCRRVI

圖 5、不同物種間之咖啡醃輔酶 A 甲基轉移酶胺基酸比較

Fig. 5. Alignment of caffeoyl CoA *O*-methyltransferase (CCoAOMT) amino acid sequences between the samples of *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2 CCoAOMT; CoCCoAOMT, ABB87185), *C. formosensis* Matsum. (RH60/1 CCoAOMT; CfCCoAOMT, ABB89956), *Taiwania cryptomerioides* Hayata (TcCCoAOMT, ABB87184), *Pinus pinaster* Aiton (PpCCoAOMT, CBL95257), *Picea abies* (L.) H. Karst. (PaCCoAOMT, CAK18782), *Pinus taeda* L. (PtCCoAOMT, AAD02050), *Cunninghamia lanceolata* Hook. (CiCCoAOMT, AFX98065), *Eucalyptus camaldulensis* Dehnh. (EcCCoAOMT, ADI43381), *Camellia sinensis* (L.) Kuntze (CsCCoAOMT, AFY97680), *Populus trichocarpa* Torr. & A. Gray ex Hook. (PtrCCoAOMT, CAA12198), *Populus tomentosa* Carrière (PtoCCoAOMT, AAF44689). The black boxes represent conserved percentage of 100%.

OMT-RH60/1 (1) ATGGCAACCGTAGAGGCTTCCAAGGATTCAACACAGCAAGTCAAGCCGCCACCAGGAGGTGGGCCACAAGAGTCTCCTTCAGAGCGATGCTCTGTATCAGG  
 OMT-YH60/1 (1) ATGGCAACCGTAGAGGCTTCCAAGGATTCAACACAGCAAGTCAAGCCGCCACCAGGAGGTGGGCCACAAGAGTCTCCTTCAGAGCGATGCTCTGTATCAGG  
 OMT-YH60/2 (1) ATGGCAACCGTAGAGGCTTCCAAGGATTCAACACAGCAAGTCAAGCCGCCACCAGGAGGTGGGCCACAAGAGTCTCCTTCAGAGCGATGCTCTGTATCAGG  
 OMT/YH60/4 (1) ATGGCAACCGTAGAGGCTTCCAAGGATTCAACACAGCAAGTCAAGCCGCCACCAGGAGGTGGGCCACAAGAGTCTCCTTCAGAGCGATGCTCTGTATCAGG

OMT-RH60/1 (101) tttcttcttcttcttgcattttacagaggatttggcatatacattaggtttattcatgocctggattgattggatttggcaaatatacattaggtttgtttta  
 OMT-YH60/1 (101) tttcccttcttcttcttgcattttacagaggatttggcatatacattaggtttattcatgocctggattgattggatttggcaaatatacattaggtttgtttta  
 OMT-YH60/2 (101) tttcccttcttcttcttgcattttacagaggatttggcatatacattaggtttattcatgocctggattgattggatttggcaaatatacattaggtttgtttta  
 OMT/YH60/4 (101) tttcccttcttcttcttgcattttacagaggatttggcatatacattaggtttattcatgocctggattgattggatttggcaaatatacattaggtttgtttta

OMT-RH60/1 (201) tgcctagattgatttggatttggcatatacattgggttaacttttgcctggattgatttgggttattgggtatttataatggcagTATATATTGGAAACCAG  
 OMT-YH60/1 (201) tgcctagattg-----cttttgcctggattgatttgggttattgggtatttataatggcagTATATATTGGAAACCAG  
 OMT-YH60/2 (201) tgcctagattg-----cttttgcctggattgatttgggttattgggtatttataatggcagTATATATTGGAAACCAG  
 OMT/YH60/4 (201) tgcctagattg-----cttttgcctggattgatttgggttattgggtatttataatggcagTATATATTGGAAACCAG

OMT-RH60/1 (301) TGTTTATCCCCCGGAACTGAGCCAATGAGGGAGCTCAGAGAAATAACTGCCAAGCATCCATGgtaaaacccaatttctgaaaaatctatgtttgtgctac  
 OMT-YH60/1 (272) TGTTTATCCCCCGGAGCCTGAGCCAATGAGGGAGCTCAGAGAAATAACTGCTAAGCATCCATGgtaaaacccaatttctgaaaaatctatgtttgtgctac  
 OMT-YH60/2 (272) TGTTTATCCCCCGGAGCCTGAGCCAATGAGGGAGCTCAGAGAAATAACTGCTAAGCATCCATGgtaaaacccaatttctgaaaaatctatgtttgtgctac  
 OMT/YH60/4 (272) TGTTTATCCCCCGGAGCCTGAGCCAATGAGGGAGCTCAGAGAAATAACTGCTAAGCATCCATGgtaaaacccaatttctgaaaaatctatgtttgtgctac

OMT-RH60/1 (401) taattgaaaaatttatgttctgtgccactaataaactaatttcagtaaaacccaatttaggaaaaatattgttctccgttactaataatctaaagttag  
 OMT-YH60/1 (372) taattgaaaaatttatgttctgtgccactaataaactaatttcagtaaaacccaatttaggaaaaatattgttctccgttactaataatctaaagttag  
 OMT-YH60/2 (372) taattgaaaaatttatgttctgtgccactaataaactaatttcagtaaaacccaatttaggaaaaatattgttctccgttactaataatctaaagttag  
 OMT/YH60/4 (372) taattgaaaaatttatgttctgtgccactaataaactaatttcagtaaaacccaatttaggaaaaatattgttctccgttactaataatctaaagttag

OMT-RH60/1 (501) taaaacccaattttagaaaaatctgttctgtgctactaatagttcaatgtcagtaacgccccaaatttggaaaaatattatctctgtgctactaataacct  
 OMT-YH60/1 (472) taaaacccaattttagaaaaatctgttctgtg-----aaaaatttataatctctgtgctactaataacct  
 OMT-YH60/2 (472) taaaacccaattttagaaaaatctgttctgtg-----aaaaatttataatctctgtgctactaataacct  
 OMT/YH60/4 (472) taaaacccaattttagaaaaatctgttctgtg-----aaaaatttataatctctgtgctactaataacct

OMT-RH60/1 (601) aaggtcagtaaaatccagattttagaaaaatattatctctgtgctacaataatctaatcagtaataatcagtaaaacccaattttagaaaaatctaa-----tgt  
 OMT-YH60/1 (534) aaggtcagtaaaatccagattttagaaaaatattatctctgtgctacaataatctaatcagtaataatcagtaaaacccaattttagaaaaatctaa-----tgt  
 OMT-YH60/2 (534) aaggtcagtaaaatccagattttagaaaaatattatctctgtgctacaataatctaatcagtaataatcagtaaaacccaattttagaaaaatctaa-----tgt  
 OMT/YH60/4 (534) aaggtcagtaaaatccagattttagaaaaatattatctctgtgctacaataatctaatcagtaataatcagtaaaacccaattttagaaaaatctaa-----tgt

OMT-RH60/1 (696) ttgtactgact-----attttagaaaaatattatgttctgtgccactaataagcctaaatggcagtaaaacccaattt-----  
 OMT-YH60/1 (634) ttgtactgactaaacccaattttagaaaaatctatagcaaaaatctatgactctctgctactgacaaatctaatgtcagtaaaacccaattttagaaaaatctatgactactaa  
 OMT-YH60/2 (634) ttgtactgactaaacccaattttagaaaaatctatagcaaaaatctatgactctctgctactgacaaatctaatgtcagtaaaacccaattttagaaaaatctatgactactaa  
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OMT-RH60/1 (765) -----aggaaaaatctatgttctctgctactacaataatcaacgtcagtaaaacccaattttagaaaaatctctgttctgtgctactaataatgtt-----atgtgca  
 OMT-YH60/1 (734) caatttaggaaaaatctatgttctctgctactacaataatcaacgtcagtaaaacccaattttagaaaaatctct-----tctgtgctactaataatgttcaatgtgca  
 OMT-YH60/2 (734) caatttaggaaaaatctatgttctctgctactacaataatcaacgtcagtaaaacccaattttagaaaaatctct-----tctgtgctactaataatgttcaatgtgca  
 OMT/YH60/4 (734) caatttaggaaaaatctatgttctctgctactacaataatcaacgtcagtaaaacccaattttagaaaaatctct-----tctgtgctactaataatgttcaatgtgca

OMT-RH60/1 (858) gtaaaacccaattttagaaaaatattatctctgctactaataacctaaggtcagtaaaacccaattttagaaaaatattatctctgtgctacaataatg  
 OMT-YH60/1 (832) gtaaaacccaattttagaaaaatattatctctgctactaataacctaaggtcagtaaaacccaattttagaaaaatattatctctgtgctacaataatg  
 OMT-YH60/2 (832) gtaaaacccaattttagaaaaatattatctctgctactaataacctaaggtcagtaaaacccaattttagaaaaatattatctctgtgctacaataatg  
 OMT/YH60/4 (832) gtaaaacccaattttagaaaaatattatctctgctactaataacctaaggtcagtaaaacccaattttagaaaaatattatctctgtgctacaataatg


OMT-RH60/1 (958) taatcagtaaaacccaattttagaaaaatctttatttctgtgctagtcatagccctaaatgtcaggtttccatggtctgaagtatttcttccacatttctt  
 OMT-YH60/1 (932) tgaatcagtaaaacccaattttagaaaaatctttatttctgtgctagtcatagccctaaatgtcaggtttccatggtctgaagtatttcttccacatttctt  
 OMT-YH60/2 (932) tgaatcagtaaaacccaattttagaaaaatctttatttctgtgctagtcatagccctaaatgtcaggtttccatggtctgaagtatttcttccacatttctt  
 OMT/YH60/4 (932) tgaatcagtaaaacccaattttagaaaaatctttatttctgtgctagtcatagccctaaatgtcaggtttccatggtctgaagtatttcttccacatttctt

OMT-RH60/1 (1057) aaaaatgocctctgatttttttacagGAATTTGATGACTACTTCGGCTGATGAGGGCCAATTTTACATCTGTTGTTGAAGCTCATCAATGCCAAGAAGCACC  
 OMT-YH60/1 (1031) aaaaatgocctcttttttttacagGAATTTGATGACTACTTCGGCTGATGAGGGCCAATTTTACATCTGTTGTTGAAGCTCATCAATGCCAAGAAGCACC  
 OMT-YH60/2 (1031) aaaaatgocctcttttttttacagGAATTTGATGACTACTTCGGCTGATGAGGGCCAATTTTACATCTGTTGTTGAAGCTCATCAATGCCAAGAAGCACC  
 OMT/YH60/4 (1031) aaaaatgocctcttttttttacagGAATTTGATGACTACTTCGGCTGATGAGGGCCAATTTTACATCTGTTGTTGAAGCTCATCAATGCCAAGAAGCACC

(continued)

圖 6、臺灣扁柏與紅檜之咖啡醯輔酶 A 甲基轉移酶基因序列比對

Fig. 6. Alignment of caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) genomic DNA sequences in *Chamaecyparis formosensis* Matsum. (RH60/1) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2). The black boxes represent conserved percentage of 75%. The gray boxes represent conserved percentage of 25% and 50%, respectively. The hyphens between sequences mean deletion. Exons and introns are indicated by capital letters and lowercase letters, respectively.



*OMT*-RH60/1 (1157) ATGGAGATTGGTGTGATACTGGTTACTCGCTTCTCAGCACTGCTCTTGCCTACCTGATGATGGCAAGgtatacaaacgtgcacttctctgtgttttaa  
*OMT*-YH60/1 (1131) ATGGAGATTGGTGTGATACTGGTTACTCGCTTCTCAGCACTGCTCTTGCCTACCTGATGATGGCAAGgtatacaaacgtgcacttctctgtgttttaa  
*OMT*-YH60/2 (1131) ATGGAGATTGGTGTGATACTGGTTACTCGCTTCTCAGCACTGCTCTTGCCTACCTGATGATGGCAAGgtatacaaacgtgcacttctctgtgttttaa  
*OMT*/YH60/4 (1131) ATGGAGATTGGTGTGATACTGGTTACTCGCTTCTCAGCACTGCTCTTGCCTACCTGATGATGGCAAGgtatacaaacgtgcacttctctgtgttttaa  
  
*OMT*-RH60/1 (1257) tgaagcgcacaggattttaagggcgtccctctttattcagtgctttgaccttttacatattggcagatcataatcatgcattcacatgttcccttat-gggtt  
*OMT*-YH60/1 (1231) tgaagcgcacaggattttaagggcgtccctctttattcagtgctttgaccttttacatattggcagatcataatcatgcattcacatgttcccttaacggct  
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*OMT*-RH60/1 (1356) gagaaaaaattttaagggtttcccaatttacagtttactgattttcgtgtgcagATTTTAGCAATGGACATAAACAGGGGAGAACTATGAGTTGGGGTT  
*OMT*-YH60/1 (1331) gagaaaaaattttaagggtttcccaatttacagtttactgattttcgtgtgcagATTTTAGCAATGGACATAAACAGGGGAGAACTATGAGTTGGGGTT  
*OMT*-YH60/2 (1331) gagaaaaaattttaagggtttcccaatttacagtttactgattttcgtgtgcagATTTTAGCAATGGACATAAACAGGGGAGAACTATGAGTTGGGGTT  
*OMT*/YH60/4 (1331) gagaaaaaattttaagggtttcccaatttacagtttactgattttcgtgtgcagATTTTAGCAATGGACATAAACAGGGGAGAACTATGAGTTGGGGTT  
  
*OMT*-RH60/1 (1456) GCCAGTAATCAAAAAGCAGGGGTTGCCACAAAATTGACTTCAGAGAGGGCCCTGCCCTGCTTCTTGTCAAAATGTTGAAAAATgtaagcttaaat  
*OMT*-YH60/1 (1431) GCCAGTAATCAAAAAGCAGGGGTTGCCACAAAATTGACTTCAGAGAGGGCCCTGCCCTGCTTCTTGTCAAAATGTTGAAAAATgtaagcttaaat  
*OMT*-YH60/2 (1431) GCCAGTAATCAAAAAGCAGGGGTTGCCACAAAATTGACTTCAGAGAGGGCCCTGCCCTGCTTCTTGTCAAAATGTTGAAAAATgtaagcttaaat  
*OMT*/YH60/4 (1431) GCCAGTAATCAAAAAGCAGGGGTTGCCACAAAATTGACTTCAGAGAGGGCCCTGCCCTGCTTCTTGTCAAAATGTTGAAAAATgtaagcttaaat  
  
*OMT*-RH60/1 (1556) ctcatctgtatataaccagaaatgaaataactgttcaaaaatggggctattttttgtgggtagattctgaaatctagaaaaatfatgggtgtgatatgca  
*OMT*-YH60/1 (1531) ctcatctgtatataaccagaaatgaaataactgttcaaaaatggggctattttttgtgggtagattctgaaatctagaaaaatfatgggtgtgatatgca  
*OMT*-YH60/2 (1531) ctcatctgtatataaccagaaatgaaataactgttcaaaaatggggctattttttgtgggtagattctgaaatctagaaaaatfatgggtgtgatatgca  
*OMT*/YH60/4 (1531) ctcatctgtatataaccagaaatgaaataactgttcaaaaatggggctattttttgtgggtagattctgaaatctagaaaaatfatgggtgtgatatgca  
  
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*OMT*-YH60/1 (1631) gAAGGAAATGCATGGCTTCGATTCATATTCGTGGACGCAGACAAAGACAATTATCTGAATTACCACAAGCGGCTGATTGATCTGGTGAAGATTGGG  
*OMT*-YH60/2 (1631) gAAGGAAATGCATGGCTTCGATTCATATTCGTGGACGCAGACAAAGACAATTATCTGAATTACCACAAGCGGCTGATTGATCTGGTGAAGATTGGG  
*OMT*/YH60/4 (1631) gAAGGAAATGCATGGCTTCGATTCATATTCGTGGACGCAGACAAAGACAATTATCTGAATTACCACAAGCGGCTGATTGATCTGGTGAAGATTGGG  
  
*OMT*-RH60/1 (1756) GGAGTGATCGGGTACGACAATACTCTGTGGAATGGATCAGTGGTGGCTCCACCCGATGCCCAATGAGGAAATATGTGAGATATTACAGAGACTTTGTGA  
*OMT*-YH60/1 (1731) GGAGTGATCGGGTACGACAATACTCTGTGGAATGGATCAGTGGTGGCTCCACCCGATGCCCAATGAGGAAATATGTGAGATATTACAGAGACTTTGTGA  
*OMT*-YH60/2 (1731) GGAGTGATCGGGTACGACAATACTCTGTGGAATGGATCAGTGGTGGCTCCACCCGATGCCCAATGAGGAAATATGTGAGATATTACAGAGACTTTGTGA  
*OMT*/YH60/4 (1731) GGAGTGATCGGGTACGACAATACTCTGTGGAATGGATCAGTGGTGGCTCCACCCGATGCCCAATGAGGAAATATGTGAGATATTACAGAGACTTTGTGA  
  
*OMT*-RH60/1 (1856) TTGAACTGAACAAGGCCCTCGCTGCAGACCCTCGGATTGAAATCAGCCAAATTCCTGTAGGAGATGGCATCCTCTCTGCAGAAAGCTTATTTGA  
*OMT*-YH60/1 (1831) TTGAACTGAACAAGGCCCTCGCTGCAGACCCTCGGATTGAAATCAGCCAAATTCCTGTAGGAGATGGCATCCTCTCTGCAGAAAGCTTATTTGA  
*OMT*-YH60/2 (1831) TTGAACTGAACAAGGCCCTCGCTGCAGACCCTCGGATTGAAATCAGCCAAATTCCTGTAGGAGATGGCATCCTCTCTGCAGAAAGCTTATTTGA  
*OMT*/YH60/4 (1831) TTGAACTGAACAAGGCCCTCGCTGCAGACCCTCGGATTGAAATCAGCCAAATTCCTGTAGGAGATGGCATCCTCTCTGCAGAAAGCTTATTTGA

圖 6 (續)、臺灣扁柏與紅檜之咖啡醃輔酶 A 甲基轉移酶基因序列比對

Fig. 6. (continued) Alignment of caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) genomic DNA sequences in *Chamaecyparis formosensis* Matsum. (RH60/1) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2).

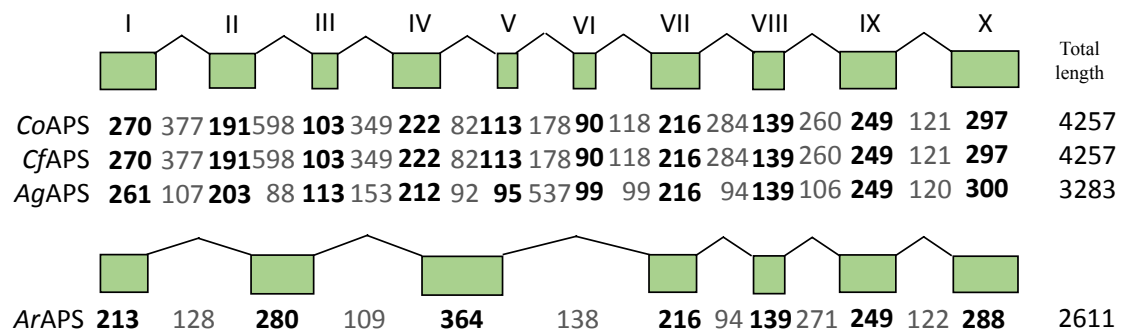


圖 7、不同物種間之蒎烯合成酶基因結構比較

Fig. 7. Schematic diagrams represent structure of genomic DNA of coding region for different pinene synthase (APS) genes. Nucleotide sequences from *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (CoAPS, YH60/2), *C. formosensis* Matsum. (CfAPS, RT31/1), *Abies grandis* Lindl. (AgAPS, U87909.1 and AF326517.1) and *Artemisia annua* L. (ArAPS, GU929215) were analyzed. Exons are indicated by boxes, introns are represented by bended lines between boxes, and the Arabic numerals under the diagrams indicate the lengths (base pairs) of the exons and introns. The Roman numerals above the boxes represent the number of exons.



*APS*-RT31/1-cDNA (1) ATGTCCTCGGTTGTATTACACCATTGGCTTCGACCTATGTTGAAACCCCAAGTAGTGAGGCCACTCATTATCATAAACCTCTTTTCACAGAAGCCTTTG  
 EU099434.1 (54) ATGTCCTCGGTTGTATTACACCATTGGCTTCGACCTATGTTGAAACCCCAAGTAGTGAGGCCACTCATTATCATAAACCTCTTTTCACAGAAGCCTTTG

*APS*-RT31/1-cDNA (103) AATAGACATACCCTAAGACCAAAATCCCTCTGCGCTCTAGAGTTGCTCAAAATCCCATTAAATGGCACTGATTACCCTGATGAAGGCATAACACGACGT  
 EU099434.1 (156) AATAGACATACCCTAAGACCAAAATCCCTCTGCGCTCTAGAGTTGCTCAAAATCCCATTAAATGGCACTGATTACCCTGATGAAGGCATAACACGACGT

*APS*-RT31/1-cDNA (205) ATTGGCAATCATCACCTAACCTTGTGGACGATGATTTTCATACAATCTCTCTCAAAGCTTATGAGGCCCTCTTATGGTGAACGCTGAGAACTGATA  
 EU099434.1 (258) ATTGGCAATCATCACCTAACCTTGTGGACGATGATTTTCATACAATCTCTCTCAAAGCTTATGAGGCCCTCTTATGGTGAACGCTGAGAACTGATA

*APS*-RT31/1-cDNA (307) AAGGATGTGAGGACATGTTCAATGCCCTTCTCTACACTCTTATCTGCAGACGATCTGATTCAACATCTTTCACTGGTTGACAGTGTAGAACGCCTGGGA  
 EU099434.1 (360) AAGGATGTGAGGACATGTTCAATGCCCTTCTCTACACTCTTATCTGCAGACGATCTGATTCAACATCTTTCACTGGTTGACAGTGTAGAACGCCTGGGA

*APS*-RT31/1-cDNA (409) ATTGACCGCCATTTTCAAATGAAATAAAAAACAGCTCTTGACTATGTTTACAGATATTGGAGCGACGCAGGCATTGGATGGTAGAGAGTACTCATGCA  
 EU099434.1 (462) ATTGACCGCCATTTTCAAATGAAATAAAAAACAGCTCTTGACTATGTTTACAGATATTGGAGCGACGCAGGCATTGGATGGTAGAGAGTACTCATGCA

*APS*-RT31/1-cDNA (511) GATCTAAACACTACAGCCTTAGGTTTTGCAATTTCTGCGCTTCATAGATATAGTGTCTGAGCAGATGTTGCAACAATTTGTTCTTAGAGATGGCCATTC  
 EU099434.1 (564) GATCTAAACACTACAGCCTTAGGTTTTGCAATTTCTGCGCTTCATAGATATAGTGTCTGAGCAGATGTTGCAACAATTTGTTCTTAGAGATGGCCATTC

*APS*-RT31/1-cDNA (613) TTGGATTCAAATAATCAGCCAAATGAAGACGATATAAAAAACATTTCAAATTTATTTTCGTGGTTCACCTCATAGCATTTCAGGGGAGAATGTTTTGGATGAT  
 EU099434.1 (666) TTGGATTCAAATAATCAGCCAAATGAAGACGATATAAAAAACATTTCAAATTTATTTTCGTGGTTCACCTCATAGCATTTCAGGGGAGAATGTTTTGGATGAT

*APS*-RT31/1-cDNA (715) GCAAAATCCTTTACAATGACATATTTAAAGCAAGTTCTACCAAGATTAGCAATTTAAATCTTTCAAGAGAGATAAAGTTCAATCTTGGATATGGTGGCAC  
 EU099434.1 (768) GCAAAATCCTTTACAATGACATATTTAAAGCAAGTTCTACCAAGATTAGCAATTTAAATCTTTCAAGAGAGATAAAGTTCAATCTTGGATATGGTGGCAC

*APS*-RT31/1-cDNA (817) ACCAATGTGCCTAGGTTGGAAGCAAGACCTATATTGATATATATGGAGAAGATAGTTCATGGGCTTCCAAAAGCATTAAACAATATCTTCTATACAAAACCT  
 EU099434.1 (870) ACCAATGTGCCTAGGTTGGAAGCAAGACCTATATTGATATATATGGAGAAGATAGTTCATGGGCTTCCAAAAGCATTAAACAATATCTTCTATACAAAACCT

*APS*-RT31/1-cDNA (919) CTAGAATTGGCAAAGTTGGACTTCAACATAATTCAGTCACTACAACAACAAGAGCTTCAAATCTTCAAGATGGTGGATGGAGTCTGATTTGGTAAAGTA  
 EU099434.1 (972) CTAGAATTGGCAAAGTTGGACTTCAACATAATTCAGTCACTACAACAACAAGAGCTTCAAATCTTCAAGATGGTGGATGGAGTCTGATTTGGTAAAGTA

*APS*-RT31/1-cDNA (1021) GATTTTGCTCGCCATCGCCATGTGGAGTATTACCTTTGGGACGCTACAGGGTGTATTGAGCCCAAATATTGAGCTTTTGAATTTGGTTTTGCAAAGTTGTCT  
 EU099434.1 (1074) GATTTTGCTCGCCATCGCCATGTGGAGTATTACCTTTGGGACGCTACAGGGTGTATTGAGCCCAAATATTGAGCTTTTGAATTTGGTTTTGCAAAGTTGTCT

*APS*-RT31/1-cDNA (1123) GCACCTGTGCACATATTTGGATGATATTTACGACACTTATGGAACCTTTTATGAGCTCAAATAATTTACAAAAGCTATTTAAAGTGGGATGCATCAATCATA  
 EU099434.1 (1176) GCACCTGTGCACATATTTGGATGATATTTACGACACTTATGGAACCTTTTATGAGCTCAAATAATTTACAAAAGCTATTTAAAGTGGGATGCATCAATCATA

*APS*-RT31/1-cDNA (1225) AAAGGACTTCCAGAATTTATGAAAGTGGCATTGAAAGCTTTTGTGAGGCTGTGAAGGACATGGCTCAAGAGGCCAAGAAAACCTCAAGGTAGAGACACACTT  
 EU099434.1 (1275) AAAGGACTTCCAGAATTTATGAAAGTGGCATTGAAAGCTTTTGTGAGGCTGTGAAGGACATGGCTCAAGAGGCCAAGAAAACCTCAAGGTAGAGACACACTT

*APS*-RT31/1-cDNA (1327) GACTATGCACGTAAGCGTGGGAAGTATATATAGATGCTTACATGAAAGAGCAGAGTGGCTTGTACTCGGTTATATGCCATCTTTAGAGGAATATTTAGAA  
 EU099434.1 (1377) GACTATGCACGTAAGCGTGGGAAGTATATATAGATGCTTACATGAAAGAGCAGAGTGGCTTGTACTCGGTTATATGCCATCTTTAGAGGAATATTTAGAA

*APS*-RT31/1-cDNA (1429) AATGGAAAAGTAAGTGCAGGGTCTCGTGTGTGACCTTGAACCTATTTTATCACTAGATGTGCCTCTTTGAGATGAATTTCTCAAAGAAATCGATTATCCA  
 EU099434.1 (1479) AATGGAAAAGTAAGTGCAGGGTCTCGTGTGTGACCTTGAACCTATTTTATCACTAGATGTGCCTCTTTGAGATGAATTTCTCAAAGAAATCGATTATCCA

*APS*-RT31/1-cDNA (1531) TCAAGGTTTGTGAGTTATTATGCTTAACCCCTAAGATTAAGAGGTGACACAAAGGACTTTTAAAGGCTGAAGCAGATCGTGGAGAAGTGGTATCATGTATAACA  
 EU099434.1 (1581) TCAAGGTTTGTGAGTTATTATGCTTAACCCCTAAGATTAAGAGGTGACACAAAGGACTTTTAAAGGCTGAAGCAGATCGTGGAGAAGTGGTATCATGTATAACA

*APS*-RT31/1-cDNA (1633) TGTACATGAAAGACCATCTGGATCCAATGAAGAAGATGCATTAATTTATCTCAATTTCTTGGATTGATGAAAGACTCAAAGAAATAAATTTGGGAATATTTA  
 EU099434.1 (1683) TGTACATGAAAGACCATCTGGATCCAATGAAGAAGATGCATTAATTTATCTCAATTTCTTGGATTGATGAAAGACTCAAAGAAATAAATTTGGGAATATTTA

*APS*-RT31/1-cDNA (1735) AAAAATGATAATGTCCCAATAATTAGCAAAGGTAATGCCTATAAATTTATCAAAGGCTCCAACCTTTGTATAAGGAAAGAGATGGATTTACGGTCTCTAGC  
 EU099434.1 (1785) AAAAATGATAATGTCCCAATAATTAGCAAAGGTAATGCCTATAAATTTATCAAAGGCTCCAACCTTTGTATAAGGAAAGAGATGGATTTACGGTCTCTAGC

*APS*-RT31/1-cDNA (1837) ATTGAGACAAAAAATTTATTTACAGAATGATGATTGGATCAATTTCAATA  
 EU099434.1 (1887) ATTGAGACAAAAAATTTATTTACAGAATGATGATTGGATCAATTTCAATA

圖 8、紅檜之蒎烯合成酶基因編碼區序列比較

Fig. 8. Alignment of cDNA sequence for alpha pinene synthase (*APS*) genes between two different individual of *Chamaecyparis formosensis* Matsum. (RT31/1, EU099434.1). The black boxes represent the different cDNA sequence.

APS-RT31/1 (1) ATGTCTCTCGGTTGTATTACACCATTGGCTTCCACTATGTTTGAACCCAAGTTAGTGAGGCCACTCATTATCATATAAGCCCTCTTTTTCATCACAAGCCTT  
 APS-RT31/6 (1) ATGTCTCTCGGTTGTATTACACCATTGGCTTCCACTATGTTTGAACCCAAGTTAGTGAGGCCACTCATTATCATATAAGCCCTCTTTTTCATCACAAGCCTT  
 APS-YH60/2 (1) ATGTCTCTCGGTTGTATTACACCATTGGCTTCCACTATGTTTGAACCCAAGTTAGTGAGGCCACTCATTATCATATAAGCCCTCTTTTTCATCACAAGCCTT  
 APS-YT31/6 (1) ATGTCTCTCGGTTGTATTACACCATTGGCTTCCACTATGTTTGAACCCAAGTTAGTGAGGCCACTCATTATCATATAAGCCCTCTTTTTCATCACAAGCCTT  
  
 APS-RT31/1 (101) TGAATAGAGCATACCCTAAGACCAAAATCCCTCTGCGCTCTAGAGTTGCTCAAAATCCCATTAAATATGGCACTGATTACCACTGATGAAGGCATAAACAGG  
 APS-RT31/6 (101) TGAATAGAGCATACCCTAAGACCAAAATCCCTCTGCGCTCTAGAGTTGCTCAAAATCCCATTAAATATGGCACTGATTACCACTGATGAAGGCATAAACAGG  
 APS-YH60/2 (101) TGAATAGAGCATACCCTAAGACCAAAATCCCTCTGCGCTCTAGAGTTGCTCAAAATCCCATTAAATATGGCACTGATTACCACTGATGAAGGCATAAACAGG  
 APS-YT31/6 (101) TGAATAGAGCATACCCTAAGACCAAAATCCCTCTGCGCTCTAGAGTTGCTCAAAATCCCATTAAATATGGCACTGATTACCACTGATGAAGGCATAAACAGG  
  
 APS-RT31/1 (201) ACGTATTGGCAATCATCACCTAACTTGTGGGACGATGATTTTCATACAATCTCTCTCAAAAGCTTATGAGgtacatcctagtttactatcatcttgagc  
 APS-RT31/6 (201) ACGTATTGGCAATCATCACCTAACTTGTGGGACGATGATTTTCATACAATCTCTCTCAAAAGCTTATGAGgtacatcctagtttactatcatcttgagc  
 APS-YH60/2 (201) ACGTATTGGCAATCATCACCTAACTTGTGGGACGATGATTTTCATACAATCTCTCTCAAAAGCTTATGAGgtacatcctagtttactatcatcttgagc  
 APS-YT31/6 (201) ACGTATTGGCAATCATCACCTAACTTGTGGGACGATGATTTTCATACAATCTCTCTCAAAAGCTTATGAGgtacatcctagtttactatcatcttgagc  
  
 APS-RT31/1 (301) tcagttaaatcactaaatagttcgttctagttcttttggattagtttatgggattttatTTTTccatgttgggattatcgttcatgaatgaacaaagag  
 APS-RT31/6 (301) tcagttaaatcactaaatagttcgttctagttcttttggattagtttatgggattttatTTTTccatgttgggattatcgttcatgaatgaacaaagag  
 APS-YH60/2 (301) tcagttaaatcactaaatagttcgttctagttcttttggattagtttatgggattttatTTTTccatgttgggattatcgttcatgaatgaacaaagag  
 APS-YT31/6 (301) tcagttaaatcactaaatagttcgttctagttcttttggattagtttatgggattttatTTTTccatgttgggattatcgttcatgaatgaacaaagag  
  
 APS-RT31/1 (401) tcataaaagt tactatccctgcataaattgttgtgcttaacctatcttcttaatttactatcttctgaactgaatttaagtagtgcaatattgaatg  
 APS-RT31/6 (401) tcataaaagt tactatccctgcataaattgttgtgcttaacctatcttcttaatttactatcttctgaactgaatttaagtagtgcaatattgaatg  
 APS-YH60/2 (401) tcataaaagt tactatccctgcataaattgttgtgcttaacctatcttcttaatttactatcttctgaactgaatttaagtagtgcaatattgaatg  
 APS-YT31/6 (401) tcataaaagt tactatccctgcataaattgttgtgcttaacctatcttcttaatttactatcttctgaactgaatttaagtagtgcaatattgaatg  
  
 APS-RT31/1 (501) tttcataatggattttgtgagtgctctattgatttaagttttagaccatgttttagaacaaacaaagaaaaagagaaaagaaaagtgtactaaag  
 APS-RT31/6 (501) tttcataatggattttgtgagtgctctattgatttaagttttagaccatgttttagaacaaacaaagaaaaagagaaaagaaaagtgtactaaag  
 APS-YH60/2 (501) tttcataatggattttgtgagtgctctattgatttaagttttagaccatgttttagaacaaacaaagaaaaagagaaaagaaaagtgtactaaag  
 APS-YT31/6 (501) tttcataatggattttgtgagtgctctattgatttaagttttagaccatgttttagaacaaacaaagaaaaagagaaaagaaaagtgtactaaag  
  
 APS-RT31/1 (601) aatctgaagttctgatctctttttgcatctgtgatggccggtcacagGCCCCCTCTTATGGTGAACGTGCTGAGAAGCTGATAAAGGATGTCAGGGACAT  
 APS-RT31/6 (601) aatctgaagttctgatctctttttgcatctgtgatggccggtcacagGCCCCCTCTTATGGTGAACGTGCTGAGAAGCTGATAAAGGATGTCAGGGACAT  
 APS-YH60/2 (601) aatctgaagttctgatctctttttgcatctgtgatggccggtcacagGCCCCCTCTTATGGTGAACGTGCTGAGAAGCTGATAAAGGATGTCAGGGACAT  
 APS-YT31/6 (601) aatctgaagttctgatctctttttgcatctgtgatggccggtcacagGCCCCCTCTTATGGTGAACGTGCTGAGAAGCTGATAAAGGATGTCAGGGACAT  
  
 APS-RT31/1 (701) GTTCAATGCCCTTCCTCTACACTCTTCATCTGCAGCAGTCTGATTCAACATCTTTCCTGGTTGACAGTGTAGAAGCCTGGGAATTGACCGCCATTTT  
 APS-RT31/6 (701) GTTCAATGCCCTTCCTCTACACTCTTCATCTGCAGCAGTCTGATTCAACATCTTTCCTGGTTGACAGTGTAGAAGCCTGGGAATTGACCGCCATTTT  
 APS-YH60/2 (701) GTTCAATGCCCTTCCTCTACACTCTTCATCTGCAGCAGTCTGATTCAACATCTTTCCTGGTTGACAGTGTAGAAGCCTGGGAATTGACCGCCATTTT  
 APS-YT31/6 (701) GTTCAATGCCCTTCCTCTACACTCTTCATCTGCAGCAGTCTGATTCAACATCTTTCCTGGTTGACAGTGTAGAAGCCTGGGAATTGACCGCCATTTT  
  
 APS-RT31/1 (801) CAAAATGAAATAAAAACAGCTCTTGACTATGTTTACAGgtttgctgttttctaaagctctcggatgagtgattatgtttttgcatatgttatgaaa  
 APS-RT31/6 (801) CAAAATGAAATAAAAACAGCTCTTGACTATGTTTACAGgtttgctgttttctaaagctctcggatgagtgattatgtttttgcatatgttatgaaa  
 APS-YH60/2 (801) CAAAATGAAATAAAAACAGCTCTTGACTATGTTTACAGgtttgctgttttctaaagctctcggatgagtgattatgtttttgcatatgttatgaaa  
 APS-YT31/6 (801) CAAAATGAAATAAAAACAGCTCTTGACTATGTTTACAGgtttgctgttttctaaagctctcggatgagtgattatgtttttgcatatgttatgaaa  
  
 APS-RT31/1 (901) aagacttttccctagtgtaaacctatgtttcaagtaaacagtgatcagatatttaataagaaatatacacttgatttatgatagaatttaacaacata  
 APS-RT31/6 (901) aagacttttccctagtgtaaacctatgtttcaagtaaacagtgatcagatatttaataagaaatatacacttgatttatgatagaatttaacaacata  
 APS-YH60/2 (901) aagacttttccctagtgtaaacctatgtttcaagtaaacagtgatcagatatttaataagaaatatacacttgatttatgatagaatttaacaacata  
 APS-YT31/6 (901) aagacttttccctagtgtaaacctatgtttcaagtaaacagtgatcagatatttaataagaaatatacacttgatttatgatagaatttaacaacata  
  
 APS-RT31/1 (1001) ttttatgtttgatttgttaggtatcctaactgagatcatalaattagcctgatttcagatgatttatttgggtgattacggtgtctttggcaaacac  
 APS-RT31/6 (1001) ttttatgtttgatttgttaggtatcctaactgagatcatalaattagcctgatttcagatgatttatttgggtgattacggtgtctttggcaaacac  
 APS-YH60/2 (1001) ttttatgtttgatttgttaggtatcctaactgagatcatalaattagcctgatttcagatgatttatttgggtgattacggtgtctttggcaaacac  
 APS-YT31/6 (1001) ttttatgtttgatttgttaggtatcctaactgagatcatalaattagcctgatttcagatgatttatttgggtgattacggtgtctttggcaaacac  
  
 APS-RT31/1 (1101) tgtttatgtgaaaaatgagtcctcaagtaaacagagtaaacgaccataaaaaagcattttgtgctcgtatgtgtgtggaactaggggttggttct  
 APS-RT31/6 (1101) tgtttatgtgaaaaatgagtcctcaagtaaacagagtaaacgaccataaaaaagcattttgtgctcgtatgtgtgtggaactaggggttggttct  
 APS-YH60/2 (1101) tgtttatgtgaaaaatgagtcctcaagtaaacagagtaaacgaccataaaaaagcattttgtgctcgtatgtgtgtggaactaggggttggttct  
 APS-YT31/6 (1101) tgtttatgtgaaaaatgagtcctcaagtaaacagagtaaacgaccataaaaaagcattttgtgctcgtatgtgtgtggaactaggggttggttct

(continued)

圖 9、臺灣扁柏與紅檜之蒎烯合成酶基因序列比對

Fig. 9. Alignment of alpha pinene synthase (APS) genomic DNA sequences in *Chamaecyparis formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6). The black boxes represent conserved percentage of 25%. The dark and light gray boxes represent conserved percentage of 50% and 75%, respectively. The hyphens between sequences mean deletion. Exons and introns are indicated by capital letters and lowercase letters, respectively.

*APS-RT31/1* (1201) actgaataataactaacttttgatttatlgagacgattcatgtaaaaatgtaataacatagaatttaacttttgatttatlgagacgattcatgtaaaa  
*APS-RT31/6* (1201) actgaataataactaacttttgatttatlgagacgattcatgtaaaaatgtaataacatagaatttaacttttgatttatlgagacgattcatgtaaaa  
*APS-YH60/2* (1201) actgaataataactaacttttgatttatlgagacgattcatgtaaaaatgtaataacatagaatttaacttttgatttatlgagacgattcatgtaaaa  
*APS-YT31/6* (1201) actgaataataactaacttttgatttatlgagacgattcatgtaaaaatgtaataacatagaatttaacttttgatttatlgagacgattcatgtaaaa  
  
*APS-RT31/1* (1301) ttgtaataacatagaatttttaaggaaaaatgtacaatttttaaaaaaaatataaatttagatgtacttttgaaatcaaaagtctatccattag  
*APS-RT31/6* (1301) ttgtaataacatagaatttttaaggaaaaatgtacaatttttaaaaaaaatataaatttagatgtacttttgaaatcaaaagtctatccattag  
*APS-YH60/2* (1301) ttgtaataacatagaatttttaaggaaaaatgtacaatttttaaaaaaaatataaatttagatgtacttttgaaatcaaaagtctatccattag  
*APS-YT31/6* (1301) ttgtaataacatagaatttttaaggaaaaatgtacaatttttaaaaaaaatataaatttagatgtacttttgaaatcaaaagtctatccattag  
  
*APS-RT31/1* (1401) aacaattctcatatcttggaaatatttaataaacagATATTGGAGCGACGCAGGCATTGGATGGTAGAGAGAGTACTCATGCAGATCTAAACACTACA  
*APS-RT31/6* (1401) aacaattctcatatcttggaaatatttaataaacagATATTGGAGCGACGCAGGCATTGGATGGTAGAGAGAGTACTCATGCAGATCTAAACACTACA  
*APS-YH60/2* (1401) aacaattctcatatcttggaaatatttaataaacagATATTGGAGCGACGCAGGCATTGGATGGTAGAGAGAGTACTCATGCAGATCTAAACACTACA  
*APS-YT31/6* (1401) aacaattctcatatcttggaaatatttaataaacagATATTGGAGCGACGCAGGCATTGGATGGTAGAGAGAGTACTCATGCAGATCTAAACACTACA  
  
*APS-RT31/1* (1501) GCCTTAGGTTTTCGAATTCCTGCCTTCATAGATATAGTgtctcttcaggtaatgtctgcagttactgaatcagatttttttcatttaagttctgaatt  
*APS-RT31/6* (1501) GCCTTAGGTTTTCGAATTCCTGCCTTCATAGATATAGTgtctcttcaggtaatgtctgcagttactgaatcagatttttttcatttaagttctgaatt  
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*APS-YT31/6* (1501) GCCTTAGGTTTTCGAATTCCTGCCTTCATAGATATAGTgtctcttcaggtaatgtctgcagttactgaatcagatttttttcatttaagttctgaatt  
  
*APS-RT31/1* (1601) tagaatttatagaaaagattgagatgactataaactctatttagatcacagagatgggtggcaaaagtatctgttctgcaataatgacgggtgccaatga  
*APS-RT31/6* (1601) tagaatttatagaaaagattgagatgactataaactctatttagatcacagagatgggtggcaaaagtatctgttctgcaataatgacgggtgccaatga  
*APS-YH60/2* (1601) tagaatttatagaaaagattgagatgactataaactctatttagatcacagagatgggtggcaaaagtatctgttctgcaataatgacgggtgccaatga  
*APS-YT31/6* (1601) tagaatttatagaaaagattgagatgactataaactctatttagatcacagagatgggtggcaaaagtatctgttctgcaataatgacgggtgccaatga  
  
*APS-RT31/1* (1701) aatttcagatgtaagacaatatggaaaaatgaaatgaaatcattgtgtctcatcatagaatcggttatgtttagatcaaaaattttatatttacttc  
*APS-RT31/6* (1701) aatttcagatgtaagacaatatggaaaaatgaaatgaaatcattgtgtctcatcatagaatcggttatgtttagatcaaaaattttatatttacttc  
*APS-YH60/2* (1701) aatttcagatgtaagacaatatggaaaaatgaaatgaaatcattgtgtctcatcatagaatcggttatgtttagatcaaaaattttatatttacttc  
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*APS-RT31/1* (1801) ttaaaaagaaatttataataaaatataatatttctattttatattctgtataaaaattttaaattgtttagtttttaataagaacttatGTCATGGCAGAT  
*APS-RT31/6* (1801) ttaaaaagaaatttataataaaatataatatttctattttatattctgtataaaaattttaaattgtttagtttttaataagaacttatGTCATGGCAGAT  
*APS-YH60/2* (1801) ttaaaaagaaatttataataaaatataatatttctattttatattctgtataaaaattttaaattgtttagtttttaataagaacttatGTCATGGCAGAT  
*APS-YT31/6* (1801) ttaaaaagaaatttataataaaatataatatttctattttatattctgtataaaaattttaaattgtttagtttttaataagaacttatGTCATGGCAGAT  
  
*APS-RT31/1* (1901) GTGTTGCAACAATTTGTTCTTAGAGATGGGCCATTCTGGATTCAAATAATCAGCCAAATGAAGACGATATAAAAAACATCTAAATTTATTCGTGGTT  
*APS-RT31/6* (1901) GTGTTGCAACAATTTGTTCTTAGAGATGGGCCATTCTGGATTCAAATAATCAGCCAAATGAAGACGATATAAAAAACATCTAAATTTATTCGTGGTT  
*APS-YH60/2* (1901) GTGTTGCAACAATTTGTTCTTAGAGATGGGCCATTCTGGATTCAAATAATCAGCCAAATGAAGACGATATAAAAAACATCTAAATTTATTCGTGGTT  
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Fig. 9. (continued) Alignment of alpha pinene synthase (*APS*) genomic DNA sequences in *Chamaecyparis formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6).



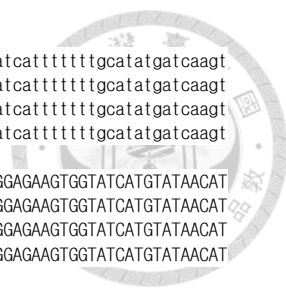
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*APS-YH60/2* (2501) TTCTAGAATTGGCAAAGTTGGACTTCAACATAAATTCAGTCACTACAACAACAAGAGCTTCAAATTTCTCTCAAGGtgagaat t t t t a c g t t a t g a a a t a t a t  
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*APS-RT31/6* (2601) t c t t t a a a t t t a a g g c g t a a a t c t t t t a c a a t a t a a t a t g t a t t g g a g a t t g a a a a a t c a a g t t t a c t a a g c a a t g t a t g t t c a t t t t a g A T G G T G G A T  
*APS-YH60/2* (2601) t c t t t a a a t t t a a g g c g t a a a t c t t t t a c a a t a t a a t a t g t a t t g g a g a t t g a a a a a t c a a g t t t a c t a a g c a a t g t a t g t t c a t t t t a g A T G G T G G A T  
*APS-YT31/6* (2601) t c t t t a a a t t t a a g g c g t a a a t c t t t t a c a a t a t a a t a t g t a t t g g a g a t t g a a a a a t c a a g t t t a c t a a g c a a t g t a t g t t c a t t t t a g A T G G T G G A T  
  
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*APS-RT31/1* (2801) A G A A T T G G G T T T G C A A A G T T G T C T G C A C T T G T C A C A T A T T T G G A T A T T A C G A C A C T T A T G G A A C T T T T G A T G A G C T C A A A A T A T T C A C A A A A G C T A  
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*APS-RT31/1* (2901) T T A A A A G g t a t t c a t t t a t t g t t a t t g g t a a a t a g a t t g a t a t g c t a g a a a a g t t a a a t a t c t a g t t a g t t a t c t a g g t a c a t c a a t c t t a g t g c a g g  
*APS-RT31/6* (2901) T T A A A A G g t a t t c a t t t a t t g t t a t t g g t a a a t a g a t t g a t a t g c t a g a a a a g t t a a a t a t c t a g t t a g t t a t c t a g g t a c a t c a a t c t t a g t g c a g g  
*APS-YH60/2* (2901) T T A A A A G g t a t t c a t t t a t t g t t a t t g g t a a a t a g a t t g a t a t g c t a g a a a a g t t a a a t a t c t a g t t a g t t a t c t a g g t a c a t c a a t c t t a g t g c a g g  
*APS-YT31/6* (2901) T T A A A A G g t a t t c a t t t a t t g t t a t t g g t a a a t a g a t t g a t a t g c t a g a a a a g t t a a a t a t c t a g t t a g t t a t c t a g g t a c a t c a a t c t t a g t g c a g g  
  
*APS-RT31/1* (3001) a a a t t c a c a t g t t g a c t a t a a t t c a t t g t g g t t a a t t g t a g g a a a a c a t a a a t g g t t t t a a t t t t c t a t g t t c t t g t a c t t a c t a a a c a c a t t t c t c t a  
*APS-RT31/6* (3001) a a a t t c a c a t g t t g a c t a t a a t t c a t t g t g g t t a a t t g t a g g a a a a c a t a a a t g g t t t t a a t t t t c t a t g t t c t t g t a c t t a c t a a a a c a c a t t t c t c t a  
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*APS-RT31/1* (3101) g a c a t t g a a a t a t a a t a a a t a a a t t a t t a a t a a a t t t c t a a a t t t a t a t a t a a a a t t t a g t a a t a a a t t a g t t t c t t c a t g t a a g t g g g a t g c  
*APS-RT31/6* (3101) g a c a t t g a a a t a t a a t a a a t a a a t t a t t a a t a a a t t t t a a a t t t a t a t a t a a a a t t t a g t a a t a a a t t a g t t t c t t c a t g t a a g t g g g a t g c  
*APS-YH60/2* (3101) g a c a t t g a a a t a t a a t a a a t a a a t t a t t a a t a a a t t t t a a a t t t a t a t a t a a a a t t t a g t a a t a a a t t a g t t t c t t c a t g t a a g t g g g a t g c  
*APS-YT31/6* (3101) g a c a t t g a a a t a t a a t a a a t a a a t t a t t a a t a a a t t t t a a a t t t a t a t a t a a a a t t t a g t a a t a a a t t a g t t t c t t c a t g t a a g t g g g a t g c  
  
*APS-RT31/1* (3201) A T C A A T C A T A A A A G G A C T T C C A G A A T T A T G A A A G T G G C A T T C G A A G C T T T T G A T G A A G C T G T G A A G G A C A T G G C T C A A G A G C C A A G A A A A C T C A A G G T  
*APS-RT31/6* (3201) A T C A A T C A T A A A A G G A C T T C C A G A A T T A T G A A A G T G G C A T T C G A A G C T T T T G A T G A A G C T G T G A A G G A C A T G G C T C A A G A G C C A A G A A A A C T C A A G G T  
*APS-YH60/2* (3201) A T C A A T C A T A A A A G G A C T T C C A G A A T T A T G A A A G T G G C A T T C G A A G C T T T T G A T G A A G C T G T G A A G G A C A T G G C T C A A G A G C C A A G A A A A C T C A A G G T  
*APS-YT31/6* (3201) A T C A A T C A T A A A A G G A C T T C C A G A A T T A T G A A A G T G G C A T T C G A A G C T T T T G A T G A A G C T G T G A A G G A C A T G G C T C A A G A G C C A A G A A A A C T C A A G G T  
  
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*APS-RT31/6* (3301) A G A G A C A C A C T T G A C T A T G C A C G T A A A G C G t a a g g a t t a t t a a t t g t a t g t g t t t a a t a a a t a t a a t a g a t g t c t a t a a t a t a a t g t g t g t g t  
*APS-YH60/2* (3301) A G A G A C A C A C T T G A C T A T G C A C G T A A A G C G t a a g g a t t a t t a a t t g t a t g t g t t t a a t a a a t a t a a t a g a t g t c t a t a a t a t a a t g t g t g t g t  
*APS-YT31/6* (3301) A G A G A C A C A C T T G A C T A T G C A C G T A A A G C G t a a g g a t t a t t a a t t g t a t g t g t t t a a t a a a t a t a a t a g a t g t c t a t a a t a t a a t g t g t g t g t  
  
*APS-RT31/1* (3401) g t g t g t g t g t g t g t g t a a t c t a c t t t t c c c t t g a c a c t g a t g a a a t t g a a t t a g a t t t t a a a a a a a t t a a a t a t a g a t a t t t g g c a a t a t  
*APS-RT31/6* (3401) g t g t g t g t g t g t g t g t g t a a t c t a c t t t t c c c t t g a c a c t g a t g a a a t t g a a t t a g a t t t t a a a a a a a t t a a a t a t a g a t a t t t g g c a a t a t  
*APS-YH60/2* (3401) g t g t g t g t g t g t g t g t a a t c t a c t t t t c c c t t g a c a c t g a t g a a a t t g a a t t a g a t t t t a a a a a a a t t a a a t a t a g a t a t t t g g c a a t a t  
*APS-YT31/6* (3401) g t g t g t g t g t g t g t g t a a t c t a c t t t t c c c t t g a c a c t g a t g a a a t t g a a t t a g a t t t t a a a a a a a t t a a a t a t a g a t a t t t g g c a a t a t  
  
*APS-RT31/1* (3501) t t a a c c t a g g g c t t g g t t t c g t t a g a t a t t c a t g t t a a t a t c a t g a a a t a t a g t a t t a a t t a t a a t a t c t t t g a a t g a g T G G G A A G T A T  
*APS-RT31/6* (3501) t t a a c c t a g g g c t t g g t t t c g t t a g a t a t t c a t g t t a a t a t c a t g a a a t a t a g t a t t a a t t a t a a t a t c t t t g a a t g a g T G G G A A G T A T  
*APS-YH60/2* (3501) t t a a c c t a g g g c t t g g t t t c g t t a g a t a t t c a t g t t a a t a t c a t g a a a t a t a g t a t t a a t t a t a a t a t c t t t g a a t g a g T G G G A A G T A T  
*APS-YT31/6* (3501) t t a a c c t a g g g c t t g g t t t c g t t a g a t a t t c a t g t t a a t a t c a t g a a a t a t a g t a t t a a t t a t a a t a t c t t t g a a t g a g T G G G A A G T A T  
  
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*APS-RT31/1* (3701) T G T T G T G A C C T T G C A A C C T A T T T T A C A C T A G A T G T G C C T C T T C A G A T G A A A T T C T C A A A G A A A T C G A T T A T C C A T C A A G G T T T G A T G A G T T A T T A T G C  
*APS-RT31/6* (3701) T G T T G T G A C C T T G C A A C C T A T T T T A C A C T A G A T G T G C C T C T T C A G A T G A A A T T C T C A A A G A A A T C G A T T A T C C A T C A A G G T T T G A T G A G T T A T T A T G C  
*APS-YH60/2* (3701) T G T T G T G A C C T T G C A A C C T A T T T T A C A C T A G A T G T G C C T C T T C A G A T G A A A T T C T C A A A G A A A T C G A T T A T C C A T C A A G G T T T G A T G A G T T A T T A T G C  
*APS-YT31/6* (3701) T G T T G T G A C C T T G C A A C C T A T T T T A C A C T A G A T G T G C C T C T T C A G A T G A A A T T C T C A A A G A A A T C G A T T A T C C A T C A A G G T T T G A T G A G T T A T T A T G C

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Fig. 9. (continued) Alignment of alpha pinene synthase (*APS*) genomic DNA sequences in *Chamaecyparis formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6).





*APS-RT31/1* (3801) TTAACCCCTAAGATTAAGAGGTGACACAAGGACTTTTAAAGgtatgatataaaattgaaactttaactttacaacatcattttttgcatatgatcaagt  
*APS-RT31/6* (3801) TTAACCCCTAAGATTAAGAGGTGACACAAGGACTTTTAAAGgtatgatataaaattgaaactttaactttacaacatcattttttgcatatgatcaagt  
*APS-YH60/2* (3801) TTAACCCCTAAGATTAAGAGGTGACACAAGGACTTTTAAAGgtatgatataaaattgaaactttaactttacaacatcattttttgcatatgatcaagt  
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*APS-YT31/6* (3901) aacatagattcaagatgagtatatttgagaacttataaataactaatatgtttttgaagGCTGAAGCAGATCGTGGAGAAGTGGTATCATGTATAACAT  
  
*APS-RT31/1* (4001) GTTACATGAAAGACCATCCTGGATCCAATGAAGAAGATGCATTAATTTATCTCAATTCCTTTGATTGATGAAAGACTCAAAGAATTAATTTGGGAATATTT  
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*APS-YH60/2* (4001) GTTACATGAAAGACCATCCTGGATCCAATGAAGAAGATGCATTAATTTATCTCAATTCCTTTGATTGATGAAAGACTCAAAGAATTAATTTGGGAATATTT  
*APS-YT31/6* (4001) GTTACATGAAAGACCATCCTGGATCCAATGAAGAAGATGCATTAATTTATCTCAATTCCTTTGATTGATGAAAGACTCAAAGAATTAATTTGGGAATATTT  
  
*APS-RT31/1* (4101) AAAAAATGATAATGTCCCAATAATTAGCAAAGGTAATGCCTATAATTTATCAAAGGTCTCCAACCTTTGTATAAGGAAAAGAGATGGATTTACGGTCTCT  
*APS-RT31/6* (4101) AAAAAATGATAATGTCCCAATAATTAGCAAAGGTAATGCCTATAATTTATCAAAGGTCTCCAACCTTTGTATAAGGAAAAGAGATGGATTTACGGTCTCT  
*APS-YH60/2* (4101) AAAAAATGATAATGTCCCAATAATTAGCAAAGGTAATGCCTATAATTTATCAAAGGTCTCCAACCTTTGTATAAGGAAAAGAGATGGATTTACGGTCTCT  
*APS-YT31/6* (4101) AAAAAATGATAATGTCCCAATAATTAGCAAAGGTAATGCCTATAATTTATCAAAGGTCTCCAACCTTTGTATAAGGAAAAGAGATGGATTTACGGTCTCT  
  
*APS-RT31/1* (4201) AGCATTGAGACAAAAAATCTTATCTACAGAATGATGATTGGATCAATTCCAATA  
*APS-RT31/6* (4201) AGCATTGAGACAAAAAATCTTATCTACAGAATGATGATTGGATCAATTCCAATA  
*APS-YH60/2* (4201) AGCATTGAGACAAAAAATCTTATCTACAGAATGATGATTGGATCAATTCCAATA  
*APS-YT31/6* (4201) AGCATTGAGACAAAAAATCTTATCTACAGAATGATGATTGGATCAATTCCAATA

圖 9 (續)、臺灣扁柏與紅檜之蒎烯合成酶基因序列比對

Fig. 9. (continued) Alignment of alpha pinene synthase (*APS*) genomic DNA sequences in *Chamaecyparis formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6).

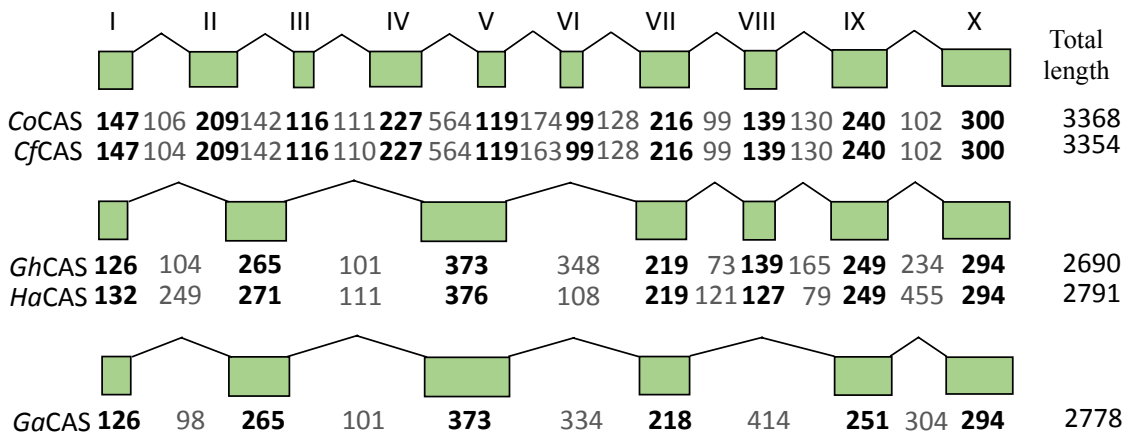


圖 10、不同物種間之杜松烯合成酶基因結構比較

Fig. 10. Schematic diagrams represent structure of genomic DNA of coding region for different cadinene synthase (CAS) genes. Nucleotide sequences from *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (CoCAS, YH60/2), *C. formosensis* Matsum. (CfCAS, RT31/1), *Gossypium hirsutum* L. (GhCAS, AY800106), *Helianthus annuus* L. (HaCAS, EU443250), and *Gossypium arboreum* L. (GaCAS, AF174294) were analyzed. Exons are indicated by boxes, introns are represented by bended lines between boxes, and the Arabic numerals under the diagrams indicate the lengths (base pairs) of the exons and introns. The Roman numerals above the boxes represent the number of exons.

JN715077 (1) ATGGCTGAAGTTGGTCTCTCTCAAATTCATATGCATCCGCAATCATGACAAGAAATCAGAGCAACAAATCAGGCGAAGGGTAGCAGAATTTTCATCCAA  
CAS-RT31/1 (1) ATGGCTGAAGTTGGTCTCTCTCAAATTCATATGCATCCGCAATCATGACAAGAAATCAGAGCAACAAATCAGGCGAAGGGTAGCAGAATTTTCATCCAA  
CAS-RT31/5 (1) ATGGCTGAAGTTGGTCTCTCTCAAATTCATATGCATCCGCAATCATGACAAGAAATCAGAGCAACAAATCAGGCGAAGGGTAGCAGAATTTTCATCCAA  
CAS-RT31/7 (1) ATGGCTGAAGTTGGTCTCTCTCAAATTCATATGCATCCGCAATCATGACAAGAAATCAGAGCAACAAATCAGGCGAAGGGTAGCAGAATTTTCATCCAA  
CAS-YH60/2 (1) ATGGCTGAAGTTGGTCTCTCTCAAATTCATATGCATCCGCAATCATGACAAGAAATCAGAGCAACAAATCAGGCGAAGGGTAGCAGAATTTTCATCCAA  
CAS-YT31/1 (1) ATGGCTGAAGTTGGTCTCTCTCAAATTCATATGCATCCGCAATCATGACAAGAAATCAGAGCAACAAATCAGGCGAAGGGTAGCAGAATTTTCATCCAA

JN715077 (101) ACGTTTGGGAGTACGAATTCCTGCAGTCCCTTTCATCGCCATATGGG  
CAS-RT31/1 (101) ACGTTTGGGAGTACGAATTCCTGCAGTCCCTTTCATCGCCATATGGGgaagtaaggttaacttactgcaagttc-ttaatatagtttttttctt  
CAS-RT31/5 (101) ACGTTTGGGAGTACGAATTCCTGCAGTCCCTTTCATCGCCATATGGGgaagtaaggttaacttactgcaagttc-ttaatatagtttttttctt  
CAS-RT31/7 (101) ACGTTTGGGAGTACGAATTCCTGCAGTCCCTTTCATCGCCATATGGGgaagtaaggttaacttactgcaagttc-ttaatatagtttttttctt  
CAS-YH60/2 (101) ACGTTTGGGAGTACGAATTCCTGCAGTCCCTTTCATCGCCATATGGGgaagtaaggttaacttactgcaagttc-ttaatatagtttttttctt  
CAS-YT31/1 (101) ACGTTTGGGAGTACGAATTCCTGCAGTCCCTTTCATCGCCATATGGGgaagtaaggttaacttactgcaagttc-ttaatatagtttttttctt

JN715077 (148) GCACCTTCGTAAGTGGGAGATTAAACATCTTAATTGAGGAGATTAA  
CAS-RT31/1 (199) tctcttttctgtaccgtttccaagctaaatctcatatctatgaatgacagGCACCTTCGTAAGTGGGAGATTAAACATCTTAATTGAGGAGATTAA  
CAS-RT31/5 (199) tctcttttctgtaccgtttccaagctaaatctcatatctatgaatgacagGCACCTTCGTAAGTGGGAGATTAAACATCTTAATTGAGGAGATTAA  
CAS-RT31/7 (199) tctcttttctgtaccgtttccaagctaaatctcatatctatgaatgacagGCACCTTCGTAAGTGGGAGATTAAACATCTTAATTGAGGAGATTAA  
CAS-YH60/2 (201) tctcttttctgtaccgtttccaagctaaatctcatatctatgaatgacagGCACCTTCGTAAGTGGGAGATTAAACATCTTAATTGAGGAGATTAA  
CAS-YT31/1 (201) tctcttttctgtaccgtttccaagctaaatctcatatctatgaatgacagGCACCTTCGTAAGTGGGAGATTAAACATCTTAATTGAGGAGATTAA

JN715077 (195) GATGGATATATTTGATGGTCTGGTTGGAGATGGAGAAAAGAATATGAATCCGTCTGCTTATGACCTTCTTAAAGGTTCTTCGTTGTTGATATTCTGCAG  
CAS-RT31/1 (299) GATGGATATATTTGATGGTCTGGTTGGAGATGGAGAAAAGAATATGAATCCGTCTGCTTATGACCTTCTTAAAGGTTCTTCGTTGTTGATATTCTGCAG  
CAS-RT31/5 (299) GATGGATATATTTGATGGTCTGGTTGGAGATGGAGAAAAGAATATGAATCCGTCTGCTTATGACCTTCTTAAAGGTTCTTCGTTGTTGATATTCTGCAG  
CAS-RT31/7 (299) GATGGATATATTTGATGGTCTGGTTGGAGATGGAGAAAAGAATATGAATCCGTCTGCTTATGACCTTCTTAAAGGTTCTTCGTTGTTGATATTCTGCAG  
CAS-YH60/2 (301) GATGGATATATTTGATGGTCTGGTTGGAGATGGAGAAAAGAATATGAATCCGTCTGCTTATGACCTTCTTAAAGGTTCTTCGTTGTTGATATTCTGCAG  
CAS-YT31/1 (301) GATGGATATATTTGATGGTCTGGTTGGAGATGGAGAAAAGAATATGAATCCGTCTGCTTATGACCTTCTTAAAGGTTCTTCGTTGTTGATATTCTGCAG

JN715077 (295) AGCCTGGGAATTGAACGCCATTTAAAAAAGAAATAAAAGCAGTCTAGATTATACTTACAA  
CAS-RT31/1 (399) AGCCTGGGAATTGAACGCCATTTAAAAAAGAAATAAAAGCAGTCTAGATTATACTTACAAgaagttggtttatataaaaaaaccttaaatctaaaa  
CAS-RT31/5 (399) AGCCTGGGAATTGAACGCCATTTAAAAAAGAAATAAAAGCAGTCTAGATTATACTTACAAgaagttggtttatataaaaaaaccttaaatctaaaa  
CAS-RT31/7 (399) AGCCTGGGAATTGAACGCCATTTAAAAAAGAAATAAAAGCAGTCTAGATTATACTTACAAgaagttggtttatataaaaaaaccttaaatctaaaa  
CAS-YH60/2 (401) AGCCTGGGAATTGAACGCCATTTAAAAAAGAAATAAAAGCAGTCTAGATTATACTTACAAgaagttggtttatataaaaaaaccttaaatctaaaa  
CAS-YT31/1 (401) AGCCTGGGAATTGAACGCCATTTAAAAAAGAAATAAAAGCAGTCTAGATTATACTTACAAgaagttggtttatataaaaaaaccttaaatctaaaa

JN715077 (357)  
CAS-RT31/1 (499) gacgttaaatggtttggtttatcaaaagtgtttacagatataaactggtttggttcatacaaaaaatggtttacatTTTTCTTatcatctacatctctt  
CAS-RT31/5 (499) gacgttaaatggtttggtttatcaaaagtgtttacagatataaactggtttggttcatacaaaaaatggtttacatTTTTCTTatcatctacatctctt  
CAS-RT31/7 (499) gacgttaaatggtttggtttatcaaaagtgtttacagatataaactggtttggttcatacaaaaaatggtttacatTTTTCTTatcatctacatctctt  
CAS-YH60/2 (501) gacgttaaatggtttggtttatcaaaagtgtttacagatataaactggtttggttcatacaaaaaatggtttacatTTTTCTTatcatctacatctctt  
CAS-YT31/1 (501) gacgttaaatggtttggtttatcaaaagtgtttacagatataaactggtttggttcatacaaaaaatggtttacatTTTTCTTatcatctacatctctt

JN715077 (357) ATATTGGAATGATGAAAAGGGTATTTCTTGGCGAGCGGAAACCTTATTGTTGATCTCAACACTAATGCTTTGGGATTCAAAGTCTTCGCCTTAA  
CAS-RT31/1 (599) tcagATATTGGAATGATGAAAAGGGTATTTCTTGGCGAGCGGAAACCTTATTGTTGATCTCAACACTAATGCTTTGGGATTCAAAGTCTTCGCCTTAA  
CAS-RT31/5 (599) tcagATATTGGAATGATGAAAAGGGTATTTCTTGGCGAGCGGAAACCTTATTGTTGATCTCAACACTAATGCTTTGGGATTCAAAGTCTTCGCCTTAA  
CAS-RT31/7 (599) tcagATATTGGAATGATGAAAAGGGTATTTCTTGGCGAGCGGAAACCTTATTGTTGATCTCAACACTAATGCTTTGGGATTCAAAGTCTTCGCCTTAA  
CAS-YH60/2 (601) tcagATATTGGAATGATGAAAAGGGTATTTCTTGGCGAGCGGAAACCTTATTGTTGATCTCAACACTAATGCTTTGGGATTCAAAGTCTTCGCCTTAA  
CAS-YT31/1 (601) tcagATATTGGAATGATGAAAAGGGTATTTCTTGGCGAGCGGAAACCTTATTGTTGATCTCAACACTAATGCTTTGGGATTCAAAGTCTTCGCCTTAA

JN715077 (453) TGAATATTACGTGTCTCCAG  
CAS-RT31/1 (699) TGAATATTACGTGTCTCCAGgttgttacttattcttttagcttaactcgatattataaatgtagaagtttagatfaagtgatcagttttttatggttg  
CAS-RT31/5 (699) TGAATATTACGTGTCTCCAGgttgttacttattcttttagcttaactcgatattataaatgtagaagtttagatfaagtgatcagttttttatggttg  
CAS-RT31/7 (699) TGAATATTACGTGTCTCCAGgttgttacttattcttttagcttaactcgatattataaatgtagaagtttagatfaagtgatcagttttttatggttg  
CAS-YH60/2 (701) TGAATATTACGTGTCTCCAGgttgttacttattcttttagcttaactcgatattataaatgtagaagtttagatfaagtgatcagttttttatggttg  
CAS-YT31/1 (701) TGAATATTACGTGTCTCCAGgttgttacttattcttttagcttaactcgatattataaatgtagaagtttagatfaagtgatcagttttttatggttg

(continued)

圖 11、臺灣扁柏與紅檜之杜松烯合成酶基因序列比對

Fig. 11. Alignment of cadinene synthase (*CAS*) cDNA sequences in *Chamaecyparis formosensis* Matsum. (JN715077) and genomic DNA sequences in *C. formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* var. *formosana* *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6). The black boxes represent conserved percentage of 15 to 50%. The gray boxes represent conserved percentage of 60% to 83%. The hyphens between sequences mean deletion. Exons and introns are indicated by capital letters and lowercase letters, respectively.

JN715077 (473) ATGTTTTTCAGAACTCCAAGATGAAATGGGACAATTTATTGATCTCGGAACCTTTAAAGAAGATGAAT  
CAS-RT31/1 (798) caattgtatttctaataatgttgccttaacagATGTTTTTCAGAACTCCAAGATGAAATGGGACAATTTATTGATCTCGGAACCTTTAAAGAAGATGAAT  
CAS-RT31/5 (798) caattgtatttctaataatgttgccttaacagATGTTTTTCAGAACTCCAAGATGAAATGGGACAATTTATTGATCTCGGAACCTTTAAAGAAGATGAAT  
CAS-RT31/7 (798) caattgtatttctaataatgttgccttaacagATGTTTTTCAGAACTCCAAGATGAAATGGGACAATTTATTGATCTCGGAACCTTTAAAGAAGATGAAT  
CAS-YH60/2 (801) caattgtatttctaataatgttgccttaacagATGTTTTTCAGAACTCCAAGATGAAATGGGACAATTTATTGATCTCGGAACCTTTAAAGAAGATGAAT  
CAS-YT31/1 (800) caattgtatttctaataatgttgccttaacagATGTTTTTCAGAACTCCAAGATGAAATGGGACAATTTATTGATCTCGGAACCTTTAAAGAAGATGAAT

JN715077 (542) CCAAATTAAGAAGTCTATTGAGTTTATATCGAGCTTCAGAGATTTGTTTTCCAGAGGAGAATATCTTGAACAGGCCAAAATGTTGCTTCTACATGCCT  
CAS-RT31/1 (898) CCAAATTAAGAAGTCTATTGAGTTTATATCGAGCTTCAGAGATTTGTTTTCCAGAGGAGAATATCTTGAACAGGCCAAAATGTTGCTTCTACATGCCT  
CAS-RT31/5 (898) CCAAATTAAGAAGTCTATTGAGTTTATATCGAGCTTCAGAGATTTGTTTTCCAGAGGAGAATATCTTGAACAGGCCAAAATGTTGCTTCTACATGCCT  
CAS-RT31/7 (898) CCAAATTAAGAAGTCTATTGAGTTTATATCGAGCTTCAGAGATTTGTTTTCCAGAGGAGAATATCTTGAACAGGCCAAAATGTTGCTTCTACATGCCT  
CAS-YH60/2 (901) CCAAATTAAGAAGTCTATTGAGTTTATATCGAGCTTCAGAGATTTGTTTTCCAGAGGAGAATATCTTGAACAGGCCAAAATGTTGCTTCTACATGCCT  
CAS-YT31/1 (900) CCAAATTAAGAAGTCTATTGAGTTTATATCGAGCTTCAGAGATTTGTTTTCCAGAGGAGAATATCTTGAACAGGCCAAAATGTTGCTTCTACATGCCT

JN715077 (642) TAGACAAGCAATTGAAGAAAATCGAGAGTTGGTGAACAAAAGTCAACTTATAATAGAG  
CAS-RT31/1 (998) ACAACAAGCAATTGAAGAAAATCGAGAGTTGGTGAACAAAAGTCAACTTATAATAGAGgcaagcattccatttaactataaaaaaacttttagtagtccattta  
CAS-RT31/5 (998) ACAACAAGCAATTGAAGAAAATCGAGAGTTGGTGAACAAAAGTCAACTTATAATAGAGgcaagcattccatttaactataaaaaaacttttagtagtccattta  
CAS-RT31/7 (998) ACAACAAGCAATTGAAGAAAATCGAGAGTTGGTGAACAAAAGTCAACTTATAATAGAGgcaagcattccatttaactataaaaaaacttttagtagtccattta  
CAS-YH60/2 (1001) ACAACAAGCAATTGAAGAAAATCGAGAGTTGGTGAACAAAAGTCAACTTATAATAGAGgcaagcattccatttaactataaaaaaacttttagtagtccattta  
CAS-YT31/1 (1000) ACAACAAGCAATTGAAGAAAATCGAGAGTTGGTGAACAAAAGTCAACTTATAATAGAGgcaagcattccatttaactataaaaaaacttttagtagtccattta

JN715077 (700)  
CAS-RT31/1 (1098) taaggttgcgcattaaatagacctgtaatatctaactcaatgatgtgggtttcaaaaatftaagcattactagattaaatataaaaaatagtaag  
CAS-RT31/5 (1098) taaggttgcgcattaaatagacctgtaatatctaactcaatgatgtgggtttcaaaaatftaagcattactagattaaatataaaaaatagtaag  
CAS-RT31/7 (1098) taaggttgcgcattaaatagacctgtaatatctaactcaatgatgtgggtttcaaaaatftaagcattactagattaaatataaaaaatagtaag  
CAS-YH60/2 (1101) taaggttgcgcattaaatagacctgtaatatctaactcaatgatgtgggtttcaaaaatftaagcattactagattaaatataaaaaatagtaag  
CAS-YT31/1 (1100) taaggttgcgcattaaatagacctgtaatatctaactcaatgatgtgggtttcaaaaatftaagcattactagattaaatataaaaaatagtaag

JN715077 (700)  
CAS-RT31/1 (1198) atttatctgtacacacatagacacaatttttagatgacatatttttggtaattttgttaagttgactctgtaggaaaaaactactagtttagtccatg  
CAS-RT31/5 (1198) atttatctgtacacacatagacacaatttttagatgacatatttttggtaattttgttaagttgactctgtaggaaaaaactactagtttagtccatg  
CAS-RT31/7 (1198) atttatctgtacacacatagacacaatttttagatgacatatttttggtaattttgttaagttgactctgtaggaaaaaactactagtttagtccatg  
CAS-YH60/2 (1201) atttatctgtacacacatagacacaatttttagatgacatatttttggtaattttgttaagttgactctgtaggaaaaaactactagtttagtccatg  
CAS-YT31/1 (1200) atttatctgtacacacatagacacaatttttagatgacatatttttggtaattttgttaagttgactctgtaggaaaaaactactagtttagtccatg

JN715077 (700)  
CAS-RT31/1 (1298) tgaataaaagtctcaaggatttttgaattagatcacacatattttctctcaagtgatccatcgatcattttaaaatttgagattgtaattgattcag  
CAS-RT31/5 (1298) tgaataaaagtctcaaggatttttgaattagatcacacatattttctctcaagtgatccatcgatcattttaaaatttgagattgtaattgattcag  
CAS-RT31/7 (1298) tgaataaaagtctcaaggatttttgaattagatcacacatattttctctcaagtgatccatcgatcattttaaaatttgagattgtaattgattcag  
CAS-YH60/2 (1301) tgaataaaagtctcaaggatttttgaattagatcacacatattttctctcaagtgatccatcattttaaaatttgagattgtaattgattcag  
CAS-YT31/1 (1300) tgaataaaagtctcaaggatttttgaattagatcacacatattttctctcaagtgatccatcattttaaaatttgagattgtaattgattcag

JN715077 (700)  
CAS-RT31/1 (1398) tgtggggtctcttttaaatatttttaaaaaatatttttagggcttggacctctcaccctgtcataataatcaattcaactctgtcgttgaccaaatatgt  
CAS-RT31/5 (1398) tgtggggtctcttttaaatatttttaaaaaatatttttagggcttggacctctcaccctgtcataataatcaattcaactctgtcgttgaccaaatatgt  
CAS-RT31/7 (1398) tgtggggtctcttttaaatatttttaaaaaatatttttagggcttggacctctcaccctgtcataataatcaattcaactctgtcgttgaccaaatatgt  
CAS-YH60/2 (1401) tgtggggtctcttttaaatatttttaaaaaatatttttagggcttggacctctcaccctgtcataataatcaattcaactctgtcgttgaccaaatatgt  
CAS-YT31/1 (1400) tgtggggtctcttttaaatatttttaaaaaatatttttagggcttggacctctcaccctgtcataataatcaattcaactctgtcgttgaccaaatatgt


JN715077 (700)  
CAS-RT31/1 (1498) ggctcttaaataaggcttattttatctctcaacttgtagtttgtacatgctttcttgagtcatttaattttagtcaataatgttaaagttaataataatc  
CAS-RT31/5 (1498) ggctcttaaataaggcttattttatctctcaacttgtagtttgtacatgctttcttgagtcatttaattttagtcaataatgttaaagttaataataatc  
CAS-RT31/7 (1498) ggctcttaaataaggcttattttatctctcaacttgtagtttgtacatgctttcttgagtcatttaattttagtcaataatgttaaagttaataataatc  
CAS-YH60/2 (1501) ggctcttaaataaggcttattttatctctcaacttgtagtttgtacatgctttcttgagtcatttaattttagtcaataatgttaaagttaataataatc  
CAS-YT31/1 (1500) ggctcttaaataaggcttattttatctctcaacttgtagtttgtacatgctttcttgagtcatttaattttagtcaataatgttaaagttaataataatc

JN715077 (700) GTTGAATATATTATGAAATATCCATGGACGTGTAGAGTCCCTAGATGGGAGGTATGGAACCTATATCAAAATATTTAGA  
CAS-RT31/1 (1598) tcatctaattctaattgtatagGTTGAATATATTATGAAATATCCATGGACGTGTAGAGTCCCTAGATGGGAGGTATGGAACCTATATCAAAATATTTAGA  
CAS-RT31/5 (1598) tcatctaattctaattgtatagGTTGAATATATTATGAAATATCCATGGACGTGTAGAGTCCCTAGATGGGAGGTATGGAACCTATATCAAAATATTTAGA  
CAS-RT31/7 (1598) tcatctaattctaattgtatagGTTGAATATATTATGAAATATCCATGGACGTGTAGAGTCCCTAGATGGGAGGTATGGAACCTATATCAAAATATTTAGA  
CAS-YH60/2 (1601) tcatctaattctaattgtatagGTTGAATATATTATGAAATATCCATGGACGTGTAGAGTCCCTAGATGGGAGGTATGGAACCTATATCAAAATATTTAGA  
CAS-YT31/1 (1600) tcatctaattctaattgtatagGTTGAATATATTATGAAATATCCATGGACGTGTAGAGTCCCTAGATGGGAGGTATGGAACCTATATCAAAATATTTAGA

(continued)

圖 11 (續)、臺灣扁柏與紅檜之杜松烯合成酶基因序列比對

Fig. 11. (continued) Alignment of cadinene synthase (CAS) cDNA sequences in *Chamaecyparis formosensis* Matsum. (JN715077) and genomic DNA sequences in *C. formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* var. *formosana* *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6).



  
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JN715077 (778) GGAGACATTCATGCTTCAATGTGCATGAAGGGAGTTTATGA  
 CAS-RT31/1 (1698) GGAGACATTCATGCTTCAATGTGCATGAAGGGAGTTTATGAgtaagtcocaaacatcatctcatgtatacctttcttaataatgcatagtagaagaactca  
 CAS-RT31/5 (1698) GGAGACATTCATGCTTCAATGTGCATGAAGGGAGTTTATGAgtaagtcocaaacatcatctcatgtatacctttcttaataatgcatagtagaagaactca  
 CAS-RT31/7 (1698) GGAGACATTCATGCTTCAATGTGCATGAAGGGAGTTTATGAgtaagtcocaaacatcatctcatgtatacctttcttaataatgcatagtagaagaactca  
 CAS-YH60/2 (1701) GGAGACATTCATGCTTCAATGTGCATGAAGGGAGTTTATGAgtaagtcocaaacatcatctcatgtatacctttcttaataatgcatagtagaagaactca  
 CAS-YT31/1 (1700) GGAGACATTCATGCTTCAATGTGCATGAAGGGAGTTTATGAgtaagtcocaaacatcatctcatgtatacctttcttaataatgcatagtagaagaactca  
  
 JN715077 (819) \_\_\_\_\_  
 CAS-RT31/1 (1798) ttcaattatgcttaataataaattatttatttctccactcactgcaagaacatcatatgtctaaatggctaaat\_\_\_\_\_agtgataaattattt  
 CAS-RT31/5 (1798) ttcaattatgcttaataataaattatttatttctccactcactgcaagaacatcatatgtctaaatggctaaat\_\_\_\_\_agtgataaattattt  
 CAS-RT31/7 (1798) ttcaattatgcttaataataaattatttatttctccactcactgcaagaacatcatatgtctaaatggctaaat\_\_\_\_\_agtgataaattattt  
 CAS-YH60/2 (1801) ttcaattatgcttaataataaattatttatttctccactcactgcaagaacatcatatgtctaaatggctaaat\_\_\_\_\_agtgataaattattt  
 CAS-YT31/1 (1800) ttcaattatgcttaataataaattatttatttctccactcactgcaagaacatcatatgtctaaatggctaaat\_\_\_\_\_agtgataaattattt  
 JN715077 (819) AATGCCAAGTGACAAAAGAACGAAGATTTTGGAAATGGCAACTACTAGACTTTAACATTTTGAAGATCAACACCATAATGAGCTC  
 CAS-RT31/1 (1887) tactttttctttcagAATGCCAAGTGACAAAAGAACGAAGATTTTGGAAATGGCAACTACTAGACTTTAACATTTTGAAGATCAACACCATAATGAGCTC  
 CAS-RT31/5 (1887) tactttttctttcagAATGCCAAGTGACAAAAGAACGAAGATTTTGGAAATGGCAACTACTAGACTTTAACATTTTGAAGATCAACACCATAATGAGCTC  
 CAS-RT31/7 (1887) tactttttctttcagAATGCCAAGTGACAAAAGAACGAAGATTTTGGAAATGGCAACTACTAGACTTTAACATTTTGAAGATCAACACCATAATGAGCTC  
 CAS-YH60/2 (1901) tactttttctttcagAATGCCAAGTGACAAAAGAACGAAGATTTTGGAAATGGCAACTACTAGACTTTAACATTTTGAAGATCAACACCATAATGAGCTC  
 CAS-YT31/1 (1900) tactttttctttcagAATGCCAAGTGACAAAAGAACGAAGATTTTGGAAATGGCAACTACTAGACTTTAACATTTTGAAGATCAACACCATAATGAGCTC  
  
 JN715077 (904) AAAATTTTATCCAA  
 CAS-RT31/1 (1987) AAAATTTTATCCAAgt aagggaaactatatagtat tttgtccttattttaaataattcaatttatcttgtttaggatcatatgttctttaaagaac  
 CAS-RT31/5 (1987) AAAATTTTATCCAAgt aagggaaactatatagtat tttgtccttattttaaataattcaatttatcttgtttaggatcatatgttctttaaagaac  
 CAS-RT31/7 (1987) AAAATTTTATCCAAgt aagggaaactatatagtat tttgtccttattttaaataattcaatttatcttgtttaggatcatatgttctttaaagaac  
 CAS-YH60/2 (2001) AAAATTTTATCCAAgt aagggaaactatatagtat tttgtccttattttaaataattcaatttatcttgtttaggatcatatgttctttaaagaac  
 CAS-YT31/1 (2000) AAAATTTTATCCAAgt aagggaaactatatagtat tttgtccttattttaaataattcaatttatcttgtttaggatcatatgttctttaaagaac  
  
 JN715077 (918) \_\_\_\_\_ ATGGTGAATGAGACAAAAGTGAAGGAACATAAATTTCTTCAGGCAACGACATGTGGAG  
 CAS-RT31/1 (2087) taatagggtcaaatat tttggattgggatatgaataaataagATGGTGAATGAGACAAAAGTGAAGGAACATAAATTTCTTCAGGCAACGACATGTGGAG  
 CAS-RT31/5 (2087) taatagggtcaaatat tttggattgggatatgaataaataagATGGTGAATGAGACAAAAGTGAAGGAACATAAATTTCTTCAGGCAACGACATGTGGAG  
 CAS-RT31/7 (2087) taatagggtcaaatat tttggattgggatatgaataaataagATGGTGAATGAGACAAAAGTGAAGGAACATAAATTTCTTCAGGCAACGACATGTGGAG  
 CAS-YH60/2 (2101) taatagggtcaaatat tttggattgggatatgaataaataagATGGTGAATGAGACAAAAGTGAAGGAACATAAATTTCTTCAGGCAACGACATGTGGAG  
 CAS-YT31/1 (2100) taatagggtcaaatat tttggattgggatatgaataaataagATGGTGAATGAGACAAAAGTGAAGGAACATAAATTTCTTCAGGCAACGACATGTGGAG  
  
 JN715077 (976) TTCTACTTTTTGTATGCATGTGGACTTATGAGAAAAGAGCTCTCTGCAACTAGACTTTGCTTTGCTAAAGTGGGTGCACTCATTAECTCTTCTTGATGATA  
 CAS-RT31/1 (2187) TTCTACTTTTTGTATGCATGTGGACTTATGAGAAAAGAGCTCTCTGCAACTAGACTTTGCTTTGCTAAAGTGGGTGCACTCATTAECTCTTCTTGATGATA  
 CAS-RT31/5 (2187) TTCTACTTTTTGTATGCATGTGGACTTATGAGAAAAGAGCTCTCTGCAACTAGACTTTGCTTTGCTAAAGTGGGTGCACTCATTAECTCTTCTTGATGATA  
 CAS-RT31/7 (2187) TTCTACTTTTTGTATGCATGTGGACTTATGAGAAAAGAGCTCTCTGCAACTAGACTTTGCTTTGCTAAAGTGGGTGCACTCATTAECTCTTCTTGATGATA  
 CAS-YH60/2 (2201) TTCTACTTTTTGTATGCATGTGGACTTATGAGAAAAGAGCTCTCTGCAACTAGACTTTGCTTTGCTAAAGTGGGTGCACTCATTAECTCTTCTTGATGATA  
 CAS-YT31/1 (2200) TTCTACTTTTTGTATGCATGTGGACTTATGAGAAAAGAGCTCTCTGCAACTAGACTTTGCTTTGCTAAAGTGGGTGCACTCATTAECTCTTCTTGATGATA  
  
 JN715077 (1076) TTTTGTGACACTTATGGAACCATGTGAGCTTGTACCTTTTGCAACAGCTTTGATCAA  
 CAS-RT31/1 (2287) TTTTGTGACACTTATGGAACCATGTGAGCTTGTACCTTTTGCAACAGCTTTGATCAAgtatgttaatatgttatacaatatattatgataaccttt  
 CAS-RT31/5 (2287) TTTTGTGACACTTATGGAACCATGTGAGCTTGTACCTTTTGCAACAGCTTTGATCAAgtatgttaatatgttatacaatatattatgataaccttt  
 CAS-RT31/7 (2287) TTTTGTGACACTTATGGAACCATGTGAGCTTGTACCTTTTGCAACAGCTTTGATCAAgtatgttaatatgttatacaatatattatgataaccttt  
 CAS-YH60/2 (2301) TTTTGTGACACTTATGGAACCATGTGAGCTTGTACCTTTTGCAACAGCTTTGATCAAgtatgttaatatgttatacaatatattatgataaccttt  
 CAS-YT31/1 (2300) TTTTGTGACACTTATGGAACCATGTGAGCTTGTACCTTTTGCAACAGCTTTGATCAAgtatgttaatatgttatacaatatattatgataaccttt  
  
 JN715077 (1134) \_\_\_\_\_ GTGGGACATGTCAATAATGAATCACCTCCAGAATATATGAAA  
 CAS-RT31/1 (2387) cagtgatatgttgatattttgtgtatatttattataaattttaatgttagGTGGGACATGTCAATAATGAATCACCTCCAGAATATATGAAA  
 CAS-RT31/5 (2387) cagtgatatgttgatattttgtgtatatttattataaattttaatgttagGTGGGACATGTCAATAATGAATCACCTCCAGAATATATGAAA  
 CAS-RT31/7 (2387) cagtgatatgttgatattttgtgtatatttattataaattttaatgttagGTGGGACATGTCAATAATGAATCACCTCCAGAATATATGAAA  
 CAS-YH60/2 (2401) cagtgatatgttgatattttgtgtatatttattataaattttaatgttagGTGGGACATGTCAATAATGAATCACCTCCAGAATATATGAAA  
 CAS-YT31/1 (2400) cagtgatatgttgatattttgtgtatatttattataaattttaatgttagGTGGGACATGTCAATAATGAATCACCTCCAGAATATATGAAA  
  
 JN715077 (1177) ACATGTTTCCAATTTGCGTACAAAACATACATGGAATAGCTACAGAGGCAGAGAAGATACATGGTCCATGTGTGCAAAAAGTGGATGCATGACACT  
 CAS-RT31/1 (2487) ACATGTTTCCAATTTGCGTACAAAACATACATGGAATAGCTACAGAGGCAGAGAAGATACATGGTCCATGTGTGCAAAAAGTGGATGCATGACACTgttaa  
 CAS-RT31/5 (2487) ACATGTTTCCAATTTGCGTACAAAACATACATGGAATAGCTACAGAGGCAGAGAAGATACATGGTCCATGTGTGCAAAAAGTGGATGCATGACACTgttaa  
 CAS-RT31/7 (2487) ACATGTTTCCAATTTGCGTACAAAACATACATGGAATAGCTACAGAGGCAGAGAAGATACATGGTCCATGTGTGCAAAAAGTGGATGCATGACACTgttaa  
 CAS-YH60/2 (2501) ACATGTTTCCAATTTGCGTACAAAACATACATGGAATAGCTACAGAGGCAGAGAAGATACATGGTCCATGTGTGCAAAAAGTGGATGCATGACACTgttaa  
 CAS-YT31/1 (2500) ACATGTTTCCAATTTGCGTACAAAACATACATGGAATAGCTACAGAGGCAGAGAAGATACATGGTCCATGTGTGCAAAAAGTGGATGCATGACACTgttaa

(continued)

圖 11 (續)、臺灣扁柏與紅檜之杜松烯合成酶基因序列比對

Fig. 11. (continued) Alignment of cadinene synthase (CAS) cDNA sequences in *Chamaecyparis formosensis* Matsum. (JN715077) and genomic DNA sequences in *C. formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* var. *formosana* *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6).



JN715077 (1273) TGAAGACTATTATTTGGCACAACCTCAAGATGCAGAGTGGATTGCTAACAAATATCTCCCAAGTTTGACTGA

CAS-RT31/1 (2587) gttat t t t g t t g c g a c a a a a t t a g t t t a g a t t t a a a t a t c t a a a a a a a t a g t a a a t t t t t t t t g t t c a a g a a a t t g t t g t t g t t g t a a a a c t a a t

CAS-RT31/5 (2587) gttat t t t g t t g c g a c a a a a t t a g t t t a g a t t t a a a t a t c t a a a a a a a t a g t a a a t t t t t t t t g t t c a a g a a a t t g t t g t t g t t g t a a a a c t a a t

CAS-RT31/7 (2587) gttat t t t g t t g c g a c a a a a t t a g t t t a g a t t t a a a t a t c t a a a a a a a t a g t a a a t t t t t t t t g t t c a a g a a a t t g t t g t t g t t g t a a a a c t a a t

CAS-YH60/2 (2601) gttat t t t g t t g c g a c a a a a t t a g t t t a g a t t t a a a t a t c t a a a a a a a t a g t a a a t t t t t t t t g t t c a a g a a a t t g t t g t t g t t g t a a a a c t a a t

CAS-YT31/1 (2600) gttat t t t g t t g c g a c a a a a t t a g t t t a g a t t t a a a t a t c t a a a a a a a t a g t a a a t t t t t t t t g t t c a a g a a a t t g t t g t t g t t g t a a a a c t a a t

JN715077 (1273) TGAAGACTATTATTTGGCACAACCTCAAGATGCAGAGTGGATTGCTAACAAATATCTCCCAAGTTTGACTGA

CAS-RT31/1 (2687) ggtat at t c t t g a t t t a a a t t g a a g TGAAGACTATTATTTGGCACAACCTCAAGATGCAGAGTGGATTGCTAACAAATATCTCCCAAGTTTGACTGA

CAS-RT31/5 (2687) ggtat at t c t t g a t t t a a a t t g a a g TGAAGACTATTATTTGGCACAACCTCAAGATGCAGAGTGGATTGCTAACAAATATCTCCCAAGTTTGACTGA

CAS-RT31/7 (2687) ggtat at t c t t g a t t t a a a t t g a a g TGAAGACTATTATTTGGCACAACCTCAAGATGCAGAGTGGATTGCTAACAAATATCTCCCAAGTTTGACTGA

CAS-YH60/2 (2701) ggtat at t c t t g a t t t a a a t t g a a g TGAAGACTATTATTTGGCACAACCTCAAGATGCAGAGTGGATTGCTAACAAATATCTCCCAAGTTTGACTGA

CAS-YT31/1 (2700) ggtat at t c t t g a t t t a a a t t g a a g TGAAGACTATTATTTGGCACAACCTCAAGATGCAGAGTGGATTGCTAACAAATATCTCCCAAGTTTGACTGA

JN715077 (1347) ATATTTAGAAAAGTTCTGTACCATCTACAACAGTCCAGTACTCTCATTATTTCCATGCTTTAATTGACACAATTTCCCTGATGATATATTGAGAAA

CAS-RT31/1 (2787) ATATTTAGAAAAGTTCTGTACCATCTACAACAGTCCAGTACTCTCATTATTTCCATGCTTTAATTGACACAATTTCCCTGATGATATATTGAGAAA

CAS-RT31/5 (2787) ATATTTAGAAAAGTTCTGTACCATCTACAACAGTCCAGTACTCTCATTATTTCCATGCTTTAATTGACACAATTTCCCTGATGATATATTGAGAAA

CAS-RT31/7 (2787) ATATTTAGAAAAGTTCTGTACCATCTACAACAGTCCAGTACTCTCATTATTTCCATGCTTTAATTGACACAATTTCCCTGATGATATATTGAGAAA

CAS-YH60/2 (2801) ATATTTAGAAAAGTTCTGTACCATCTACAACAGTCCAGTACTCTCATTATTTCCATGCTTTAATTGACACAATTTCCCTGATGATATATTGAGAAA

CAS-YT31/1 (2800) ATATTTAGAAAAGTTCTGTACCATCTACAACAGTCCAGTACTCTCATTATTTCCATGCTTTAATTGACACAATTTCCCTGATGATATATTGAGAAA

JN715077 (1447) ATTACTAAGTCCAAAGTTGTGTTGCATGGGGTTGTCGACTGGTTGATGACTCCAAAGATTTTCAG

CAS-RT31/1 (2887) ATTACTAAGTCCAAAGTTGTGTTGCATGGGGTTGTCGACTGGTTGATGACTCCAAAGATTTTCAGgtacatat t t t a c t a t t a t g t a a a t a t t a t g a t t

CAS-RT31/5 (2887) ATTACTAAGTCCAAAGTTGTGTTGCATGGGGTTGTCGACTGGTTGATGACTCCAAAGATTTTCAGgtacatat t t t a c t a t t a t g t a a a t a t t a t g a t t

CAS-RT31/7 (2887) ATTACTAAGTCCAAAGTTGTGTTGCATGGGGTTGTCGACTGGTTGATGACTCCAAAGATTTTCAGgtacatat t t t a c t a t t a t g t a a a t a t t a t g a t t

CAS-YH60/2 (2901) ATTACTAAGTCCAAAGTTGTGTTGCATGGGGTTGTCGACTGGTTGATGACTCCAAAGATTTTCAGgtacatat t t t a c t a t t a t g t a a a t a t t a t g a t t

CAS-YT31/1 (2900) ATTACTAAGTCCAAAGTTGTGTTGCATGGGGTTGTCGACTGGTTGATGACTCCAAAGATTTTCAGgtacatat t t t a c t a t t a t g t a a a t a t t a t g a t t

JN715077 (1513) GATGAGAAAGAGCATGGAGAGAGTGCATCATG

CAS-RT31/1 (2987) t g t c t c t a t g a a g t a t a c a t t c t a a t a g a a a a t g a c a t a a a t a t t a t t a c a a t g g t t t t t g a a g GATGAGAAAGAGCATGGAGAGAGTGCATCATG

CAS-RT31/5 (2987) t g t c t c t a t g a a g t a t a c a t t c t a a t a g a a a a t g a c a t a a a t a t t a t t a c a a t g g t t t t t g a a g GATGAGAAAGAGCATGGAGAGAGTGCATCATG

CAS-RT31/7 (2987) t g t c t c t a t g a a g t a t a c a t t c t a a t a g a a a a t g a c a t a a a t a t t a t t a c a a t g g t t t t t g a a g GATGAGAAAGAGCATGGAGAGAGTGCATCATG

CAS-YH60/2 (3001) t g t c t c t a t g a a g t a t a c a t t c t a a t a g a a a a t g a c a t a a a t a t t a t t a c a a t g g t t t t t g a a g GATGAGAAAGAGCATGGAGAGAGTGCATCATG

CAS-YT31/1 (3000) t g t c t c t a t g a a g t a t a c a t t c t a a t a g a a a a t g a c a t a a a t a t t a t t a c a a t g g t t t t t g a a g GATGAGAAAGAGCATGGAGAGAGTGCATCATG

JN715077 (1545) GATAGAGTGTTATATGAAGGAAAATCCTGGAAC TACAAGGAAGCAAGCTTTAGATCATGCCAATATGCTTATTGAGTCAAATTTGAAGAATTTGATCAAA

CAS-RT31/1 (3087) GATAGAGTGTTATATGAAGGAAAATCCTGGAAC TACAAGGAAGCAAGCTTTAGATCATGCCAATATGCTTATTGAGTCAAATTTGAAGAATTTGATCAAA

CAS-RT31/5 (3087) GATAGAGTGTTATATGAAGGAAAATCCTGGAAC TACAAGGAAGCAAGCTTTAGATCATGCCAATATGCTTATTGAGTCAAATTTGAAGAATTTGATCAAA

CAS-RT31/7 (3087) GATAGAGTGTTATATGAAGGAAAATCCTGGAAC TACAAGGAAGCAAGCTTTAGATCATGCCAATATGCTTATTGAGTCAAATTTGAAGAATTTGATCAAA

CAS-YH60/2 (3101) GATAGAGTGTTATATGAAGGAAAATCCTGGAAC TACAAGGAAGCAAGCTTTAGATCATGCCAATATGCTTATTGAGTCAAATTTGAAGAATTTGATCAAA

CAS-YT31/1 (3100) GATAGAGTGTTATATGAAGGAAAATCCTGGAAC TACAAGGAAGCAAGCTTTAGATCATGCCAATATGCTTATTGAGTCAAATTTGAAGAATTTGATCAAA

JN715077 (1645) CATCGATATTCTACGAGTATTGCATACCAAGTACTTGAAGAGATTATATTTTGATATGTATAGGTCGGTAGCTTTTCATTTTCAAGGATATTGATGGAT

CAS-RT31/1 (3187) CATCGATATTCTACGAGTATTGCATACCAAGTACTTGAAGAGATTATATTTTGATATGTATAGGTCGGTAGCTTTTCATTTTCAAGGATATTGATGGAT

CAS-RT31/5 (3187) CATCGATATTCTACGAGTATTGCATACCAAGTACTTGAAGAGATTATATTTTGATATGTATAGGTCGGTAGCTTTTCATTTTCAAGGATATTGATGGAT

CAS-RT31/7 (3187) CATCGATATTCTACGAGTATTGCATACCAAGTACTTGAAGAGATTATATTTTGATATGTATAGGTCGGTAGCTTTTCATTTTCAAGGATATTGATGGAT

CAS-YH60/2 (3201) CATCGATATTCTACGAGTATTGCATACCAAGTACTTGAAGAGATTATATTTTGATATGTATAGGTCGGTAGCTTTTCATTTTCAAGGATATTGATGGAT

CAS-YT31/1 (3200) CATCGATATTCTACGAGTATTGCATACCAAGTACTTGAAGAGATTATATTTTGATATGTATAGGTCGGTAGCTTTTCATTTTCAAGGATATTGATGGAT

JN715077 (1745) TTTCAAAATCTAGCAAGGCTATTAGAGATGACATAAAGAAGATTCTAGTTGAACCTATATATTTT TAG

CAS-RT31/1 (3287) TTTCAAAATCTAGCAAGGCTATTAGAGATGACATAAAGAAGATTCTAGTTGAACCTATATATTTT TAG

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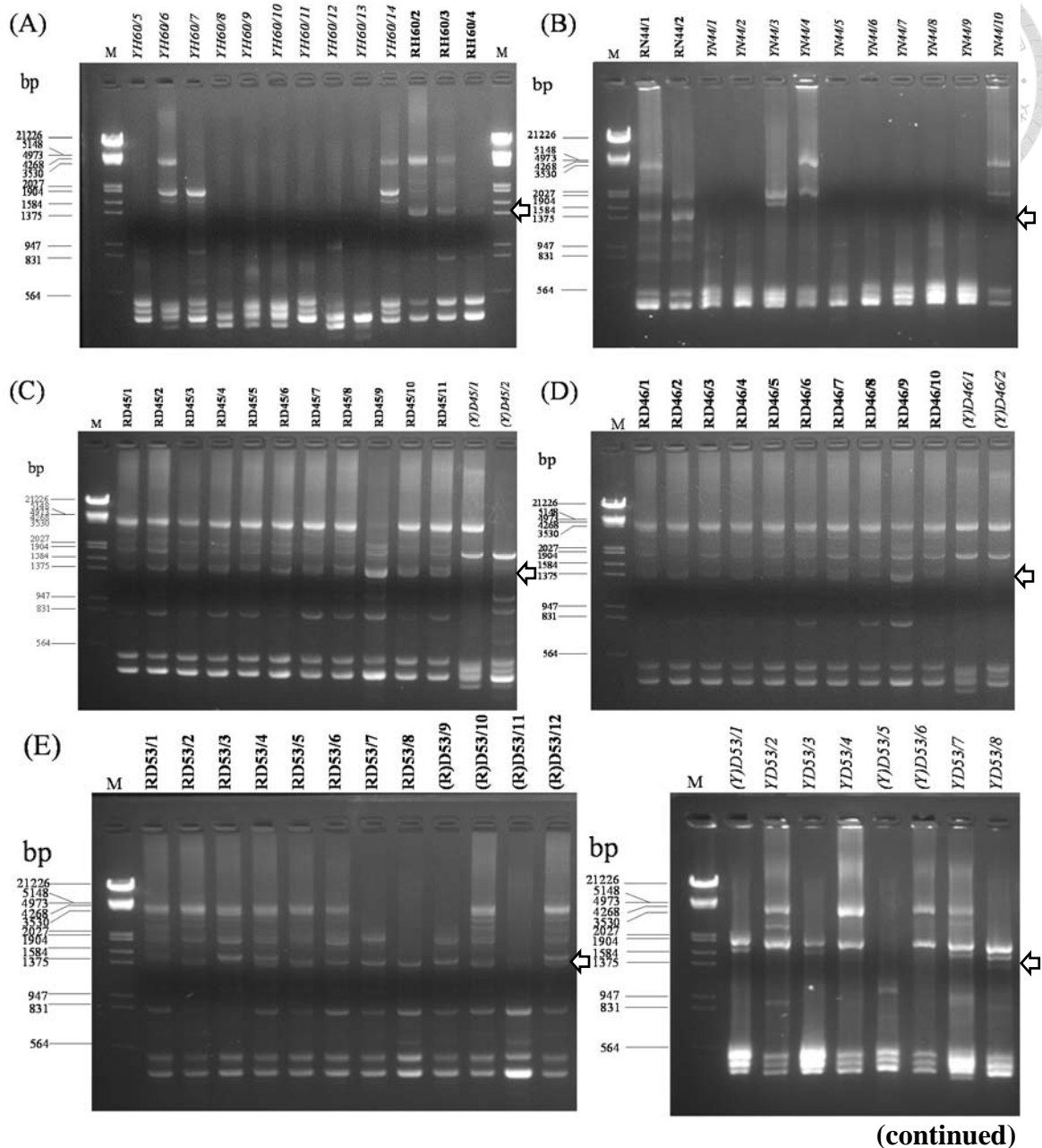
CAS-RT31/7 (3287) TTTCAAAATCTAGCAAGGCTATTAGAGATGACATAAAGAAGATTCTAGTTGAACCTATATATTTT TAG

CAS-YH60/2 (3301) TTTCAAAATCTAGCAAGGCTATTAGAGATGACATAAAGAAGATTCTAGTTGAACCTATATATTTT TAG

CAS-YT31/1 (3300) TTTCAAAATCTAGCAAGGCTATTAGAGATGACATAAAGAAGATTCTAGTTGAACCTATATATTTT TAG

圖 11 (續)、臺灣扁柏與紅檜之杜松烯合成酶基因序列比對

Fig. 11. (continued) Alignment of cadinene synthase (CAS) cDNA sequences in *Chamaecyparis formosensis* Matsum. (JN715077) and genomic DNA sequences in *C. formosensis* Matsum. (RT31/1、RT31/6) and *C. obtusa* var. *formosana* *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2、YT31/6).



(continued)

圖 12、以 OMT/MF-1 及 OMT/MR-1 為引子之臺灣檜木葉子樣品電泳分析圖

Fig. 12. The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers for caffeoyl CoA *O*-methyltransferase gene as molecular markers from the leaves of selected cypress accessions collected from (A) Ho Ping working circle 60 forest compartment (H60); (B) Nan Ao working circle 44 forest compartment (N44); (C), (D), (E) Dasi working circle 45, 46, 53 forest compartment, respectively (D45, D46, D53). M= marker. PCR with the primers OMT/MF-1 and OMT/MR-1. The bold and italic word of ID indicate they belong to red and yellow cypress, respectively. Arrows beside the figure indicate the specific bands only found in red cypress.

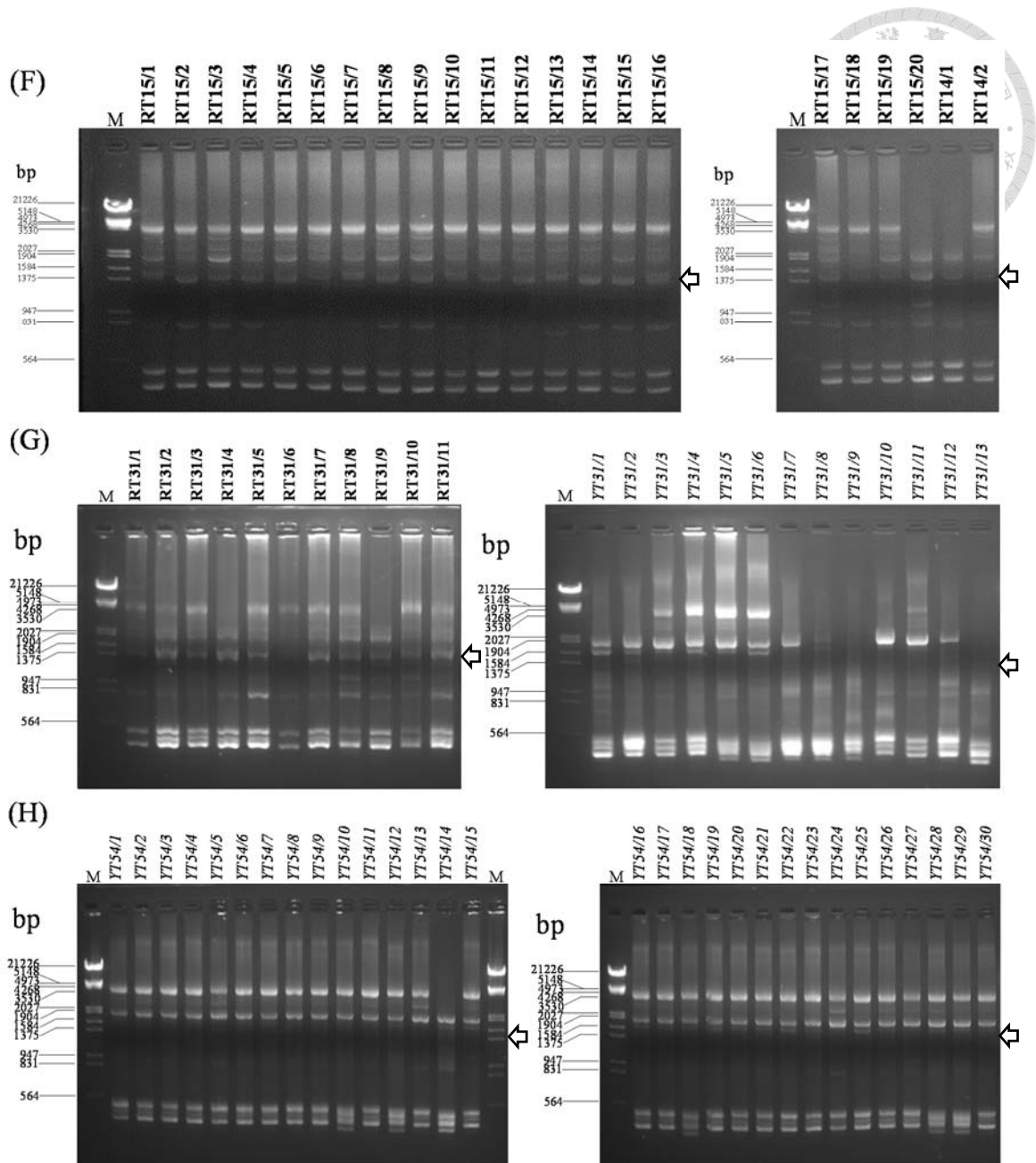


圖 12 (續)、以 OMT/MF-1 及 OMT/MR-1 為引子之臺灣檜木葉子樣品電泳分析圖  
 Fig. 12. (continued) The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers for caffeoyl CoA *O*-methyltransferase gene as molecular markers from the leaves of selected cypress accessions collected from (F) Taipingshan working circle 14, 15 forest compartment (T14, T15); (G), (H) Taipingshan working circle 31, 54 forest compartment, respectively (T31, T54). Arrows beside the figure indicate the specific bands only found in red cypress.



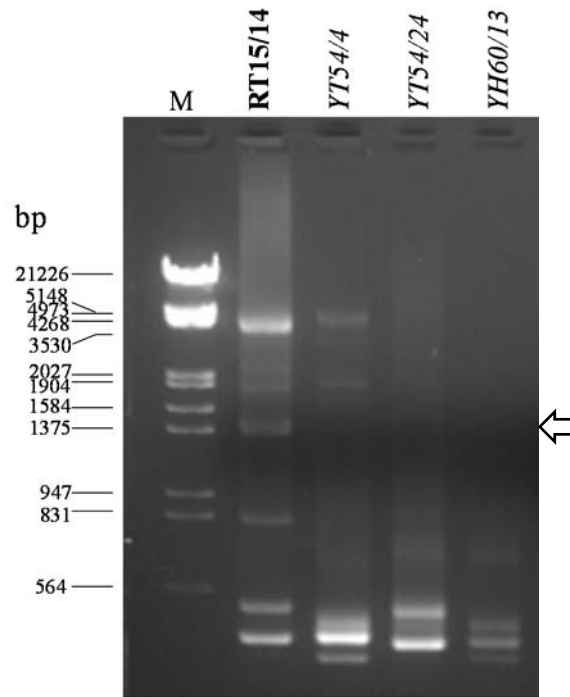


圖 13、以 OMT/MF-1 及 OMT/MR-1 為引子之臺灣檜木木材樣品電泳分析圖

Fig. 13. The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers OMT/MF-1 and OMT/MR-1 for caffeoyl CoA *O*-methyltransferase gene as molecular markers for the wood samples collected from *Chamaecyparis formosensis* Matsum. (RT15/14) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT54/4, YT54/24, YH60/13). The bold and italic word of ID indicate they belong to red and yellow cypress, respectively. M= marker. Arrows beside the figure indicate the specific bands only found in red cypress.

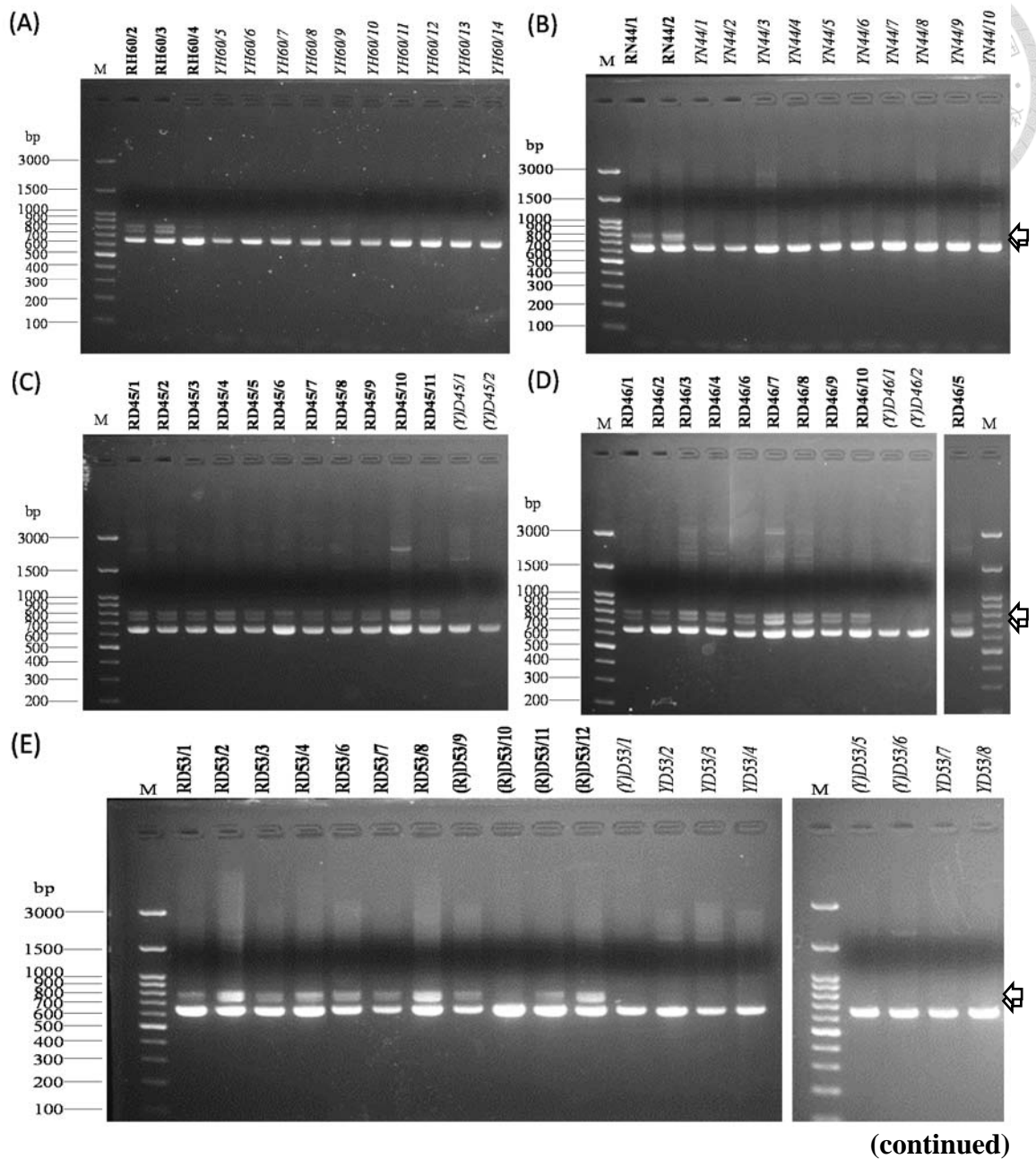


圖 14、以 CAS5 及 CAS/MR-2 為引子之臺灣檜木葉片樣品電泳分析圖

Fig. 14. The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers for cadinene synthase gene as molecular markers from the leaves samples of selected cypress accessions collected from (A) Ho Ping working circle 60 forest compartment (H60); (B) Nan Ao working circle 44 forest compartment (N44); (C), (D), (E) Dasi working circle 45, 46, 53 forest compartment, respectively (D45, D46, D53). M= marker. PCR with the primers CAS5 and CAS/MR-2. The bold and italic word of ID indicate they belong to red and yellow cypress, respectively. Arrows beside the figure indicate the specific bands only found in red cypress.

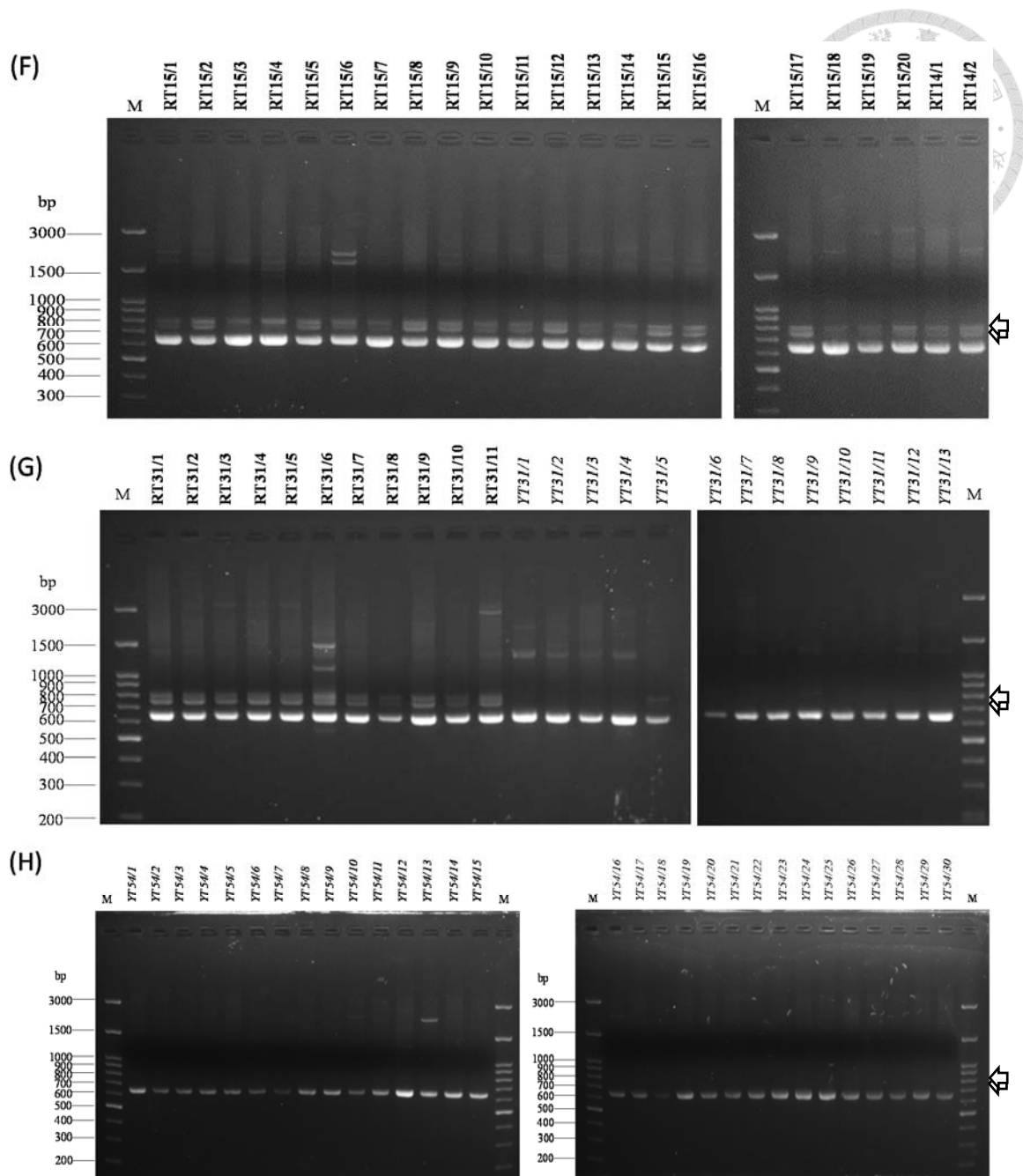


圖 14 (續)、以 CAS5 及 CAS/MR-2 為引子之臺灣檜木葉片樣品電泳分析圖

Fig. 14. (continued) The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers for cadinene synthase gene as molecular markers from the leaves samples of selected cypress accessions collected from (F) Taipingshan working circle 14, 15 forest compartment (T14, T15); (G), (H) Taipingshan working circle 31, 54 forest compartment, respectively (T31, T54). Arrows beside the figure indicate the specific bands only found in red cypress.



*CAS-C5R-RT31/1-b* (1) ATGGCTGAAGTTGGTCTCTTCAAAAATTCATATGCCTCTGGCAATCATGACAAGATATCAGAGCAACAAATCAGTCGAAGGGTAGCAGAAATTCATCCAA  
*CAS-C5R-RT31/1-a* (1) ATGGCTGAAGTTGGTCTCTTCAAAAATTCATATGCCTCTGGCAATCATGACAAGATATCAGAGCAACAAATCAGTCGAAGGGTAGCAGAAATTCATCCAA  
*CAS-C5R-RT31/1* (1) ATGGCTGAAGTTGGTCTCTTCAAAAATTCATATGCCTCTGGCAATCATGACAAGATATCAGAGCAACAAATCAGTCGAAGGGTAGCAGAAATTCATCCAA  
*CAS-C5R-YT31/6* (1) ATGGCTGAAGTTGGTCTCTTCAAAAATTCATATGCCTCTGGCAATCATGACAAGATATCAGAGCAACAAATCAGTCGAAGGGTAGCAGAAATTCATCCAA  
  
*CAS-C5R-RT31/1-b* (101) ACGTTTGGGGTACGAATTCCTGCAGTCCCTTTTCATCGCCATATGGGGTAAGTAAAGGT-----A-----GTATTATATTTCCCT  
*CAS-C5R-RT31/1-a* (101) ACGTTTGGGGTACGAATTCCTGCAGTCCCTTTTCATCGCCATATGGGGTAAGTAAAGGT-----A-----GTATTATATTTCCCT  
*CAS-C5R-RT31/1* (101) ACGTTTGGGGTACGAATTCCTGCAGTCCCTTTTCATCGCCATATGGGGTAAGTAAAGGTTAATTAAGTCTGCAAAAGTTCTTAAATATTTGATTTTATTTCCCT  
*CAS-C5R-YT31/6* (101) ACGTTTGGGGTACGAATTCCTGCAGTCCCTTTTCATCGCCATATGGGGTAAGTAAAGGTTAATTAAGTCTGCAAAAGTTCTTAAATATTTGATTTTATTTCCCT  
  
*CAS-C5R-RT31/1-b* (175) TCTCTTTTCTGTACCGTTTCCAAGCAAAAATCTCATATGACTGCAACCATGATTTTAT-----CAGGCTCCTTGTACTGCGAGAGGTTAACACCTTAATTG  
*CAS-C5R-RT31/1-a* (175) TCTCTTTTCTGTACCGTTTCCAAGCAAAAATCTCATATGACTGCAACCATGATTTTAT-----CAGGCTCCTTGTACTGCGAGAGGTTAACACCTTAATTG  
*CAS-C5R-RT31/1* (199) TCTCTTTTCTGTACCGTTTCCAAGCAAAAATCTCATAT-----ATCTATTGAATGACAGGACCTTGTACTGCGAGAGGTTAACACCTTAATTG  
*CAS-C5R-YT31/6* (201) TCTCTTTTCTGTACCGTTTCCAAGCAAAAATCTCATAT-----ATCTATTGAATGACAGGACCTTGTACTGCGAGAGGTTAACACCTTAATTG  
  
*CAS-C5R-RT31/1-b* (273) AGGAGATTAAGATGGATATATTTGATGGTCTGGTGGAGATGGAGAAAAGAATATGAATCCGCTGCTTATGACCTTCTTGAAGGTTCTTCTGTTGTA  
*CAS-C5R-RT31/1-a* (273) AGGAGATTAAGATGGATATATTTGATGGTCTGGTGGAGATGGAGAAAAGAATATGAATCCGCTGCTTATGACCTTCTTGAAGGTTCTTCTGTTGTA  
*CAS-C5R-RT31/1* (289) AGGAGATTAAGATGGATATATTTGATGGTCTGGTGGAGATGGAGAAAAGAATATGAATCCGCTGCTTATGACCTTCTTGAAGGTTCTTCTGTTGTA  
*CAS-C5R-YT31/6* (291) AGGAGATTAAGATGGATATATTTGATGGTCTGGTGGAGATGGAGAAAAGAATATGAATCCGCTGCTTATGACCTTCTTGAAGGTTCTTCTGTTGTA  
  
*CAS-C5R-RT31/1-b* (373) TATTATGCAGAGCCTGGGAATTCATCGCCATTTTCAAAAAGAAATAAAAGCAGTTCTAGATTATGCTTACAAGTAAGTTCGTTTATATTACTTTGCTA  
*CAS-C5R-RT31/1-a* (373) TATTATGCAGAGCCTGGGAATTCATCGCCATTTTCAAAAAGAAATAAAAGCAGTTCTAGATTATGCTTACAAGTAAGTTCGTTTATATTACTTTGCTA  
*CAS-C5R-RT31/1* (389) TATT-----  
*CAS-C5R-YT31/6* (391) TATT-----  
  
*CAS-C5R-RT31/1-b* (473) ATTATACAATAGAATCATTGTTGTTGTTGTA AAAACTAATGGTATATCTTGATATTCTGCAGAGCCTGGGAATTGAACGCCATTTTAAAAAGAAATAA  
*CAS-C5R-RT31/1-a* (473) ATTATACAATAGAATCATTGTTGTTGTTGTA AAAACTAATGGTATATCTTGATATTCTGCAGAGCCTGGGAATTGAACGCCATTTTAAAAAGAAATAA  
*CAS-C5R-RT31/1* (393) -----CTGCAGAGCCTGGGAATTGAACGCCATTTTAAAAAGAAATAA  
*CAS-C5R-YT31/6* (395) -----CTGCAGAGCCTGGGAATTGAACGCCATTTTAAAAAGAAATAA  
  
*CAS-C5R-RT31/1-b* (573) AAGCAGTTCTAGATTACTTACAAGTAAGTTGGTTATATATAAAAACCTTAAATCTAAAAGACGTTAAATCGTTTGGTTATCAAAGTTGTTACAG  
*CAS-C5R-RT31/1-a* (573) AAGCAGTTCTAGATTACTTACAAGTAAGTTGGTTATATATAAAAACCTTAAATCTAAAAGACGTTAAATCGTTTGGTTATCAAAGTTGTTACAG  
*CAS-C5R-RT31/1* (436) AAGCAGTTCTAGATTACTTACAAGTAAGTTGGTTATATATAAAAACCTTAAATCTAAAAGACGTTAAATCGTTTGGTTATCAAAGTTGTTACAG  
*CAS-C5R-YT31/6* (438) AAGCAGTTCTAGATTACTTACAAGTAAGTTGGTTATATATAAAAACCTTAAATCTAAAAGACGTTAAATCGTTTGGTTATCAAAGTTGTTACAG  
  
*CAS-C5R-RT31/1-b* (673) TATTAACCTGGTTTGTTCATATAAAAA-----TTAT-----TCAGATATTGGAATGATGAAAAGGGTATTTCTTGGGG  
*CAS-C5R-RT31/1-a* (673) TATTAACCTGGTTTGTTCATATAAAAA-----TTAT-----TCAGATATTGGAATGATGAAAAGGGTATTTCTTGGGG  
*CAS-C5R-RT31/1* (536) TATTAACCTGGTTTGTTCATATAAAAA-----TTAT-----TCAGATATTGGAATGATGAAAAGGGTATTTCTTGGGG  
*CAS-C5R-YT31/6* (538) TATTAACCTGGTTTGTTCATATAAAAA-----TTAT-----TCAGATATTGGAATGATGAAAAGGGTATTTCTTGGGG  
  
*CAS-C5R-RT31/1-b* (741) GAGCGGAAACCTTATTGT  
*CAS-C5R-RT31/1-a* (773) GAGCGGAAACCTTATTGT  
*CAS-C5R-RT31/1* (636) GAGCGGAAACCTTATTGT  
*CAS-C5R-YT31/6* (638) GAGCGGAAACCTTATTGT

圖 15、臺灣紅檜於 *CAS-C5R* 分子標誌中之特異片段序列分析。

Fig. 15. Alignment of about 0.75 kb (b), 0.8 kb (a) sequence in *Chamaecyparis formosensis* Matsum. (RT31/1), part of cadinene synthase (*CAS*) genomic DNA sequences in *C. formosensis* Matsum. (RT31/1) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT31/6). The fragments a, b synthesized by primer CAS5 and CAS/MR-2 are showed in Fig. 13, 14 and only found in *C. formosensis* Matsum. The black boxes represent conserved percentage of 100%. The hyphen between sequences mean deletion.

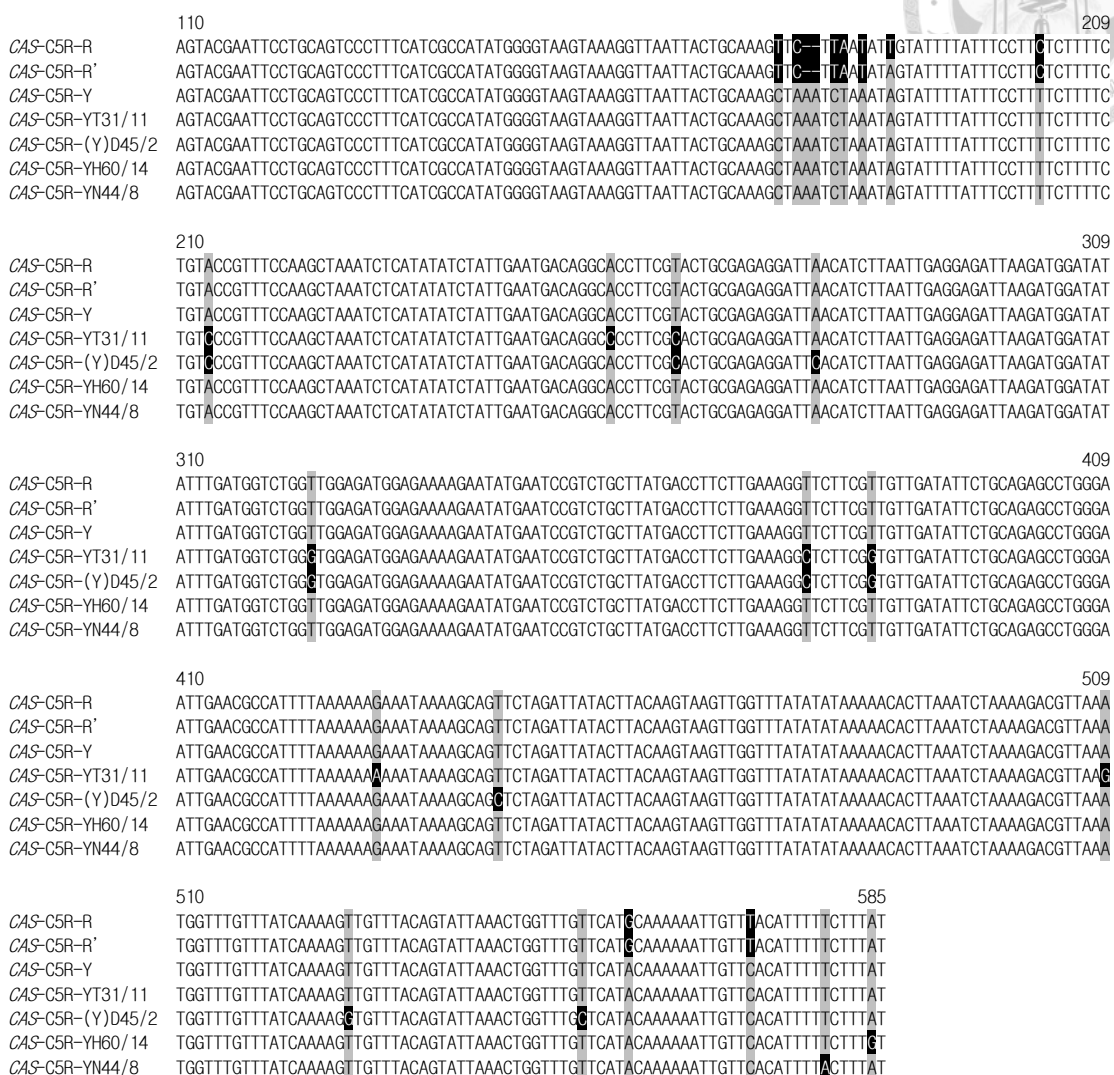


圖 16、臺灣扁柏與紅檜葉子樣品之 CAS-C5R 分子標誌序列比對

Fig. 16. Alignment of the major sequence amplified with Indel-flanking primers CAS5 and CAS/MR-2 for leaves samples in *Chamaecyparis formosensis* Matsum. (R, R') and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (Y, YT31/11, (Y)D45/2, YH60/14, YN44/8). The black boxes represent conserved percentage of 20% to 40%. The gray boxes represent conserved percentage of 60% to 80%. Analysis about 150 different individuals, all red cypress show the sequence either R or R'. And all yellow cypress show the sequence as Y, but have some exception indicated in this figure. The hyphen between sequences mean deletion. Positions refer to the genomic sequence of cadinene synthase gene for *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT31/6).

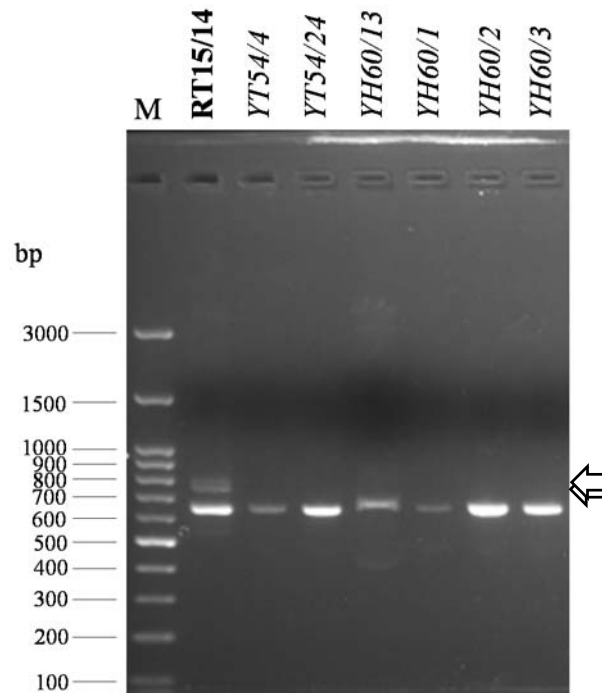


圖 17、以CAS5 及CAS/MR-2為引子之臺灣檜木木材樣品電泳分析圖

Fig. 17. The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers CAS5 and CAS/MR-2 for cadinene synthase gene as molecular markers for the wood samples collected from *Chamaecyparis formosensis* Matsum. (RT15/14) and *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT54/4, YT54/24, YH60/13, YH60/1, YH60/2, YH60/3). The bold and italic word of ID indicate they belong to red and yellow cypress, respectively. M= marker. Arrows beside the figure indicate the specific bands only found in red cypress.

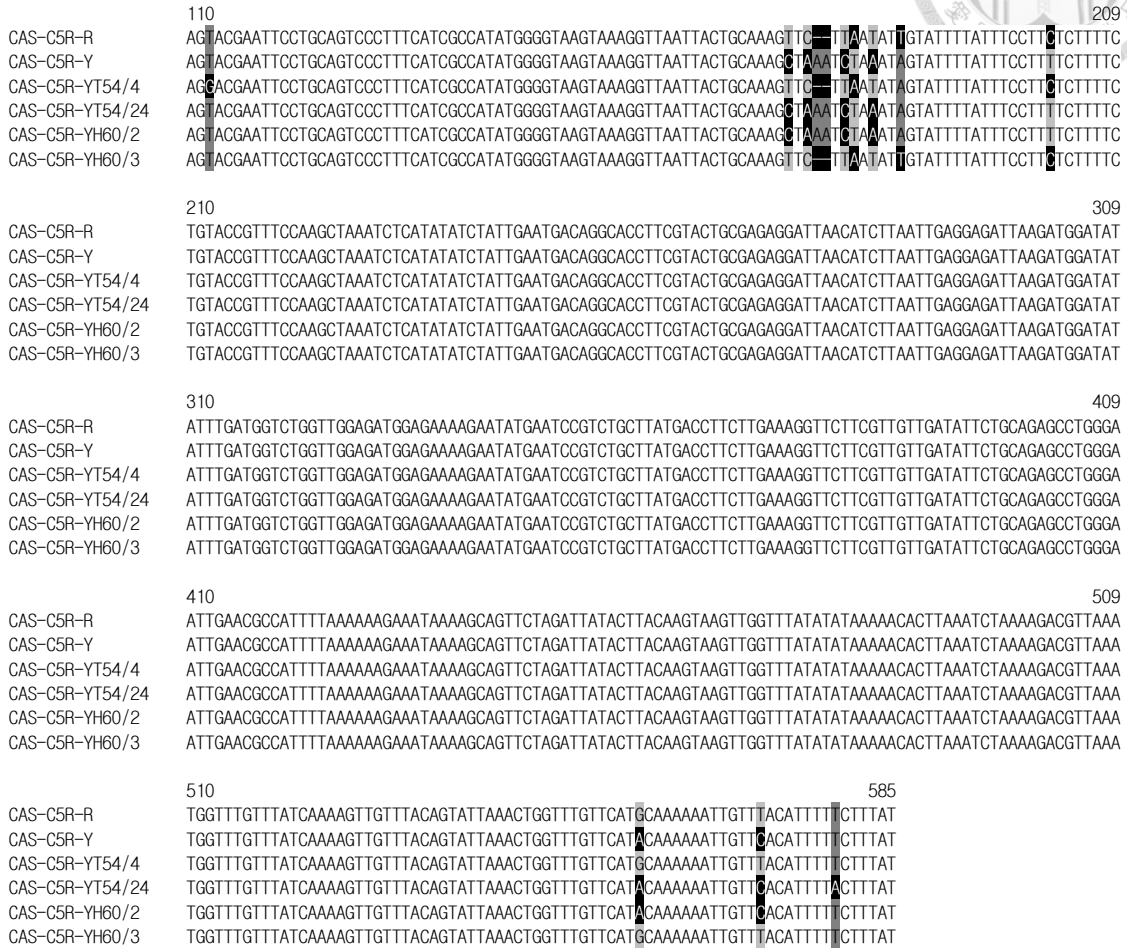


圖 18、臺灣扁柏木材樣品之 CAS-C5R 分子標誌序列比對

Fig. 18. Alignment of the major sequence amplified with Indel-flanking primers CAS5 and CAS/MR-2 for leaves samples in *Chamaecyparis formosensis* Matsum. (R), *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (Y) and for wood samples in *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT54/4, YT54/24, YH60/2, YH60/3). The black boxes represent conserved percentage lower than 50%. The gray boxes represent conserved percentage higher than 60%. The hyphen between sequences mean deletion. Positions refer to the genomic sequence of cadinene synthase gene for *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT31/6).

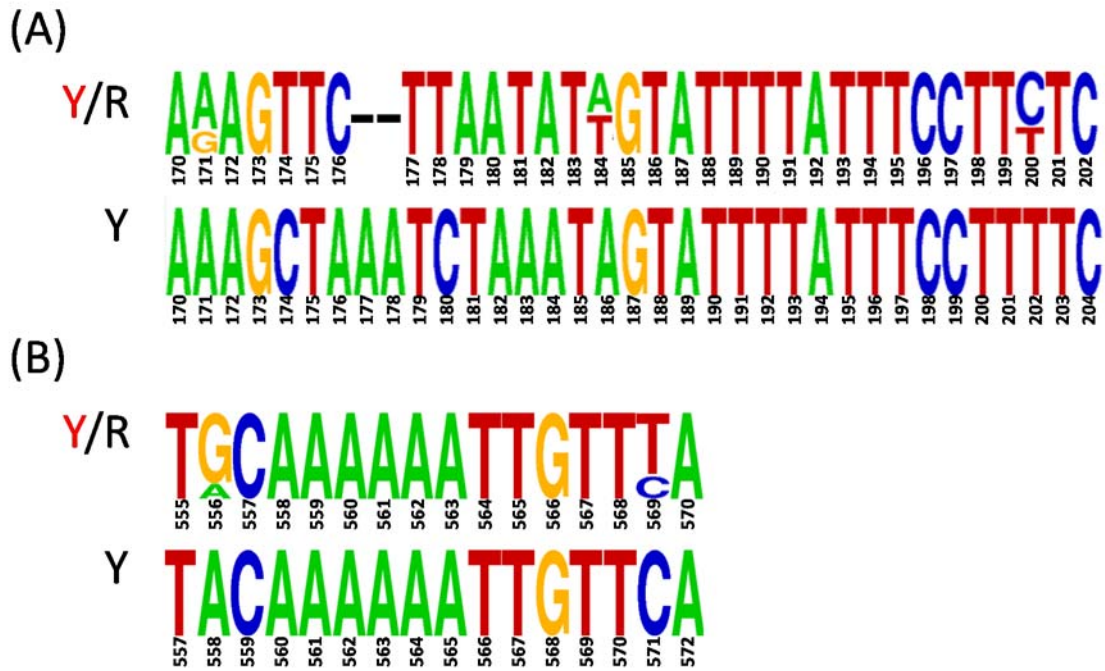


圖 19、臺灣扁柏與紅檜於 CAS-C5R 分子標誌中 650 bp DNA 序列差異圖

Fig. 19. DNA sequence variation of the 650 bp PCR fragments amplified with Indel-flanking primers (CAS5 and CAS/MR-2) of cadinene synthase gene for the selected cypress accessions of about 150 different individuals collected from Loudong forestry district. The numbers for the nucleotides are shown below the sequence. The first nucleotide of CAS5 primer is position 1. There are sequence variation of the 650 bp fragments in the position of 170 to 204 (A), and 555 to 572 (B). R= red cypress, Y= yellow cypress. Red “Y” means the sequence be found only in wood sample of yellow cypress in this study.



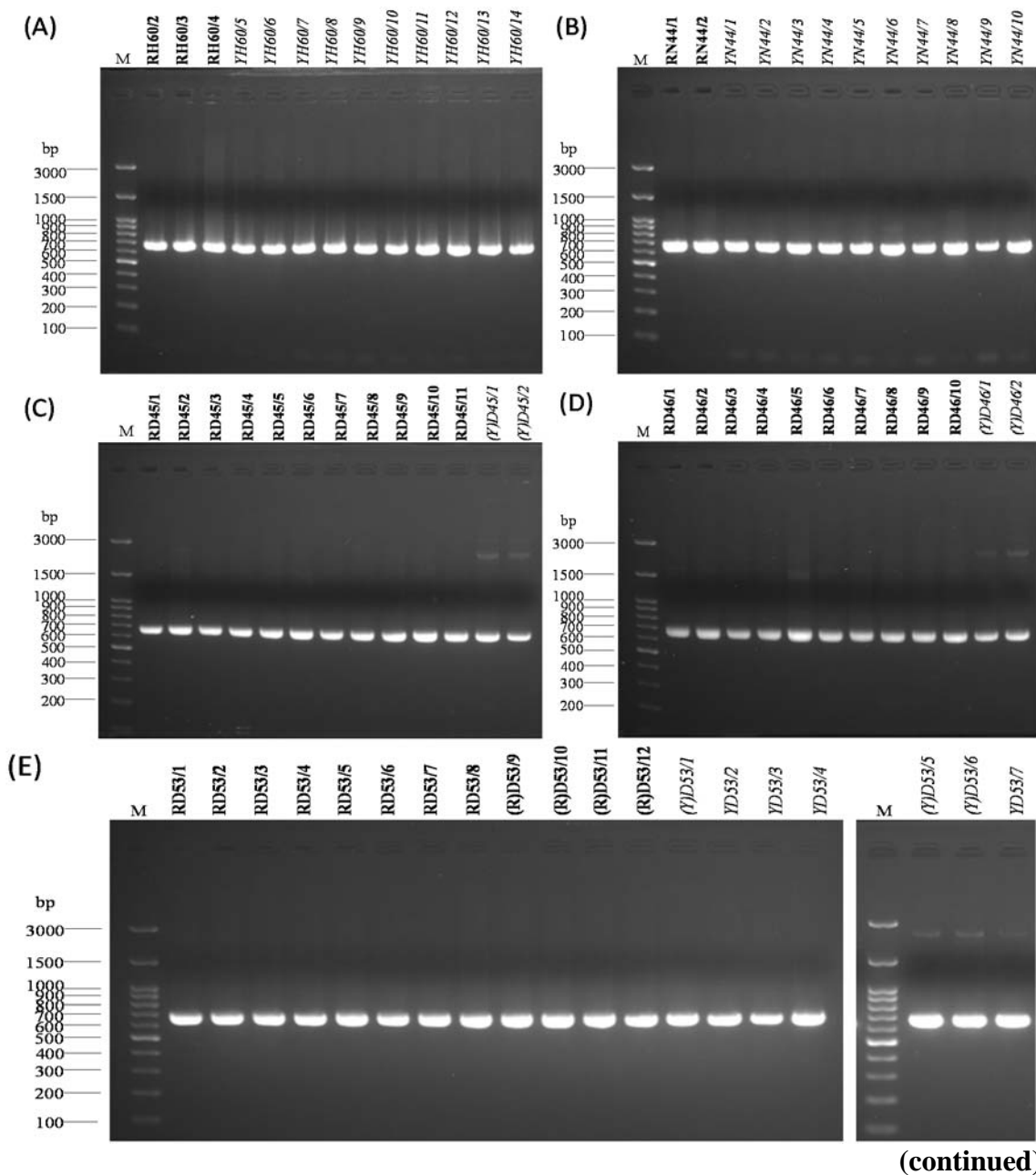


圖 20、以 CAS/MF-1 及 CAS/MR-1 為引子之臺灣檜木葉片樣品電泳分析圖

Fig. 20. The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers for cadinene synthase gene as molecular markers from the leaves samples of selected cypress accessions collected from (A) Ho Ping working circle 60 forest compartment (H60); (B) Nan Ao working circle 44 forest compartment (N44); (C), (D), (E) Dasi working circle 45, 46, 53 forest compartment, respectively (D45, D46, D53). M= marker. PCR with the primers CAS/MF-1 and CAS/MR-1. The bold and italic word of ID indicate they belong to red and yellow cypress, respectively.

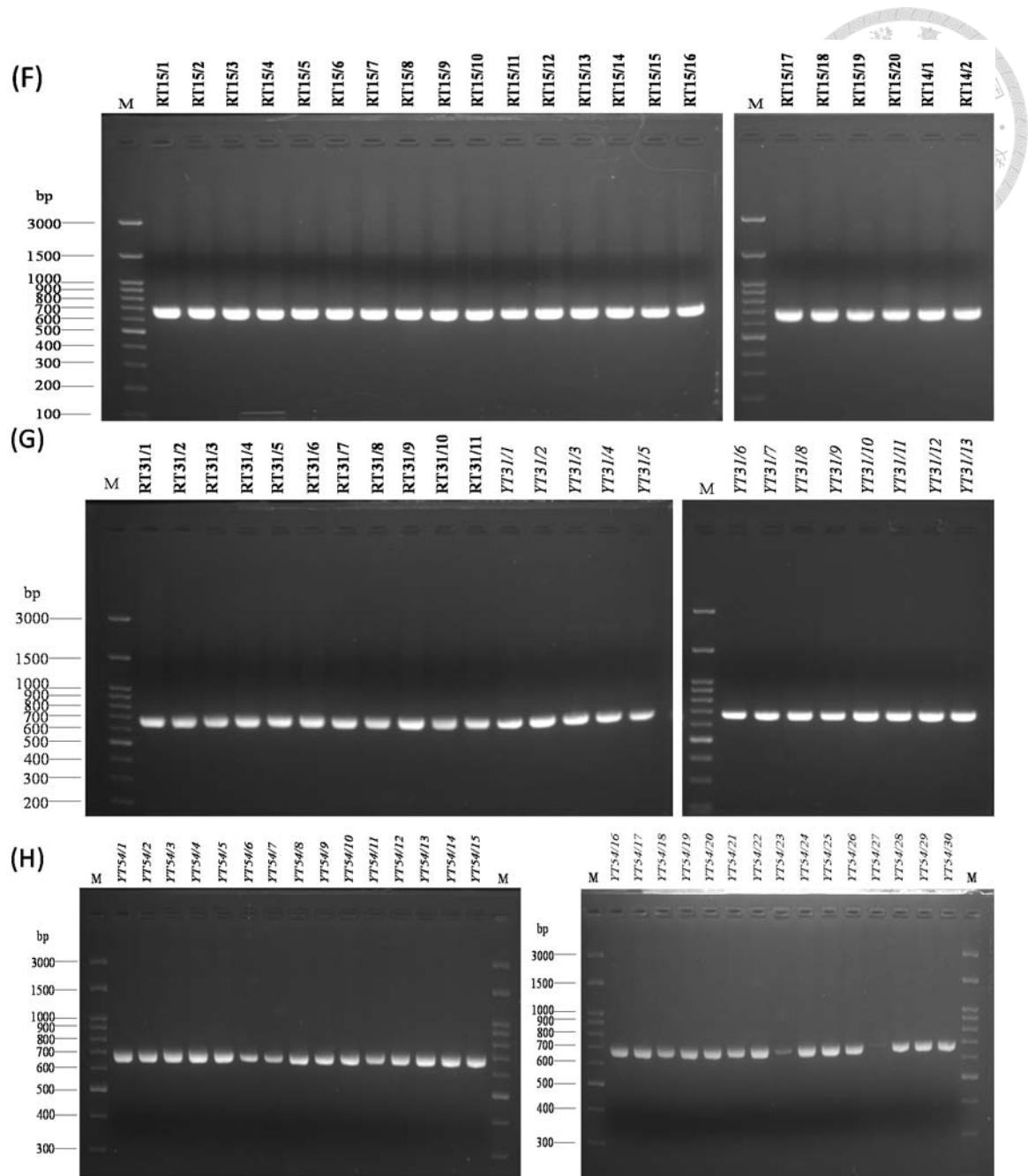


圖 20 (續)、以 CAS/MF-1 及 CAS/MR-1 為引子之臺灣檜木葉片樣品電泳分析圖  
 Fig. 20. (continued) The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers for cadinene synthase gene as molecular markers from the leaves samples of selected cypress accessions collected from (F) Taipingshan working circle 14, 15 forest compartment (T14, T15); (G), (H) Taipingshan working circle 31, 54 forest compartment, respectively (T31, T54).

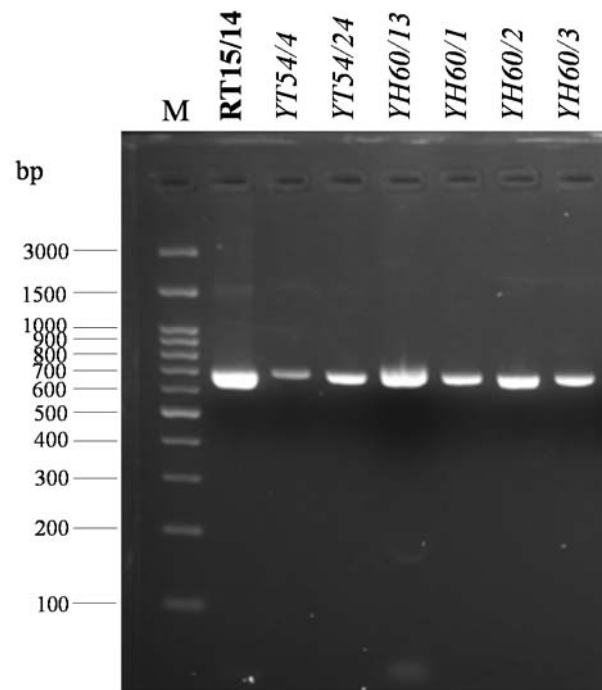


圖 21、以 CAS/MF-1 及 CAS/MR-1 為引子之臺灣檜木木材樣品電泳分析圖

Fig. 21. The graph of 2% agarose gel electrophoresis of PCR fragments amplified with Indel-flanking primers CAS/MF-1 and CAS/MR-1 for cadinene synthase gene as molecular markers for the wood samples collected from RT15/14, YT54/4, YT54/24, YH60/13, YH60/1, YH60/2, YH60/3. The bold and italic word of ID indicate they belong to red and yellow cypress, respectively.

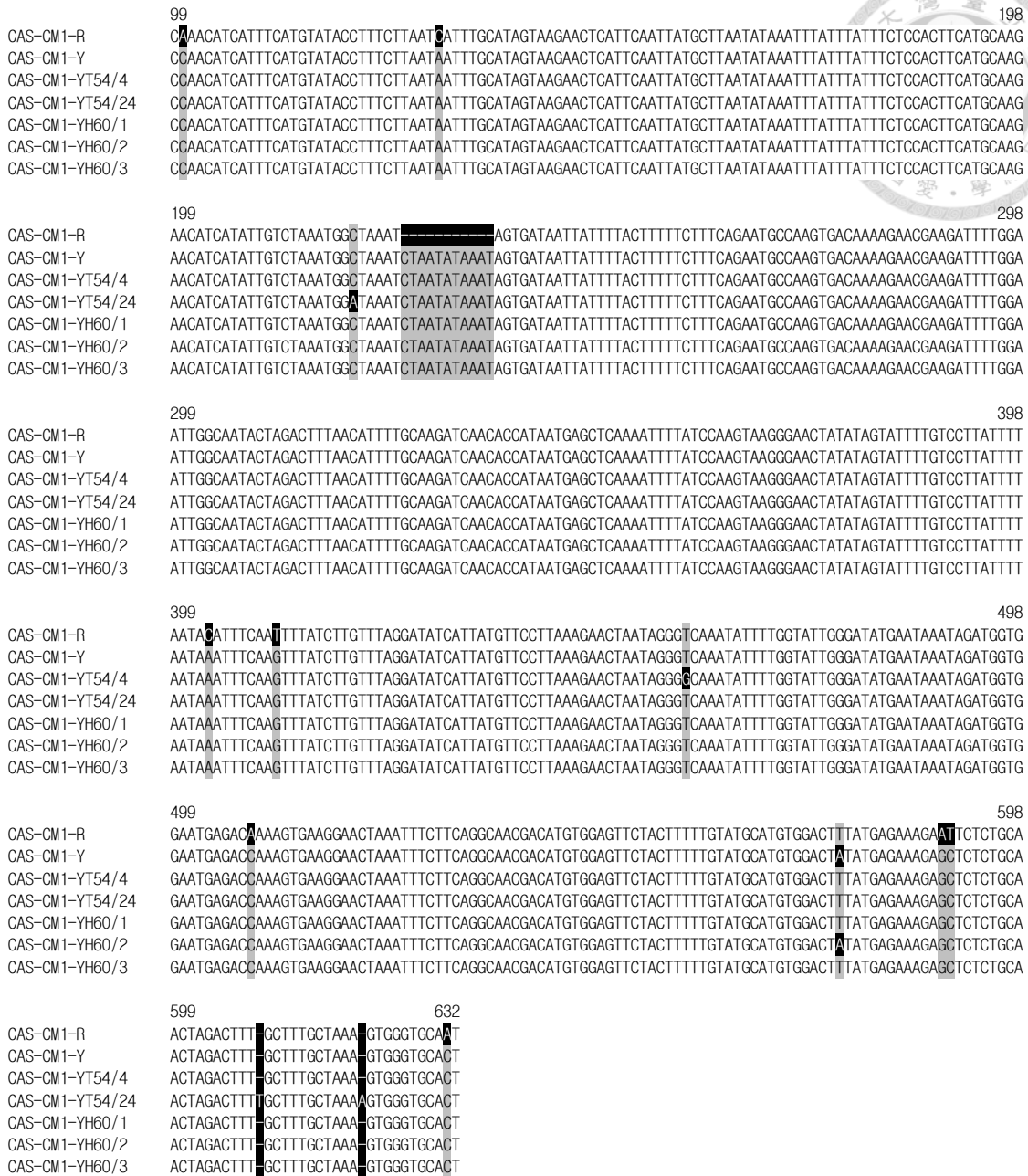


圖 22、臺灣扁柏與紅檜木材樣品之 CAS-CM1 分子標誌序列比對

Fig. 22. Alignment of the major sequence amplified with Indel-flanking primers CAS/MF-1 and CAS/MR-1 for leaves samples in *Chamaecyparis formosensis* Matsum. (R), *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (Y) and for wood samples in *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT54/4, YT54/24, YH60/1, YH60/2, YH60/3). The black boxes represent conserved percentage lower than 30%. The gray boxes represent conserved percentage of 70% to 86%. Deletions sequences are indicated by hyphen. The first nucleotide position is the 5' of CAS/MF-1 primer sequence and positions refer to sequence for *C. obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YT31/6).

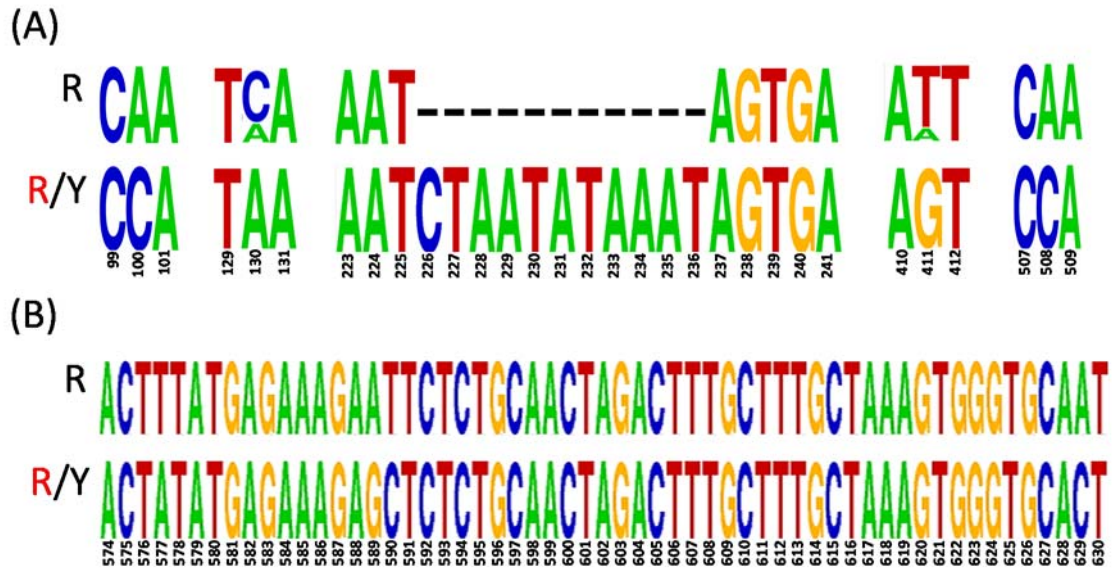
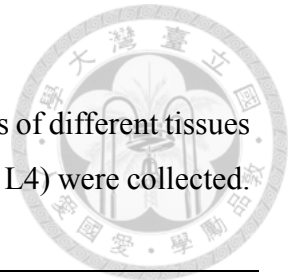


圖 23、臺灣扁柏與紅檜於 CAS-CM1 分子標誌中 DNA 序列差異圖

Fig. 23. DNA sequence variation of the 700 bp PCR fragments amplified with Indel-flanking primers (CAS/MF-1 and CAS/MR-1) for cadinene synthase gene for the selected cypress accessions of about 150 different individuals collected from Loudong forestry district. The numbers for the nucleotides are shown below the sequence. The first nucleotide of CAS/MF-1 primer is position 1. There are sequence variation of the 700 bp fragments in the different position from 99 to 509 (A), and 574 to 630 (B) of *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder. R= red cypress, Y= yellow cypress. Red “R” means the sequence be found rarely in red cypress in this study.

表 1、本研究檢驗之木材樣品其臺灣檜木族群資料

Table 1. The wood samples information of cypress population. Chunks of different tissues and different condition gathered from various depths of the log (L1 to L4) were collected. And based on the bark to the xylem direction define depth.



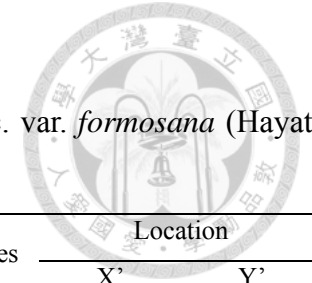
ID	Species	Location			Sample type	
		Working circle	X'	Y'	Tissue	Condition
YH60/1	<i>Co</i>	H60	304216	2709865	root	Dead
YH60/2	<i>Co</i>	H60	304216	2709868	trunk	Fresh
YH60/3	<i>Co</i>	H60	304169	2709890	trunk	Dead
YH60/4	<i>Co</i>	H60	304151	2709899	root trunk	Fresh
RH60/1	<i>Cf</i>	H60	304174	2709921	root trunk	Fresh
RT98/1	<i>Cf</i>	T98	302556	2713931	trunk	Fresh

Cypress include *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder [(*Co*) also called Taiwan yellow cypress (Y)] and *C. formosensis* Matsum. [(*Cf*), Taiwan red cypress (R)]. The samples was collected from Taipingshan working circle (T)\_forest compartment ( \_: indicates different number of area) and Ho Ping working circle (H)\_forest compartment.

X', Y' of location belong to universal transverse mercator (UTM) grid system and were calculated by global positioning system (GPS).

表 2、本研究檢驗之葉片樣品其臺灣檜木族群資料

Table 2. The leaf samples information of cypress population. Cypress include *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder [(*Co*) also called Taiwan yellow cypress (Y)] and *C. formosensis* Matsum. [(*Cf*), Taiwan red cypress (R)].



Working circle	ID	Species	Location		ID	Species	Location		ID	Species	Location	
			X'	Y'			X'	Y'			X'	Y'
H60	RH60/2	<i>Cf</i>	304213	2709852	RH60/3	<i>Cf</i>	304209	2709861	RH60/4	<i>Cf</i>	304228	2709866
	YH60/5	<i>Co</i>	304243	2709809	YH60/6	<i>Co</i>	304208	2709805	YH60/7	<i>Co</i>	304188	2709803
	YH60/8	<i>Co</i>	304213	2709858	YH60/9	<i>Co</i>	304221	2709877	YH60/10	<i>Co</i>	304232	2709872
	YH60/11	<i>Co</i>	304247	2709905	YH60/12	<i>Co</i>	304239	2709914	YH60/13	<i>Co</i>	304243	2709922
	YH60/14	<i>Co</i>	304239	2709918								
N44	RN44/1	<i>Cf</i>	311920	2712093	RN44/2	<i>Cf</i>	311718	2711687	YN44/1	<i>Co</i>	311706	2711777
	YN44/2	<i>Co</i>	311708	2711780	YN44/3	<i>Co</i>	311711	2711784	YN44/4	<i>Co</i>	311722	2711779
	YN44/5	<i>Co</i>	311724	2711788	YN44/6	<i>Co</i>	311728	2711783	YN44/7	<i>Co</i>	311727	2711805
	YN44/8	<i>Co</i>	311724	2711827	YN44/9	<i>Co</i>	311726	2711834	YN44/10	<i>Co</i>	311727	2711830
D45	RD45/1	<i>Cf</i>	294975	2726456	RD45/2	<i>Cf</i>	294977	2726470	RD45/3	<i>Cf</i>	294976	2726472
	RD45/4	<i>Cf</i>	294958	2726525	RD45/5	<i>Cf</i>	294963	2726539	RD45/6	<i>Cf</i>	294961	2726541
	RD45/7	<i>Cf</i>	294969	2726543	RD45/8	<i>Cf</i>	294978	2726557	RD45/9	<i>Cf</i>	294963	2726549
	RD45/10	<i>Cf</i>	294967	2726558	RD45/11	<i>Cf</i>	294974	2726560	(Y)D45/1*	<i>Co</i>	295059	2726255
	(Y)D45/2*	<i>Co</i>	295060	2726253								

The samples was collected from Taipingshan (T), Dasi (D), Nan Ao (N) and Ho Ping (H) working circles. And the numbers behind working circles indicate different area of forest compartment. The first word of ID means its species (R= red cypress, Y= yellow cypress), next three words mean its collected position, and the last word indicates different individual.

\*= Species identification based on molecular is opposite with the result based on phenotype character.

X', Y' of location belong to universal transverse mercator (UTM) grid system and were calculated by global positioning system (GPS).

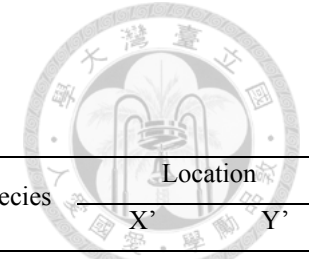


表 2 (續)、本研究檢驗之葉片樣品其臺灣檜木族群資料

Table 2 (continued). The leaf samples information of cypress population.

Working circle	ID	Species	Location		ID	Species	Location		ID	Species	Location	
			X'	Y'			X'	Y'			X'	Y'
D46	RD46/1	<i>Cf</i>	295293	2726332	RD46/2	<i>Cf</i>	295333	2726353	RD46/3	<i>Cf</i>	295397	2726249
	RD46/4	<i>Cf</i>	295393	2726251	RD46/5	<i>Cf</i>	295404	2726277	RD46/6	<i>Cf</i>	295415	2726283
	RD46/7	<i>Cf</i>	295400	2726281	RD46/8	<i>Cf</i>	295505	2726172	RD46/9	<i>Cf</i>	295553	2726114
	RD46/10	<i>Cf</i>	295541	2726108	YD46/1	<i>Co</i>	295063	2726256	YD46/2	<i>Co</i>	295269	2726307
D53	RD53/1	<i>Cf</i>	294248	2721419	RD53/2	<i>Cf</i>	294247	2721417	RD53/3	<i>Cf</i>	293350	2721175
	RD53/4	<i>Cf</i>	293338	2721179	RD53/5	<i>Cf</i>	293197	2721205	RD53/6	<i>Cf</i>	293161	2721188
	RD53/7	<i>Cf</i>	293139	2721201	RD53/8	<i>Cf</i>	293140	2721203	(R)D53/9*	<i>Cf</i>	294236	2721436
	(R)D53/10*	<i>Cf</i>	294292	2721448	(R)D53/11*	<i>Cf</i>	293211	2721206	(R)D53/12*	<i>Cf</i>	293708	2722177
	(Y)D53/1*	<i>Co</i>	293891	2722863	YD53/2	<i>Co</i>	294256	2721496	YD53/3	<i>Co</i>	294239	2721528
	YD53/4	<i>Co</i>	294210	2721477	(Y)D53/5*	<i>Co</i>	293894	2722862	(Y)D53/6*	<i>Co</i>	293887	2722829
	YD53/7	<i>Co</i>	293107	2722173	YD53/8	<i>Co</i>	293710	2722178				
T14	RT14/1	<i>Cf</i>	297855	2727419	RT14/2	<i>Cf</i>	297855	2727563				
T15	RT15/1	<i>Cf</i>	297843	2727125	RT15/2	<i>Cf</i>	297843	2727120	RT15/3	<i>Cf</i>	297838	2727111
	RT15/4	<i>Cf</i>	297892	2727083	RT15/5	<i>Cf</i>	297895	2727078	RT15/6	<i>Cf</i>	297905	2727082
	RT15/7	<i>Cf</i>	297901	2727090	RT15/8	<i>Cf</i>	297892	2727091	RT15/9	<i>Cf</i>	297902	2727106
	RT15/10	<i>Cf</i>	297881	2727107	RT15/11	<i>Cf</i>	297883	2727111	RT15/12	<i>Cf</i>	297888	2727118
	RT15/13	<i>Cf</i>	297875	2727092	RT15/14	<i>Cf</i>	297948	2727001	RT15/15	<i>Cf</i>	297946	2727021

\*= Species identification based on molecular is opposite with the result based on phenotype character.

X', Y' of location belong to universal transverse mercator (UTM) grid system and were calculated by global positioning system (GPS).



表 2 (續)、本研究檢驗之葉片樣品其臺灣檜木族群資料

Table 2. (continued) The leaf samples information of cypress population.

Working circle	ID	Species	Location		ID	Species	Location		ID	Species	Location	
			X'	Y'			X'	Y'			X'	Y'
T15	RT15/16	<i>Cf</i>	297948	2727025	RT15/17	<i>Cf</i>	297947	2727025	RT15/18	<i>Cf</i>	297952	2727028
	RT15/19	<i>Cf</i>	297903	2727102	RT15/20	<i>Cf</i>	297921	2727110				
T31	RT31/1	<i>Cf</i>	292527	2720607	RT31/2	<i>Cf</i>	292492	2720579	RT31/3	<i>Cf</i>	292480	2720575
	RT31/4	<i>Cf</i>	292456	2720575	RT31/5	<i>Cf</i>	292441	2720572	RT31/6	<i>Cf</i>	292413	2720570
	RT31/7	<i>Cf</i>	292399	2720563	RT31/8	<i>Cf</i>	292369	2720553	RT31/9	<i>Cf</i>	292281	2720557
	RT31/10	<i>Cf</i>	292249	2720587	RT31/11	<i>Cf</i>	292190	2720611	YT31/1	<i>Co</i>	292543	2720623
	YT31/2	<i>Co</i>	292553	2720629	YT31/3	<i>Co</i>	292547	2720624	YT31/4	<i>Co</i>	292525	2720597
	YT31/5	<i>Co</i>	292500	2720583	YT31/6	<i>Co</i>	292469	2720574	YT31/7	<i>Co</i>	292453	2720571
	YT31/8	<i>Co</i>	292415	2720569	YT31/9	<i>Co</i>	292401	2720562	YT31/10	<i>Co</i>	292384	2720556
	YT31/11	<i>Co</i>	292302	2720559	YT31/12	<i>Co</i>	292229	2720594	YT31/13	<i>Co</i>	292200	2720606
T54	YT54/1	<i>Co</i>	285825	2699055	YT54/2	<i>Co</i>	285814	2699063	YT54/3	<i>Co</i>	285814	2699074
	YT54/4	<i>Co</i>	285830	2699075	YT54/5	<i>Co</i>	285828	2699083	YT54/6	<i>Co</i>	285829	2699056
	YT54/7	<i>Co</i>	285820	2699052	YT54/8	<i>Co</i>	285821	2699039	YT54/9	<i>Co</i>	285824	2699044
	YT54/10	<i>Co</i>	285818	2699036	YT54/11	<i>Co</i>	285810	2699044	YT54/12	<i>Co</i>	285818	2699037
	YT54/13	<i>Co</i>	285814	2699031	YT54/14	<i>Co</i>	285792	2699017	YT54/15	<i>Co</i>	285792	2699018
	YT54/16	<i>Co</i>	285790	2699013	YT54/17	<i>Co</i>	285792	2699016	YT54/18	<i>Co</i>	285796	2699009
	YT54/19	<i>Co</i>	285788	2699009	YT54/20	<i>Co</i>	285781	2699006	YT54/21	<i>Co</i>	285783	2699000
	YT54/22	<i>Co</i>	285779	2698997	YT54/23	<i>Co</i>	285768	2699010	YT54/24	<i>Co</i>	285766	2699018
	YT54/25	<i>Co</i>	285775	2699030	YT54/26	<i>Co</i>	285748	2699013	YT54/27	<i>Co</i>	285735	2699005
	YT54/28	<i>Co</i>	285716	2699003	YT54/29	<i>Co</i>	285711	2699015	YT54/30	<i>Co</i>	285712	2699011

X', Y' of location belong to universal transverse mercator (UTM) grid system and were calculated by global positioning system (GPS).

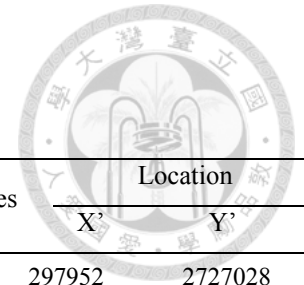
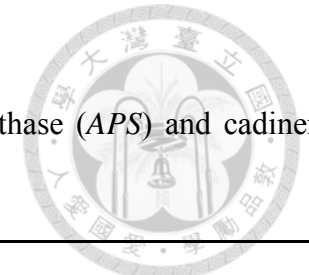


表 3、選殖臺灣檜木基因使用之引子

Table 3. Primers for PCR amplification to clone caffeoyl CoA *O*-methyltransferase (*CCoAOMT*), alpha-pinene synthase (*APS*) and cadinene synthase (*CAS*) genes. *CCoAOMT*, *APS*, *CAS* primer sequence and position are based on the YH60/2 sequence.



Primer name	Position	Sequence (5'-3')	T <sub>a</sub> (°C)
CCoAOMT5 (fwd)	(-1)-19	AATGGCAACCGTAGAGGCTA	52
CCoAOMT3 (rev)	1907-1925	YTCAAATAAYTCKTCTGCAG	
APS5 (fwd)	3-22	GTCTCTCGGTTGTATTACAC	58
APS/R-1 (rev)	2503-2523	GTCCAACCTTTGCCAATTCTAG	
APS/F-1 (fwd)	2226-2245	AATGTGCCTAGGTTGGAAGC	
APS3 (rev)	4233-4254	TATTGGAATTGATCCAATCATC	62
CAS5 (fwd)	1-20	ATGGCTGAAGTTGGTCTCTC	
CAS3 (fwd)	3341-3368	CGGCTAAAAATATATAGGTTCAACTAGAATC	

Fwd refers to forward primers which are located 5' to the DNA fragment being amplified and rev to reverse primers which are 3' to the fragment. Y= C or T, K= G or T. And T<sub>a</sub> = the optimized annealing temperature.



表 4、於聚合酶連鎖反應擴增臺灣檜木分子標誌之引子

Table 4. Primers for PCR amplification of molecular markers developed in *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder and *C. formosensis* Matsum. based on the polymorphic sequence of caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) and cadinene synthase (*CAS*) genes.

Marker Name	Primer name	Sequence (5'-3')	T <sub>a</sub> (°C)
OMT-M1	OMT/MF-1 (fwd)	CATGCCTGGATTGATTTGGA	58
	OMT/MR-1 (rev)	GGGTTTTACTGATTATACTGATTAG	
CAS-C5R	CAS5 (fwd)	ATGGCTGAAGTTGGTCTCTC	63
	CAS/MR-2 (rev)	ACAATAAGGTTTCCGCTCGC	
CAS-CM1	CAS/MF-1 (fwd)	CGTGTAGAGTCCCTAGATGG	63
	CAS/MR-1 (rev)	ACAAGCTCATCAATGGTTCC	

Fwd refers to forward primers which are located 5' to the DNA fragment being amplified and rev to reverse primers which are 3' to the fragment. T<sub>a</sub> = the optimized annealing temperature.

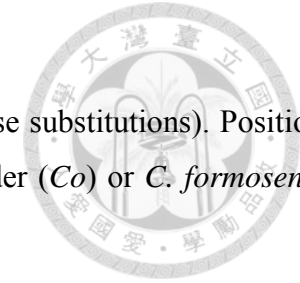


表 5、臺灣檜木咖啡醯輔酶 A 甲基轉移酶基因中之多型性序列

Table 5. Sequence variation found in the cypress caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) gene (excluding base substitutions). Positions refer to the genomic sequence of *CCoAOMT* in *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (*Co*) or *C. formosensis* Matsum. (*Cf*) from this study. The first nucleotide is the A of start codon in *CCoAOMT*.

No.	Species	Position	Length of insertion (bp)	Sequence (5'→3')
1	<i>Co</i>	212-240 (Intron 1)	29	ATTGGATTGGCATATACATTGGGTAA
2	<i>Co</i>	503-540 (Intron 2)	38	TGCTACTAATAGTTCAATGTCAGTAACGCCCAAATTGG
3	<i>Cf</i>	626-630 (Intron 2)	5	TCCAC
4	<i>Cf</i>	707-739 (Intron 2)	33	TGAGAAATTTCTGTTATGTGCTACTAACAATTT
5	<i>Co</i>	806-807 (Intron 2)	2	GT
6	<i>Cf</i>	826 (Intron 2)	1	C
7	<i>Co</i>	984 (Intron 2)	1	C
8	<i>Cf</i>	1326 (Intron 3)	1	C

表 6、臺灣檜木咖啡醯輔酶 A 甲基轉移酶基因於顯子之單一核苷酸多型性序列

Table 6. Single nucleotide polymorphisms (SNP) present in exons 1 to 5 of the cypress caffeoyl CoA *O*-methyltransferase (*CCoAOMT*) gene. Positions refer to the genomic sequence of *CCoAOMT* in *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2). The first nucleotide is the A of start codon in *CCoAOMT*.

No.	Position	Sequence (Y/R)	Amino acid*
1	39 (in Exon 1)	A/G	Gln
2	287 (in Exon 2)	G/A	Glu
3	323 (in Exon 2)	T/C	Ala
4	1488 (in Exon 4)	T/C	Ala
5	1491 (in Exon 4)	A/G	Leu
6	1494 (in Exon 4)	A/T	Pro
7	1510 (in Exon 4)	C/T	Leu
8	1646 (in Exon 5)	C/T	Gly
9	1649 (in Exon 5)	C/G	Ser
10	1914 (in Exon 5)	C/A	Arg
11	1917 (in Exon 5)	G/A	Val/Ile

\*- Polymorphisms were affecting amino acids in the *CCoAOMT* sequence.

表 7、臺灣檜木萜烯合成酶基因之單一核苷酸多型性序列

Table 7. Single nucleotide polymorphisms (SNP) present in exons and introns of the cypress alpha pinene synthase (*APS*) gene. Positions refer to the genomic sequence of *APS* for *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2). The first nucleotide is the A of start codon in *APS*.

No.	Position	Sequence	No.	Position	Sequence
1	1617 (in Intron 3)	G/T	12	1807 (in Intron 3)	G/T
2	1661 (in Intron 3)	G/A	13	1810 (in Intron 3)	T/C
3	1691 (in Intron 3)	G/T	14	2344 (in Intron 5)	T/C
4	1699 (in Intron 3)	G/A	15	2538 (in Exon 6)	G/A
5	1707 (in Intron 3)	A/C	16	2568 (in Exon 6)	C/T
6	1711 (in Intron 3)	G/A	17	2586 (in Intron 6)	C/T
7	1729 (in Intron 3)	T/G	18	2845 (in Exon 7)	C/T
8	1734 (in Intron 3)	T/G	19	3089 (in Intron 7)	T/C
9	1762 (in Intron 3)	G/A	20	3140 (in Intron 7)	T/C
10	1768 (in Intron 3)	G/A	21	3421 (in Intron 8)	A/G
11	1798 (in Intron 3)	T/A			

表 8、臺灣檜木杜松烯合成酶基因於隱子之單一核苷酸多型性序列

Table 8. Single nucleotide polymorphisms (SNP) present in introns of the cypress Cadinene Synthase (CAS) gene. Positions refer to the genomic sequence of CAS for *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2). The first nucleotide is the A of start codon in CAS.

No.	Position	Sequence (Y/R)	No.	Position	Sequence (Y/R)
1	174 (in Intron 1)	C/T	14	826 (in Intron 3)	G/T
2	176 (in Intron 1)	A/C	15	1247 (in Intron 3)	C/T
3	180 (in Intron 1)	C/T	16	1365 (in Intron 3)	A/G
4	181 (in Intron 1)	T/A	17	1426 (in Intron 3)	T/A
5	183 (in Intron 1)	A/T	18	1483 (in Intron 4)	C/T
6	186 (in Intron 1)	A/T	19	1484 (in Intron 4)	T/C
7	202 (in Intron 1)	T/C	20	1521 (in Intron 4)	G/T
8	558 (in Intron 2)	A/G	21	1551 (in Intron 4)	T/C
9	571 (in Intron 2)	C/T	22	1577 (in Intron 4)	G/A
10	771 (in Intron 3)	C/G	23	1750 (in Intron 5)	C/A
11	804 (in Intron 3)	T/A	24	2061 (in Intron 6)	G/T
12	823 (in Intron 3)	A/G	25	2419 (in Intron 7)	C/T
13	824 (in Intron 3)	T/C	26	2673 (in Intron 8)	G/A

表 9、臺灣檜木杜松烯合成酶基因於顯子之單一核苷酸多型性序列

Table 9. Single nucleotide polymorphisms (SNP) present in exons of the cypress cadinene synthase (*CAS*) gene. Positions refer to the genomic sequence of *CAS* for *Chamaecyparis obtusa* Sieb. & Zucc. var. *formosana* (Hayata) Rehder (YH60/2). The first nucleotide is the A of start codon in *CAS*.

No.	Position	Sequence (Y/R)	Amino acid*
1	41 (in Exon 1)	C/A	Ala/Asp
2	876 (in Exon 4)	A/C	Ile/Leu
3	880 (in Exon 4)	A/G	Glu/Gly
4	1032 (in Exon 4)	A/G	Met/Val
5	1710 (in Exon 5)	G/C	Asp/His
6	2158 (in Exon 7)	C/A	Thr
7	2227 (in Exon 7)	A/T	Leu/Phe
8	2239 (in Exon 7)	G/A	Glu
9	2240 (in Exon 7)	C/T	Leu/Ile
10	2279 (in Exon 7)	C/A	Leu
11	2518 (in Exon 8)	A/G	Ala
12	2595 (in Exon 8)	C/G	Thr/Ser
13	2816 (in Exon 9)	C/T	Ser
14	2884 (in Exon 9)	A/G	Asp/Gly
15	3206 (in Exon 10)	G/A	Arg

\*- Polymorphisms were affecting amino acids in the *CAS* sequence.



## 伍、討論



### 一、不同部位及木材深度之臺灣檜木 DNA 抽取量變化

木材的非法盜採及貿易破壞森林資源，不肖業者砍伐珍貴木材並製成加工品。臺灣檜木盜伐案件中之檢體，為木材或其加工製品；伏等 (2013) 指出木材 DNA 不完整，很多分子標誌在植物葉片抽取的 DNA 基礎上篩選出來，於木材 DNA 中可能不適用。故本論文以葉片 DNA 建立分子標誌的同時，也進行木材 DNA 的抽取，並利用木材 DNA 測試開發之分子標誌，確定其可應用於臺灣檜木木材之材種鑑定。不論是生立木或枯立木皆可順利抽得 DNA (圖 3)，其中生立木形成層最多，邊材次之，心材最少 (圖 3)，同 Lee 等人 (2012) 之結果。內層樹皮，韌皮部及形成層為活組織，邊材則包含活的以及死的組織 (Forest Products Laboratory, 1999)，在心材的部分皆為死細胞，但有些短片段的 DNA 仍附著於細胞壁上 (Cano, 1996)。枯立木則以第一、二深度 DNA 抽取量較高，於邊材與心材抽到少量或抽不到 DNA (圖 3) 與 Lee 等 (2012) 抽取砍下後存放約一年的木材 DNA 結果相似，於研究中僅形成層可抽出 DNA。白嶺神木 (RT98/1) 雖為生立木，但所抽取之 DNA 含量少 (圖 3A)，因其生長超過兩千年，推測採集之部分細胞可能已死亡。

### 二、臺灣檜木之基因序列分析

本論文選殖得到的臺灣扁柏 *CCoAOMT* 與林等 (2008) 選殖到的 cDNA，解碼區 (coding sequence region; CDS) 之序列長度不同，後者較前者於多了 87 個鹼基對、並分別轉譯出 249 及 278 個胺基酸 (圖 5)，本論文選殖得到的紅檜 *APS* 之 CDS 則較 Chu 等 (2009) 從紅檜選殖出的基因多了 3 個鹼基對 (圖 8)。本論文選殖得到的紅檜 *CAS* 與 Kuo 等 (2012) 選殖出的 cDNA 於 CDS 具有核苷酸序列差異。本論文之試驗材料取自羅東林區，而林等 (2008)、Chu 等 (2009) 及 Kuo 等 (2012) 之試驗材料皆取自臺灣大學實驗林溪頭營林區，可能因為地理因素影響遺傳分化 (Liu et al., 2014) 造成 *CCoAOMT*、*APS* 及 *CAS* 之 CDS 於採自羅東及溪頭的臺灣檜木中具有序列差異。目前已選殖到的 *CCoAOMT* 轉譯之胺基酸序列，

於外表性狀具有明顯差別之裸子植物與被子植物中呈高度相似 (圖 5)，在結構上均具有類似之催化構造 SAM (S-adenosyl-L-methionine) binding motif，與 SAM 結合，提供甲基轉移酶進行催化反應時所需的 methyl group，以進行甲基轉移之反應 (Ferrer et al., 2005 ; Ibdah et al., 2003)。



### 三、臺灣檜木之分子標誌分析

能顯現生物個體或群體間基因組中某種差異特徵的 DNA 片段統稱為 DNA 分子標誌 (周, 2005)，廣泛存在於基因組 DNA 各區域，並可透過電泳圖譜或定序分析區別不同基因型個體，其中以電泳圖譜檢測較為快速，定序分析則可確認多型性序列。本論文開發之 OMT/MF-1 與 OMT/MR-1 引子進行 PCR，於葉片樣品 (圖 12) 及木材樣品 (圖 13) 可利用電泳圖譜中約 1.4 kb 條帶區別同屬之臺灣扁柏及紅檜。以另一組開發之 CAS5 與 CAS/MR-2 引子，分析葉片樣品 (圖 14) 及木材樣品 (圖 17)，亦可根據電泳圖譜中條帶數目區分臺灣檜木兩物種。Park 等 (2011) 同樣利用電泳圖譜分析根據 *4-CL*、*CCoAOMT* 多型性序列設計之酶切擴增多型性序列 (cleaved amplified polymorphism sequences; CAPS)，成功區別甘蔗屬、芒屬、蔗茅屬、巴西白茅、河八王之不同屬的甘蔗。

大部分之 DNA 分子標誌，可透過電泳圖譜區分不同基因型。王等 (2007) 分析 30 個安石榴株系之 RAPD，可由 6 個引子得到 22 個多型性片段，其中由新加坡引進之品系於電泳圖譜皆擁有一個特殊片段。溫和許 (2003) 利用 RAPD 技術對桃的 29 個品種進行遺傳相似度分析，以 Operon kit 220 條隨機引子進行篩選，35 個引子於試驗樣品具有多型性，其中 OPAA3 引子進行 PCR 後，於電泳圖譜產生 705 bp 片段可鑑別雞珍桃品種。RAPD 具有不必預先知道 DNA 序列，操作簡便的優點 (周, 2005)，為早期最普及之技術，但為多條帶圖譜，部分 DNA 片段在增幅時具有較強的競爭引子與聚合酶的能力，因而會影響其他序列 (胡, 2013)，且為顯性標誌，無法分辨異型合子 (heterozygote) 及同型合子 (homozygote)。由於引子短在 PCR 反應時與模板 DNA 結合可能不完全互補，複製增殖得到的 DNA 標記在重複試驗中的再現性不佳，故 RAPD 的準確性容易受到影響。

SSR 使用的引子為根據兩側保守序列設計，在同種而不同個體間大部分相同 (周, 2005)，具有專一性及再現性均高的優點，與 RAPD 相比，準確性高，為目前

常用的 DNA 分子標誌之一。細胞核之微衛星標記 (nuclear simple sequence repeat; nSSR) 的遺傳，已用於鑑定葡萄品種親緣關係 (Lacombe et al., 2013)。利用 SSR 標記，找出 145 個不同的等位基因，顯示桃花心木族群間遺傳分化程度強，且遺傳與空間分布有明顯相關性，並根據多位點之基因型分析，已確定兩組桃花心木木材的來源地 (Degen et al., 2013)。Park 等 (2011) 於 *CCoAOMT* 同源基因找到 SSR，其中 *Saccharum L.*、*Erianthus Michx.* SSR 之不同重複次數範圍較廣，可運用於 *Saccharum L.*、*Erianthus Michx.* 屬內不同種間之鑑別。SSR 為共顯性標誌，可將同型合子和異型合子之條帶加以區分。但此技術的引子開發需要知道 SSR 兩端的鹼基序列，並非每一種物種均有此資訊可提供。

本論文開發之臺灣檜木分子標誌，期望運用在未來臺灣檜木盜伐案中，故要選擇準確性高且客觀之分子標誌進行研究分析。有別於再現性不佳之 RAPD，SSR、SNP 及 Indel 準確性高，而目前臺灣檜木於 SSR 兩端的鹼基序列相關訊息少，欲開發臺灣檜木之 SSR 所需的時間成本高。同樣為高準確性的 SNP 與 Indel 是直接比對不同個體間的 DNA 序列變異、提供客觀而明確的 DNA 資料，可隨時分析未知樣品 (胡等，2013)，故本研究利用 SNP 及 Indel 進行臺灣檜木分子標誌之開發。


本論文於臺灣檜木 *CCoAOMT* 及 *CAS* 基因發現之 SNP 可用以鑑別同屬不同種之紅檜及臺灣扁柏。與 Germano 等 (1999) 利用 SNP 技術成功區分在型態上難以區分的紅雲杉 (*Picea rubens* Sarg.)、黑雲杉 (*P. mariana* Britton, Stems & Poggenb.) 和白雲杉 (*P. glauca* Voss) 等三個近緣種結果相同，說明可用 SNP 鑑別同屬之樹種。Tsumura 等人 (2011) 發展葉綠體 DNA 資料庫以鑑別娑羅屬物種 (*Shorea* Roxb. ex C.F.Gaertn. species)，透過 DNA 序列及解剖數據 (anatomical data) 推測木材樣品可能所屬之物種。擴增稜柱木屬 (*Gonystylus* Teijsm. & Binn.) *matK*、*ropC1*、*ropB*、*accD* 和 *ndhJ* 等 5 個葉綠體基因片段，發現多個 SNP，根據該屬 7 個種共有的 SNP 設計引子，可用以區分稜柱木屬與其他屬的木材 (Ogden et al., 2008)。SNP 不僅可用於鑑別屬間及種間物種，亦可運用於個體鑑別。本論文於臺灣檜木 *CCoAOMT*、*APS*、*CAS* 基因發現之 SNP，在某些個體中，擁有獨特基因型，具有個體差異，可作為個體鑑定之依據。陳等人 (2013) 針對臺灣稜型水稻品種進行隨機型 SNP 分子標誌資料庫的初步建立，挑選在基因體組中均勻分布的 123 個 SNP 分子標誌，於 460 個樣品中具有多型性，由此可知 SNP 分子標誌鑑別不同個體的可行性。SNP 廣泛存在於基因組中，大部分同時存在於等位基因 (biallelic gene)

(Cabezas et al., 2011)，且為共顯性標誌，但須透過定序分析，所需時間較多，隨著定序技術的發展，有望縮短分析時間。

本論文利用臺灣檜木 CAS 存在的 Indel，開發可鑑別紅檜與臺灣扁柏之分子標誌。與 Paiva 等 (2011) 試驗結果相同，該試驗利用存在於大桉樹及銀葉桉 CCR 啟動子 (promoter) 的插入片段及 CAD2 第一個隱子的缺失片段，設計引子進行 PCR 分析，依據產物大小區分大桉樹及銀葉桉，說明可根據基因序列的 Indel，發展鑑別不同物種之分子標誌。相較於 SNP，較長片段之 Indel，可利用電泳圖譜進行快速檢測。

由於樹木是多年生植物，基因漂移，雜交等多種因素使得種群間存在很高的遺傳多樣性 (Austerlitz et al., 2000)。針葉樹木為異交作物，遺傳歧異度高，加上臺灣檜木為多年生植物，長時間受生長環境影響，可能造成 DNA 序列的差異。以 PCR/RFLP 及核苷酸序列分析位於棲蘭山和阿里山之紅檜與臺灣扁柏葉綠體基因組之多型性，經由 PCR 擴增出 *petG-trnP* 間之非譯碼區，經 DNA 定序可鑑別紅檜及臺灣扁柏；*trnV-trnM* 基因間區間序列在紅檜與臺灣扁柏之間有兩個 *HinfI* 酶切位置之差異，在 *petG-trnP* 基因間區間序列也發現臺灣扁柏與紅檜之間有限制片段長度及核苷酸多型性，但並無個體或族群之差異，顯示紅檜與臺灣扁柏於棲蘭山及阿里山兩族群間與族群內葉綠體 DNA 變異極小 (黃等，2000)。Matsumoto 等 (2006) 及 Nakao 等 (2001)，分析日本扁柏 (*Chamaecyparis obtusa* Siebold & Zucc.) 微衛星 DNA，開發數個高多型性 SSR 分子標誌，黃等 (2007) 利用此分子標誌，分析取自臺灣中央山脈及玉山山脈之紅檜，結果顯示紅檜不同個體擁有獨特之基因型，但在地域的部分，無法僅用單一分子標誌進行所有紅檜之來源地識別，且因同一地域分析之樣品少於 5 個，無法代表整個族群。


本論文利用臺灣檜木葉片 DNA，針對 *CCoAOMT*、CAS 多型性序列開發之分子標誌，以木材 DNA 進行測試，於 *OMT-M1* (圖 13)、*CAS-C5R* (圖 17) 及 *CAS-CM1* (圖 22) 均能成功區分同屬不同種之紅檜與臺灣扁柏，可應用在臺灣檜木木材材種之鑑別。臺灣檜木 *CCoAOMT* (圖 )、*APS* (圖 9)、*CAS* (圖 11) 基因經定序分析後，發現數個 SNP，並於某些個體擁有獨特基因型；除此之外，開發之 *CAS-C5R* (圖 16) 及 *CAS-CM1* 分子標誌，經序列分析後，同樣具有個體差異，可作為個體鑑定之依據。目前開發之三組分子標誌，僅能鑑別臺灣檜木物種及部分個體，無法達到地域識別，未能分辨採集自羅東林區的臺灣檜木，是屬於羅東林區哪個林



班。雖然無法進行小範圍的地域識別，無法區分採自不同林班之臺灣檜木，但目前於羅東林區及溪頭林區之臺灣檜木中，已發現具有地域性差異之多型性序列。本論文從羅東林區臺灣檜木中選殖的 *CCoAOMT*、*APS*、*CAS* 其 CDS 序列與選殖自臺大實驗林溪頭營林區臺灣檜木之已登錄 CDS 序列具有 SNP 及 Indel 差別，此部分可再進行研究，增加溪頭林區之臺灣檜木樣品數並進一步分析，有望開發為地域性分子標誌，分辨羅東及溪頭林區之臺灣檜木。本論文開發之分子標誌，不適用於小範圍的地域識別，無法區分採自羅東林區之臺灣檜木分別屬於哪個林班。可能與採集的地域範圍較小有關，不同林班間之臺灣檜木其遺傳分化小，基因中存在之差異序列少，甚至於遺傳差別，與 Liu (1966) 指出風媒物種紅檜與臺灣扁柏在臺灣之分布為連續性，因此種內族群之基因交流應甚為頻繁之推論相似；且根據 Lin 等 (1994) 應用同功酶之分析，探討紅檜與臺灣扁柏群分化之結果顯示，紅檜與臺灣扁柏族群間核 DNA 分化程度較低。另一可能原因推測為本試驗分析之三個基因其多型性序列，不足以區別取自 10 個不同林班之族群。

本論文成功於枯立木中抽取出 DNA 且開發之三組分子標誌，經木材 DNA 測試後，可成功運用於臺灣檜木木材材種鑑定，及部分個體之鑑別。此三組分子標誌，於未來臺灣檜木盜採案件中提供相關科學數據，進行物種鑑別，此方法操作簡便且快速，並提供客觀而明確的 DNA 資料，可減少根據外觀特性判定所造成之誤差，期望有助於遏阻臺灣檜木之非法砍伐及非法貿易。

## 陸、結語

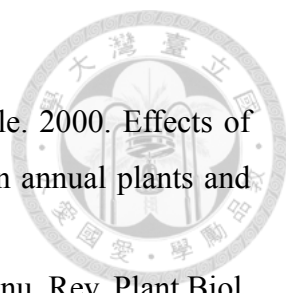


本論文開發之三組分子標誌，經木材 DNA 測試後，可用於臺灣檜木木材材種鑑定，且可進行部分個體之鑑別；除此之外，亦成功於枯立木中抽取出 DNA。針對臺灣檜木基因之 SNP 及 Indel，發展分子標誌，直接比對不同個體間的 DNA 序列變異、可提供客觀而明確的 DNA 資料。雖然三組分子標誌無法區別取自羅東林區的臺灣檜木屬於何個林班，但羅東林區的臺灣檜木與溪頭林區的臺灣檜木於 *CCoAOMT*、*APS*、*CAS* 已具有序列差異，可否作為地域性分子標誌，區別溪頭、羅東等地域分佈差距較大的臺灣檜木，仍需更進一步研究。本論文開發之 *CAS-C5R* 及 *CAS-CM1* 分子標誌，經定序分析後各具有兩種序列型態。其中臺灣扁柏於 *CAS-C5R* 除了具有與紅檜相同的序列，另有其特異序列；相反地，於 *CAS-CM1* 則是紅檜具有兩種序列型態。此部分可再進行研究，各自根據其兩種序列型態之差異處，設計臺灣扁柏與紅檜專一性引子，開發臺灣檜木物種鑑別之分子標誌。根據 DNA 的多型性，發展分子標誌，可運用於臺灣檜木木材材種及來源地的鑑別，於未來臺灣檜木盜採案件中，提供相關科學數據以供判斷分析及鑑定，期望有助於遏阻臺灣檜木木材之非法砍伐及貿易。

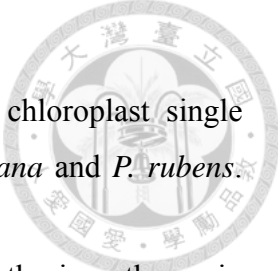
## 柒、參考文獻

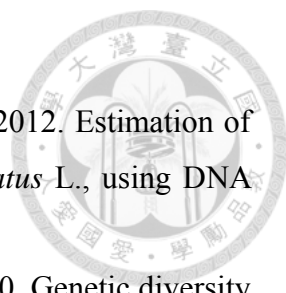


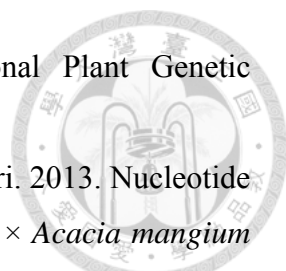
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