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Transcriptomic analysis of *Camellia ptilophylla* and identification of genes associated with flavonoid and caffeine biosynthesis

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ABSTRACT. *Camellia ptilophylla*, or cocoa tea, is naturally decaffeinated and its predominant catechins and purine alkaloids are *trans*-catechins and theobromine Regular tea [*Camellia sinensis* (L.) O. Ktze.] is evolutionarily close to cocoa tea and produces *cis*-catechins and caffeine. Here, the transcriptome of *C. ptilophylla* was sequenced using the 101-bp paired-end technique. The quality of the raw data was assessed to yield 70,227,953 cleaned reads totaling 7.09 Gbp, which were assembled *de novo* into 56,695 unique transcripts and then clustered into 44,749 unigenes. In catechin biosynthesis, leucoanthocyanidin reductase (LAR) catalyzes the transition of leucoanthocyanidin to *trans*-catechins, while anthocyanidin synthase (ANS) and anthocyanidin reductase (ANR) catalyze *cis*-catechin production. Our data demonstrate that two *LAR* genes (*CpLAR1* and *CpLAR2*) by *C. ptilophylla* may be advantageous due to the combined effects of this quantitative trait, permitting increased leucoanthocyanidin consumption for the synthesis of *trans*-catechins. In contrast, the only *ANS* gene observed in *C. sinensis* (*CsANS*) shared high identity (99.2%) to one homolog from *C. ptilophylla* (*CpANS1*), but lower identity (~80%)

to another (*CpANS2*). We hypothesized that the diverged *CpANS2* might have lost its ability to synthesize *cis*-catechins. *C. ptilophylla* and *C. sinensis* each contain two copies of ANR, which share high identity and may share the same function. Transcriptomic sequencing captured two *N*-methyl nucleosidase genes named *NMT1* and *NMT2*. *NMT2* was highly identical to three orthologous genes *TCS2*, *PCS2,* and *ICS2*, which did not undergo methylation *in vitro*; in contrast, *NMT1* was less identical to *TCS*, *PCS* and *ICS*, indicating that *NMT1* may undergo neofunctionalization.

Key words: Cocoa tea; Transcriptome; Trans-catechins biosynthesis; Theobromine biosynthesis

