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Gene Investigation into the Inner bark of Taiwania (*Taiwania cryptomerioides*)

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ABSTRACT. Taiwania (*Taiwania cryptomerioides* Hayata) is a conifer tree indigenous to Taiwan and one of the most economically important forest tree species on the island. More than 100 secondary metabolites have been isolated from this species. Essential oils and extracts from Taiwania possess many bioactivities, including antibacterial, antifungal, antitermite, antimite, antioxidiant and antitumor activities. In order to research those genes involved in biochemical synthesis and wood formation, we constructed a cDNA library from the inner bark of Taiwania. Using single-pass sequencing of cDNA clones, 973 expressed sequence tags (ESTs) were generated. A BLASTX search revealed that ESTs related to cell rescue, defense and cell aging were abundant in the inner bark library, especially genes that respond to pathogen infection. In addition, homology analysis revealed that ESTs related to cell wall structure and secondary metabolism represented about 2.3% of the clones. However, 57% of the ESTs from Taiwania showed no significant similarity to any other protein sequences in the public databases. These sequences indicate the uniqueness of Taiwania, and its consequently remarkable value.

Keywords: Expressed SequenceTags (ESTs); Inner bark; Taiwania (*Taiwania cryptomerioides* Hayata).

INTRODUCTION

Taiwania (*Taiwania cryptomerioides* Hayata) is a native species of Taiwan. Along with *Ginkgo biloba*, *Sequoiadendron giganteum* and *Metasequoia glyptostroboides,* Taiwania is a relict from the Tertiary period of the Cenozoic era. It is indigenous to the central part of Taiwan and lives at altitudes of 1,800~2,600 meters above sea level. It has been planted and flourished at heights as low as 800 meters. Taiwania is an important species economically as it provides good quality wood for construction. The wood of Taiwania has good properties, being able to withstand harsh weather, bacteria and termites. It is durable enough for long-term use (Wang et al., 1997). Large volumes of Taiwania bark residues are processed by the forest industry. The bark can be converted into fuel, chemical materials, and wood charcoal for various uses. Experiments on the chemical compounds found in Taiwania and other conifers have been conducted for years (Chang et al., 2000a).

Many researches have proved that the bioactivity of wood has a correlation with the extractives of wood. Essential oils and extractives from Taiwania heartwood, sapwood, and leaves have antibacterial, antifungal, antitermite, and antimite properties (Chang et al., 2000a). Additionally, lignans isolated from the heartwood of Taiwania have been shown to have excellent cytotoxicity against different tumor cell lines (Chang et al., 2000b). More than a hundred secondary metabolites—including terpenoids, lignans, isoflavones, and other compounds—have been isolated from this species over the past 70 years (Chang et al., 2003). The secondary metabolites of woody plants make the trees resistant to natural stresseslike drought and insects (Barnes et al., 1998). To develop a better understanding of the biochemistry of this tree, it is necessary to investigate the genes and/or enzymes, which participate in its physiological functions. Inner bark consists of sieve elements. These delicate cells are short-lived and undergo partial autolysis upon maturation, resulting in cell nuclear degradation, which is related to aging and cell death (Pia and Mart, 1998; Raver et al., 1999). Therefore, the corresponding genes are expected to be investigated in Taiwania.

A rapidly growing area of genome research is the generation of expressed sequenced tags (ESTs) in which large numbers of randomly selected cDNA clones are partially sequenced. Among forest trees, poplar (Sterky et al., 1998), loblolly pine (Allona et al., 1998), and sugi (Ujino et al., 2000) have been studied using EST analysis. cDNA libraries for these species have been constructed from wood-forming tissues, xylem and inner bark, in the expectation that the libraries would include information sequences related to secondary compounds and wood

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formation. The cDNA library of Taiwania seedling has been constructed (Chen et al., 2004). In order to collect information about cell-wall formation, secondary metabolism, defense affection and the aging of Taiwania, here we report the result of expression analysis of ESTs derived from cDNA clones isolated from the inner bark of Taiwania. The genes associated with differential expression and metabolite information will provide useful information for research on tree physiology, evolution and drug discovery.

MATERIALS AND METHODS

Plant material and cDNA library construction

The inner bark sample of a 25-year-old Taiwania was obtained from the Heshe Tract of the Experimental Forest of National Taiwan University. Total RNA for cDNA library construction was prepared following the method of Chang et al. (1993). Poly $(A)^+$ mRNA isolation was carried out with Qiagen mRNA spin-column Mini Preparation (Qiagen). First-strand cDNA was synthesized using SuperScript™ II Reverse Transcriptase (Invitrogen), and it was then converted to double-strand cDNA using PCR Plus Master Mix Kit (GeneMark). The resulting cDNA was cloned into the pGEM-T Easy vector system (Promega) following the protocol. After ligation, the mixture was used for transformation into *Escherichia coli* DH5α competent cells (Hopegen) and then plated on the Luria Bertani (LB)/ ampicillin agar plates containing IPTG and X-gal.

DNA sequencing

The white bacterial colonies were picked into 1 ml of LB broth containing ampicillin and grown for 16 h with shaking in deep 2 ml 96-well plates at 37°C. 200 µl was then removed from each well and conserved with 200 µl glycerol at -80°C for further use. Plasmid DNAs were then purified from overnight cultures with a Plasmid Miniprep Purification Kit (GeneMark). Size of insert was confirmed by PCR using vector primers. Sequencing was carried out with an ABI 377 automatic sequencer (Perkin Elmer) using T7 sequencing primers. A partial sequence from the 5′ end of each clone was obtained. These plasmids were also stored at -80°C for further use.

Sequence processing and analysis

Less than 200 bp of the nucleotide sequences were excluded, and the remaining ones were subjected to data analysis. The leading vector and poor-quality sequences were removed by either software Chromas or manually. These ESTs, after moving out poly A and poly T, were assembled into longer sequence contigs using the ContigExpress program of Vector NTI Suite 8 (InforMax, Inc.) with pairwise assembly (Huang, 1992). Two sequences with 20 bp overlapping and an identity of 0.9 would be assemble into a cluster. Others with no sequences overlapping were singleton. Translated ESTs were compared to the nonredundant (nr) database at the National Center for Biotechnology Information (NCBI) using the BLASTX algorithm (Altschul et al., 1997). In addition, individual cDNA sequences were also compared with NCBI databases using BLASTN. The databases published in the Munich Information Center for Protein Sequences (MIPS) (http://mips.gsf.de/) and with the aid of the Gene Ontology Consortium (http://www.geneontology.org) were used to identify and classify the functions of genes.

RESULTS AND DISCUSSION

Sequencing and assembly

The cDNA library of Taiwania inner bark had insert sizes of 253-964 nucleotides. Initially, a total of 1193 cDNA clones were randomly selected, sequenced, and stored at -80°C. After elimination of vector and unreadable sequences, a total of 973 high-quality clones were submitted to dbEST (DN975112-DN976084). The average read-length after vector and quality clipping was 533 nucleotides. These ESTs with at least 200 bp of high-quality sequences were assembled using Vector NTI Suit8. A total of 144 clusters were formed after assembly of 814 ESTs while 159 sequences remained as singleton ESTs, not identical to any other EST in the data set. As a result of contig analysis, we obtained a total of 303 unigene sets.

cDNA library characterization

The ESTs we obtained were compared with the nr (all non-redundant GenBank CDS translations+PDB+Swissprot+PIR+PRF) database using the BlastX algorithm. The database sequence matches were classified as either a hit (E-value $< 10^{-10}$) or no hit (E-value $\geq 10^{-10}$) (Chen et al., 2004). In addition, to minimize the rare false-positive, we chose a stringency level of > 210 for BLASX scores for filtering low-complexity sequences. BLASTX search revealed that 559 (57%) ESTs were classified as no hits, and the other 414 (43%) ESTs showed significant similarity to protein sequences described in the nr database. In the no hit category, 110 (19.6%) ESTs were singleton, and the remaining 449 (80.4%) ESTs were cluster ESTs.

The highly expressed transcripts of this cDNA library are listed in Table 1. Two clusters of abundant transcripts showed high levels of similarity to two genes related to plant defense; thaumatin-like protein (**CAA10492**) from the species of Douglas Fir (*Pseudotsuga menziesii*), and erg-1 from potato (*Solanum tuberosum*) (**AAP42136**), containing 76 and 22 ESTs, respectively. These two genes are all a result of the defense response of plant to wounding and pathogen attack (Piggott et al., 2004; Dellagi et al., 2000). However, these results differ from the ESTs derived from the inner bark of sugi with cold acclimation protein abundantly (Ujino et al., 2000).

According to their original species of putative protein matched with our sequences, these species were classified into five different organism groups which are shown in Table 2. 110 (26.5%) of the ESTs of Taiwania inner bark

Table 1. Abundant ESTs found in the inner bark cDNA library of Taiwania.

Putative function	Species	Accession number	Number of ESTs[a]	Classification
NtPRp27-like protein	*Atropa belladonna*	CAC40754	6	Cell rescue, defense and cell aging
Granule-bound starch synthase I, chloroplast precursor	*Arabidopsis thaliana*	AAN31102	6	Primary metabolism
B1056G08 13 gene product	*Oryza sativa* (japonica cultivar-group)	XP_507392	6	Protein synthesis and processing
Methionine aminopeptidase	*Arabidopsis thaliana*	AAM61284	6	Protein synthesis and processing
Ribosomal protein L8, cytosolic	*Lycopersicon esculentum*	R5TOL8	6	Protein synthesis and processing
60S ribosomal protein L24	*Oryza sativa* (japonica cultivar-group)	XP_475453	9	Protein synthesis and processing
Glycine-rich RNA-binding protein	*Picea glauca*	AAD28176	11	Transcription
Ribosomal protein L13a	*Oryza sativa* (japonica cultivar-group)	AAR01683	15	Protein synthesis and processing
Erg-1	*Solanum tuberosum*	AAP42136	22	Cell rescue, defense and cell aging
Thaumatin-like protein	*Pseudotsuga menziesii*	CAA10492	74	Cell rescue, defense and cell aging

[a]Total number of clones with the same putative protein.

Table 2. BLASTX analysis of ESTs from inner bark of Taiwania related to the original species of putative protein.

Assortment of species		Number of ESTs[a]	Example of species
Non-plant	Fungi and bacteria	9	*Schizosaccharomyces pombe, Kluyveromyces lactis*
	Animal	4	*Danio rerio, Homo sapiens, Gallus gallus*
Plant	Softwood	110	*Cryptomeria japonica, Picea glauca*
	Hardwoood	7	*Populus tremula x Populus tremuloides, Mangifera indica*
	Herb and lower plant	284	*Physcomitrella patens, Solanum tuberosum*
Total ESTs		414	

[a]Number of ESTs which were grouped by sources.

were matched with the conifers, including pine tree and fir. In addition, *Cryptomeria japonica* and Taiwania are classified to the same family. We found two clusters of ESTs that showed high levels of similarity to genes in *C. japonica* which were related to defense response protein in the databases, including putative class I chitinase and Thaumatin-like protein. Consequences of these ESTs for their putative proteins were matched with the model plants, which have already been sequenced completely, such as *Arabidopsis thaliana* and *Oryza sativa*, with the highest amount of 102 (25%) and 83 (20%), respectively. However, 13 EST sequences were similar to animal or bacteria proteins, with reliable E-values (1.0×10^{-15} to 5.0×10^{-53}) and scores (239~643), indicating these proteins had not been found in plant yet and maybe highly conserved in all organization.

Classification according to putative function

The 973 ESTs from the cDNA library of Taiwania inner bark were classified into 14 distinct groups based on their putative protein function as matched with the NCBI database as shown in Figure 1 (Sterky et al., 1998). 57% of ESTs were classed into the no hit category, that is, not significantly similar to any protein in the database of NCBI; 51 ESTs (5%) with reliable E-value (E-value < 10^{-10}) had significant homology with proteins of which the

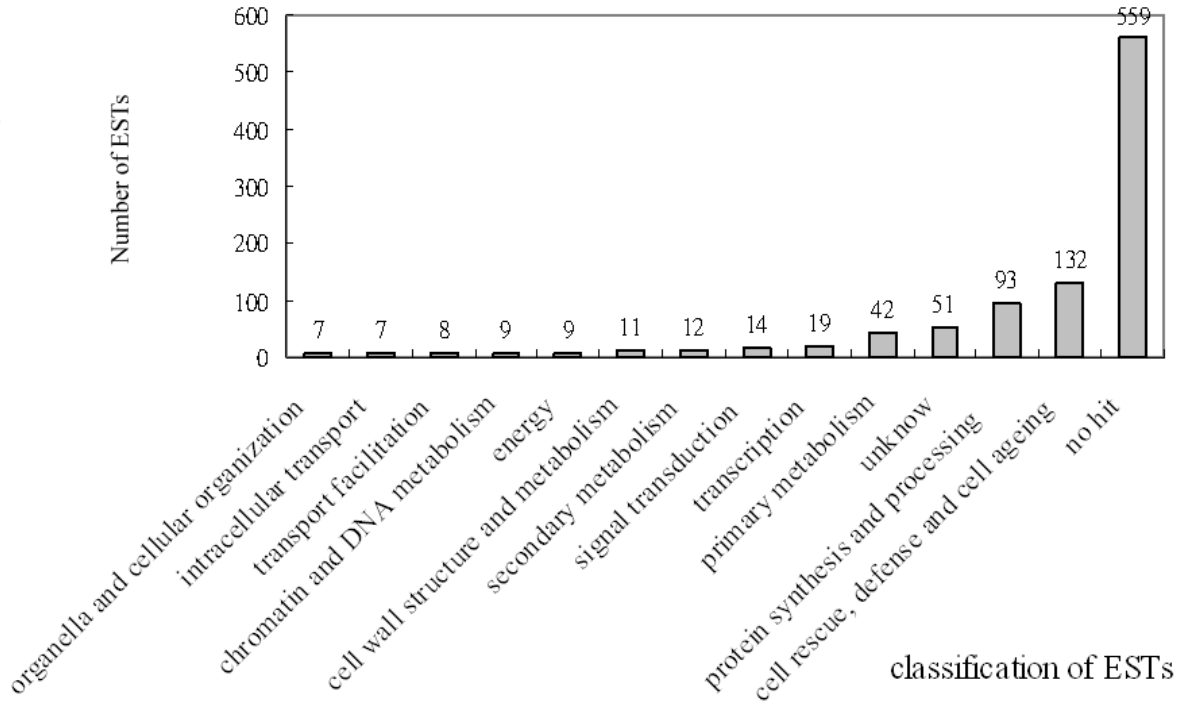

Figure 1. Functional classification of inner bark tissue ESTs from *Taiwania cryptomerioides*. ESTs with BLASTX *E*-value < 10^{-10} were classified into 12 functional categories: organelle and cellular organization, intracellular transport, transport facilitation, chromatin and DNA metabolism, energy, cell wall structure and metabolism, secondary metabolism, signal transduction, transcription, primary metabolism, protein synthesis and processing, and cell rescue, defense, andaging; Unknown: sequences similar to known sequences of uncharacterized function, and No hit: BLASTX *E*-value ≥10^{-10} and no sequence similarity to known amino acid sequences.

functions are unknown. The remaining ESTs that were matched with the database were classified into 12 groups as follows, based on their putative function; cell rescue, defense and cell aging (13.6% of total ESTs), protein synthesis and processing (9.6%), primary metabolism (4.3%), transcription (2.0%), signal transduction (1.4%), secondary metabolism (1.2%), cell wall structure and metabolism (1.1%), energy (0.9%), chromatin and DNA metabolism (0.8%), intracellular transport (0.7%), transport facilitation (0.8%), and organelle and cellular organization (0.7%).

A high percentage of expressed genes were related to cell rescue, defense, death and aging, a situation mirrored by ESTs from the sugi inner bark library. However, the varieties of the genes were not the same. Genes putatively related to this category are shown in Table 3. 132 ESTs (13.6% of the total ESTs) were related to the cell rescue, defense, and aging category. Table 3 shows ESTs of different putative proteins in the category of cell rescue, defense and aging. Thaumatin-like protein (TLP) (**CAA10492**) was the most abundant EST in this category. It is a pathogen-related protein that is a member of the PR-5 family. In infected western white pine (*Pinus monticola* D. Don) needles, thaumatin-like protein was locally induced in response to invasions of blister rust pathogen *Cronartium ribicola*. In addition, this protein was also induced by both wounding and methyl jasmonate treatments (Piggott et al., 2004). Erg-1 (**AAP42136**) was also highly expressed in the inner bark of Taiwania. In *Solanum tuberosum*, erg-1 is rapidly induced by *Erwinia carotovora* ssp. *Atroseptica*, *Phytophthora infestans*, ethylene, and salicylic acid (Dellagi et al., 2000). The presence of erg-1 and thaumatin-like proteins in the inner bark tissue suggests the general roles of these proteins in adaptation to stress and would provide a defensive response to wounding and pathogen attack. Class I chitinase was also found in the Taiwania inner bark cDNA library. Five ESTs resembled class I chitinase (**BAD02582**) from *Cryptomeria japonica*. Class I chitinase is involved in cell wall catabolism, chitin catabolism, and response to pests, pathogens and parasites. Class I chitinase isolated from the bark of Norway spruce (*Picea abies*) is induced by *Heterobasidion annosum* (Hietala et al., 2004). Three ESTs in this category were similar to cystatin (**ALL79831**), a competitive inhibitor of C1 cysteine. In plants, cysteine protease inhibitors are involved in the regulation of protein turnover and play an important role in defense against insect predation and pathogens. It has been proven that cysteine protease inhibitors can suppress hypersensitive cell death in plant cells (Belenghi et al., 2003). In addition, two ESTs from this group were similar to ubiquitin from potato and wood tobacco (**S42643** and **S28420**). In plants, the ubiquitin/proteasome pathway

Table 3. ESTs of Taiwania inner bark cDNA related to cell rescue, defense and cell aging.

Putative identification	Species	Accession number	No. of ESTs	Assortment of species
Catalase	*Glycine max*	CAA78056	1	Herb and lower plant
Cystatin	*Sandersonia aurantiaca*	AAL79831	1	Herb and lower plant
Cysteine proteinase inhibitor	*Glycine max*	T07139	1	Herb and lower plant
Cysteine proteinase inhibitor	*Arabidopsis thaliana*	AAM63160	1	Herb and lower plant
Leaf senescence-associated protein (SAG101)	*Arabidopsis thaliana*	NP_568307	1	Herb and lower plant
NOI protein	*Oryza sativa* (japonica cultivar-group)	XP_450305	1	Herb and lower plant
Phi-1-like protein	*Arabidopsis thaliana*	AAM65190	1	Herb and lower plant
Chitinase precursor	*Oryza sativa* (japonica cultivar-group)	XP_507595	1	Herb and lower plant
Nitrate-induced NOI protein	*Arabidopsis thaliana*	AAM20305	1	Herb and lower plant
NUDIX hydrolase	*Arabidopsis thaliana* (thale cress)	BAB02251	1	Herb and lower plant
Salt tolerance protein 2	*Beta vulgaris*	CAC85228	1	Herb and lower plant
Ubiquitin / ribosomal protein CEP52	*Nicotiana sylvestris*	S28420	1	Herb and lower plant
Ubiquitin / ribosomal protein S27a	*Solanum tuberosum*	S42643	1	Herb and lower plant
ER6 protein	*Lycopersicon esculentum*	AAD46412	2	Herb and lower plant
Hypersensitive-induced response protein	*Arabidopsis thaliana*	AAM63689	2	Herb and lower plant
Salt tolerance protein 3	*Beta vulgaris*	CAC85244	2	Herb and lower plant
Thaumatin-like protein	*Cryptomeria japonica*	BAC15615	3	Softwood
Thaumatin-like protein	*Cryptomeria japonica*	BAC15616	3	Softwood
Class I chitinase	*Cryptomeria japonica*	BAD02582	5	Softwood
NtPRp27-like protein	*Atropa belladonna*	CAC40754	6	Herb and lower plant
Erg-1	*Solanum tuberosum*	AAP42136	22	Herb and lower plant
Thaumatin-like protein	*Pseudotsuga menziesii*	CAA10492	74	Softwood

has been found to be involved in the cell cycle in some plants and in various signal transduction pathways, including auxin signaling, photomorphogenesis, and jasmonic acid signaling (Callis and Vierstra, 2000). Finally, one EST was similar to a leaf senescence-associated protein (SAG101) from *Arabidopsis thaliana* in the database (**NP_568307**). This protein can be classified as either related to lipid metabolism or aging (He and Gan, 2002). In Taiwania, this protein might be related to the death of sieve tubes and phloem maturation.

Based on the putative function classification, eight different kinds of protein (1.1% of the total ESTs) were related to cell wall structure and metabolism in the database (Table 4). Two ESTs were similar to cinnamoyl-CoA reductase from *Pinus taeda* (**AAL47684**), and one was similar to cinnamoyl-CoA reductase from *Populus balsamifera* subsp. *Trichocarpa* (**CAA12276**). Cinnamoyl-CoA reductase is an important reductive enzyme, involved in lignol biosynthesis (Aldwin et al., 2002). Expansin of *Glycine max* (**AAO15999**) was similar to one EST. It is a plant cell wall protein which induces extension of plant cell walls endogenously, as a cell-wall-loosening agent in *Arabidopsis thaliana* (Cho and Cosgrove, 2000). The remaining ESTs are the components of cell-wall-structure proteins.

Another category we are particularly interested in is secondary metabolism. Table 5 shows the twelve ESTs from Taiwania that are similar to nine different kinds of protein related to secondary metabolism in the database. One EST is similar to cytochrome P450 78A4 from *Pinus radiate* (**O65012**) and another to cytochrome P450 from *Panax ginseng* (**BAD15331**). Multifunctional cytochrome

Table 4. ESTs of Taiwania inner bark cDNA related to cell wall structure and metabolism.

Clone number	Putative identification	Accession number	Species	Assortment of species
CT04_65	Pectinerase protein	AAL09733	*Arabidopsis thaliana*	Herb and lower plant
CT04_79	Cinnamoyl-CoA reductase	AAL47684	*Pinus taeda*	Softwood
CT10_86	Cinnamoyl-CoA reductase	AAL47684	*Pinus taeda*	Softwood
CT06_83	Expansin	AAO15999	*Glycine max*	Herb and lower plant
CT10_38	Expansin-related protein 1 precursor	AAO64802	*Arabidopsis thaliana*	Herb and lower plant
CT08_73	Membrane-associated zinc metalloprotease	AAP31938	*Arabidopsis thaliana*	Herb and lower plant
CT10_46	Alpha-galactosidase	BAC66445	*Helianthus annuus*	Herb and lower plant
CT10_68	Alpha-galactosidase	BAC66445	*Helianthus annuus*	Herb and lower plant
CT11_82	Alpha-galactosidase	BAC66445	*Helianthus annuus*	Herb and lower plant
CT10_32	Cinnamoyl CoA reductase	CAA12276	*Populus balsamifera* subsp. *trichocarpa*	Hardwood
CT07_71	Glucan synthases	CAB81039	*Arabidopsis thaliana*	Herb and lower plant

Table 5. ESTs of Taiwania inner bark cDNA related to Secondary metabolism.

Clone number	Putative identification	Accession number	Species	Assortment of species
CT02_44	Oxidoreductase, 2OG-Fe(II) oxygenase family protein	AAN15625	*Arabidopsis thaliana*	Herb and lower plant
CT10_82	Oxidoreductase, 3OG-Fe(II) oxygenase family protein	AAN15625	*Arabidopsis thaliana*	Herb and lower plant
CT13_34	Ubiquinol--cytochrome-c reductase-like protein	AAN15706	*Arabidopsis thaliana*	Herb and lower plant
CT01_51	P450	BAB87820	*Triticum aestivum*	Herb and lower plant
CT03_42	Cytochrome P450	BAD15331	*Panax ginseng*	Herb and lower plant
CT01_79	Minor allergen (quinone reductase family protein)	CAB16805	*Arabidopsis thaliana*	Herb and lower plant
CT04_37	Minor allergen (quinone reductase family protein)	CAB16805	*Arabidopsis thaliana*	Herb and lower plant
CT04_91	Minor allergen (quinone reductase family protein)	CAB16805	*Arabidopsis thaliana*	Herb and lower plant
CT04_77	Cytochrome P450 78A4	O65012	*Pinus radiata*	Softwood
CT10_51	Cytochrome c oxidase polypeptide II	P93285	*mitochondrion Arabidopsis thaliana*	Herb and lower plant
CT10_79	Strictosidine synthase	XP_469768	*Oryza sativa* (japonica cultivar-group)	Herb and lower plant
CT13_87	Iron inhibited ABC transporter 2	XP_483817	*Oryza sativa* (japonica cultivar-group)	Herb and lower plant

P450 is involved in the biosynthesis of ginsenosides and other secondary metabolites, which were identified by using methyl jasmonate to treat ginseng hairy roots (Choi et al., 2005). P450 of *Triticum aestivum* (wheat) (**BAB87820**) catalyzes the oxidation of endogenous compounds (lauric and oleic acids) and of several herbicides (diclofop, chlortoluron, bentazon) in wheat seedlings (Forthoffer et al., 2001). Monocotyledonous crop plants are usually more resistant to herbicides than grass weeds and most dicots. Their resistance to herbicides is mediated in many cases by P450 oxygenases. Monocots thus constitute an appealing source of P450 enzymes for manipulating herbicide resistance (Batard et al., 2000). Three ESTs of this group encode for minor allergens (quinone reductase family protein) from *Arabidopsis thaliana* (**CAB16805**), which may relate to the quinone redox cycle (Jensen et al., 2002). One EST is similar to putative strictosidine synthase of *Oryza sativa* (**CAB16805**). Strictosidine synthase (STR) is the key enzyme involved in the early steps of the biosynthesis of monoterpenoid indole alkaloids. The main function of STR is to catalyze the condensation of tryptamine with secologanin into strictosidine (Inoue, 2005).

In the Taiwania inner bark cDNA library, 1.2% of the 973 ESTs were related to secondary metabolism; 1.1% were related to cell wall structure and metabolism; and 13.6% were related to cell rescue, defense, andaging. The fact that the category of cell rescue, defense and cell aging accounted for the highest percentage of these expressed genes implies that the inner bark tissues of Taiwania possess strong defense mechanisms. From the 973 clones, 57% of the ESTs had no corresponding protein in the database, and 7% were similar to proteins with unclear functions, suggesting that much remains to be discovered about Taiwania and further investigation into this species is necessary. However, the functional classification of known proteins of the inner bark provides information for further structural and functional analysis. Conserved and divergent aspects of the Taiwania genome and the biochemical effect of secondary and defensive expression of the inner bark could be studied in the future.

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臺灣杉內樹皮之基因調查

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臺灣杉為臺灣原產的重要經濟樹種之一。目前為止，超過一百種以上的臺灣杉二次代謝產物被分離純化。經實驗證實，臺灣杉的精油及二次代謝產物具有多樣化的生物活性，包括抗細菌、抗真菌、抗白蟻、抗蟎、抗氧化及抗腫瘤細胞等活性。因此，為了要了解參與臺灣杉生化合成及其木材形成的基因，於是我們構築了臺灣杉內樹皮的 cDNA 資料庫，並利用單向的序列解序，共獲得 973 筆基因表現標籤，續由 BLASXT 搜尋比對，發現這些基因多與細胞救助、防禦及老化等生理功能相關，其中以對抗病原的部份數目最多。另外，與細胞壁結構與二次代謝物相關的基因則佔 2.3%。特別的是，在此臺灣杉內樹皮基因資料庫中，約有 57% 的基因無法自目前已發表的基因中比對出相似的序列，由此顯示臺灣杉基因的唯一性與其特殊性，相當值得針對其功能進行深入的探討與研究。

關鍵詞：臺灣杉；內樹皮；基因表現標籤。