P 53 Probing Flavonoid Biosynthesis in the Young Leaves of Tea (*Camellia sinensis*) by Suppression Subtractive Hybridization

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Objectives

Tea (*Camellia sinensis*) is a commercially important plant and harvested for its polyphenols (catechins and flavonoids). To isolate genes involved in polyphenols biosynthesis from tea, 508 cDNA clones from a subtractive cDNA library from young growth stage (high catechins accumulation) were sequenced and analysed.

Materials and Methods

- Plant materials: young and old leaves of tea (*Camellia sinensis*)
- Subtractive cDNA library construction: PCR-select cDNA subtraction kit (Clontech)
- DNA sequencing: ABIPRISM 3100 DNA analyzer
- EST analysis: Compared with amino acid sequence using the BlastX program

Results and Discussion

Since high amount of polyphenols are biosynthesized and accumulated in young growth stage in green tea, a subtractive cDNA library between young and old growth stage leaves was constructed (Tester cDNA - young leaves: Driver cDNA - old leaves). A total 588 individual clones were randomly picked and sequenced from the 5′-terminus, of which 508 clones gave high-quality sequencing results. Based on the results of the BlastX comparisons, the subtractive ESTs were classified into fourteen groups according to their predicted functions. The group of ESTs of high abundance were represented by putatively assigned genes coding for enzymes of protein synthesis (16.4%), cell division and chromosome structure (8.1%), and defense and stress response (8.3%). About 11.8% of the clones were coded for enzymes involved in secondary metaboliosm with specifically high abundance of flavonoid metabolism (4.1%). These ESTs facilitate the isolation and characterization of genes of the flavonoid pathway in tea: chalcone synthase (CHS), flavone 3-hydroxylase (F3H), flavonoid 3′5′-hydroxylase (F3′5′H), flavonol synthase (FLS), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin reductase (LCR), including isoflavone reductase (IFR).

To identify growth stage-specific pattern, the structural genes of the flavonoid biosynthetic pathway were analyzed by RT-PCR between different growth stages. As expected, transcripts of F3H, DFR, LCR which related to accumulation of catechins were abundant in young leaves at the early growth stage. At the late growth stage, its accumulation was very low and specifically F3H was undetectable. The predominant expression of LCR in young leaves at the early growth stage might be corrected with the high contents of catechin in young leaves. Results of quantitative RT-PCR analysis thereby confirm that the subtracted library contains abundantly cDNA clones related to young leaves-preferential flavonoid metabolism.

The use of subtractive cDNA library has been an excellent source of ESTs related specifically to secondary metabolites biosynthesis and accumulation. In this study, we have identified many genes with potential role in secondary metabolites biosynthesis and these genes provide understanding the pathway influencing on secondary metabolites in plant. And these genes will be promising targets for genetic engineering of secondary metabolites in plants.

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