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Light-induced expression of genes involved in phenylpropanoid biosynthetic pathways in callus of tea (*Camellia sinensis* (L.) O. Kuntze)

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ABSTRACT

Tea (*Camellia sinensis* (L.) O. Kuntze) is a commercially important crop that is valued for its secondary metabolites. Light is an important environmental parameter that regulates plant growth and development and influences the phenylpropanoid metabolism in plants. To investigate the molecular mechanism by which light regulates phenylpropanoid metabolism, we established light-induced suppression subtractive hybridization (SSH) cDNA libraries of tea calli. A total of 265 clones from the library were selected, sequenced, and analyzed in this study. Nine diverse ESTs involved in phenylpropanoid biosynthesis were detected in the library. A new *CsDFR* gene (*CsDFR2*), higher increment of the expression activated by light than the previously reported *CsDFR* gene (*CsDFR1*), was cloned. The key phenylpropanoid compounds and representative genes expression analysis implied that light could be effective for activation of the biosynthesis of phenylpropanoids. Compared to the darkness control, levels of lignins, catechins, and PAs were increased 3.46, 3.00, and 1.21-fold, in light-induced calli, respectively. And lignin biosynthesis genes, involved in CCoAOMT, HCT and CCR, were identified in the light-induced SSH library. Therefore it was assumed that lignins might be the main phenylpropanoid metabolites activated by light in tea calli. In addition, our researches found that catechins, as the main secondary metabolites, significantly decreased in the tea calli compared to those in tea mature leaves, While PAs (polymer of catechins) in calli did not decrease compared to mature leaves. The data suggest that polymerization reaction might be the main pathway of flavonoid metabolism in tea callus. The SSH library established in this study represents a valuable resource for better understanding the mechanisms of light-induced secondary metabolism in tea plants.

1. Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is an important commercial crop grown in over 30 countries and consumed worldwide primarily as a beverage made from the processed leaves. As the main secondary metabolites, flavan 3-ols (catechins), along with other phenylpropanoids including flavonols, proanthocyanins (PAs), anthocyanins and lignins, are derived from multiple branches of the phenylpropanoid biosynthetic pathways (Fig. 1), one of the most-characterized secondary metabolic routes in plant systems. The metabolic genes related to catechins biosynthesis (including catechin (C), gallocatechin (GC), epicatechin (EC), and epigallocatechin (EGC)) comprise flavanone 3′-hydroxylase (*F3H*), dihydroflavonol 4-reductase (*DFR*), leucoanthocyanidin reductase (*LAR*), anthocyanidin synthase (*ANS*), and anthocyanidin reductase (*ANR*) (Tanner et al., 2003; Xie et al., 2003). The ester catechins (including epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG)) were synthesized by esterification reaction of gallic acid or proanthocyanidins (PAs) were synthesized by polymerization of the monomer catechins. In addition to phenylalanine ammonialyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), 4-coumaroyl-CoA ligase (*4CL*), and chalcone synthase (*CHS*), caffeoyl-CoA 3-O-methyltransferase (*CCoAOMT*) and hydroxycinnamoyl transferase (*HCT*) are key genes in biotechnological

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Abbreviations: ABC protein, ATP-binding cassette type membrane protein; ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; C, catechin; CAD, cinnamoyl alcohol dehydrogenase; CCH, coumaroyl-CoA 3-hydroxylase; CCoAOMT, caffeoyl-CoA 3-O-methyltransferase; CCR, cinnamoyl-CoA reductase; C3H, coumarate 3-hydroxylase; C4H, cinnamate 4-hydroxylase; CHS, chalcone synthase; CHI, chalcone isomerase; 4CL, 4-coumaroyl-CoA ligase; COMT, caffeic acid-3-O-methyltransferase; DFR, dihydroflavonol 4-reductase; EC, epicatechin; ECG, epicatechin-3-gallate; EGC, epigallocatechin; EGCG, epigallocatechin-3-gallate; FST, flavonol 4′-sulfotransferase; GC, gallocatechin; GST, glutathione S-transferase; HCT, hydroxycinnamoyl transferase; LAR, leucoanthocyanidin reductase; PAL, phenylalanine ammonialyase; SA, salicylic acid; SSH, suppression subtractive hybridization; UFGT, UDP-glucose:flavonoid 3-O-glucosyltransferase.

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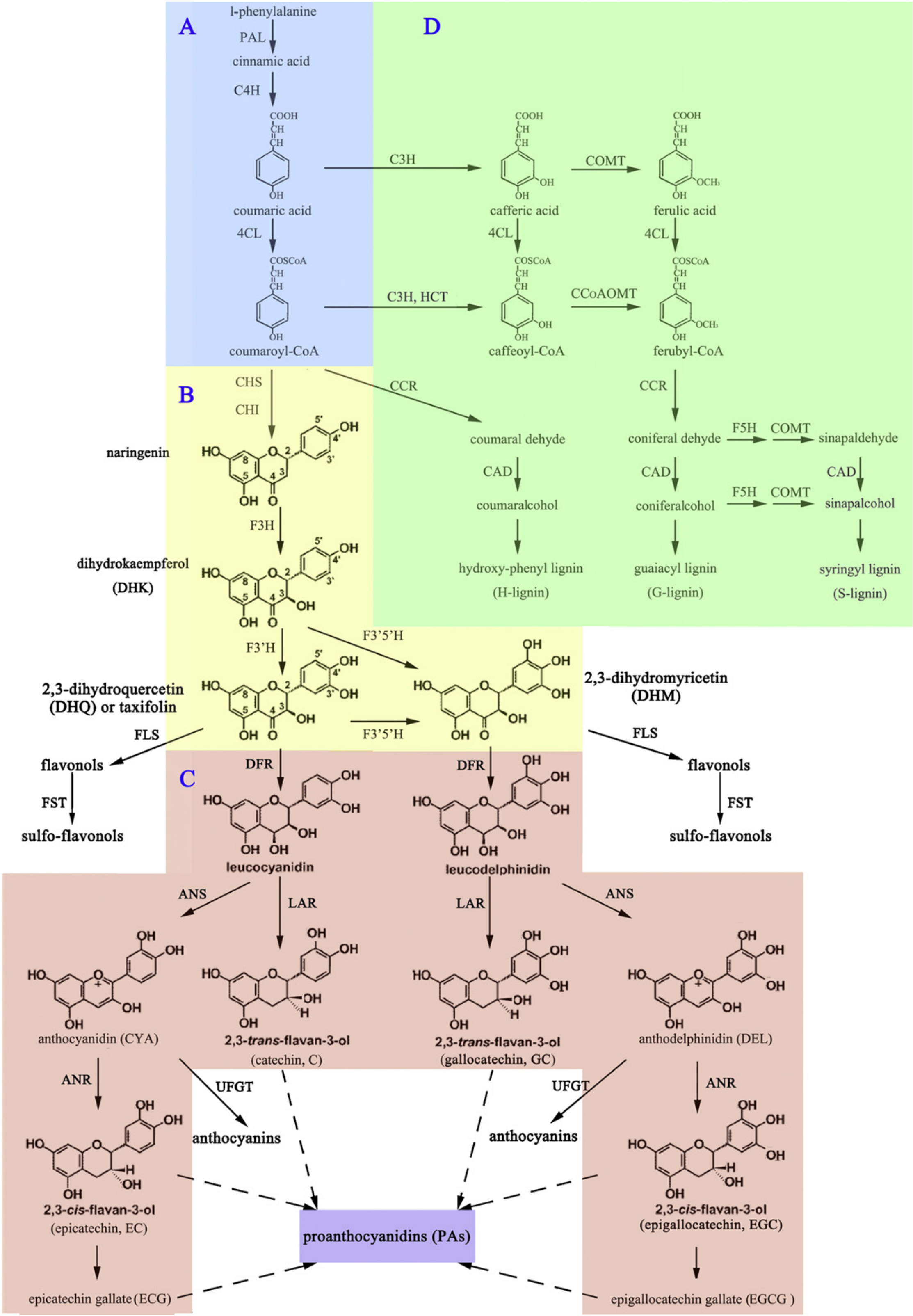

Fig. 1. Possible phenylpropanoid and flavonoid pathways in *Camellia sinensis* (L.) O. Kuntze (Punyasiri et al., 2004, Rogers and Campbell, 2004). (A) basic phenylpropanoid biosynthetic pathway; (B) early flavonoid biosynthetic pathway; (C) late flavonoid biosynthetic pathway; (D) lignin biosynthetic pathway. PAL, phenylalanine ammonialyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3′-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′5′-hydroxylase; FLS, flavonol synthase; FST, flavonol 4′-sulfo-transferase; DFR, dihydroflavonol 4-reductase; LAR, leucoanthocyanidin reductase; ANS, anthocyanidin synthase; ANR, anthocyanidin reductase; UFGT, UDP-glucose:flavonoid 3-O-glucosyltransferase; C3H, coumarate 3-hydroxylase; HCT, hydroxycinnamoyl transferase; COMT, caffeic acid-3-O-methyltransferase; CCoAOMT: caffeoyl-CoA 3-O-methyltransferase; CCH, coumaroyl-CoA 3-hydroxylase; CCR, cinnamoyl-CoA reductase; CAD, cinnamoyl alcohol dehydrogenase; F5H, ferulate 5-hydroxylase; CoA, coenzyme A.