Characterization of *O*-methyltransferases and pinoresinol reductases involved in lignin and lignan biosynthesis

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Fossil resource-based industrial society has provided us prosperity, especially in the developed countries. However, it has also brought serious negative impacts on the global environment due to the increase in the atmospheric concentration of carbon dioxide. Therefore, it is becoming more and more important to establish a sustainable and recycling-based society, which depends on renewable resources.

Wood biomass is the most abundant and potentially renewable resource and is composed mostly of secondary xylem which is formed through two metabolic events, cell-wall formation and heartwood formation. Hence, biochemical and molecular biological studies of cell-wall and heartwood formation provide the basic knowledge for establishing systems for sustainable production of wood biomass.

For the studies of cell-wall and heartwood formation, it is important to elucidate the biosynthetic mechanisms of phenylpropanoid, because the biosynthesis of phenylpropanoid is the principal metabolism of both cell-wall and heartwood formation. For example, lignin which is a phenylpropanoid polymer is one of the major cell wall components and lignans which are phenylpropanoid dimers are deposited in significant amounts in heartwood regions of trees.

Lignins and lignans are biosynthesized through the cinnamate/monolignol pathway and are closely related in terms of their chemical structures. Lignins are formed via non-enantioselective polymerization of monolignols, whereas typical lignans are formed by enantioselective coupling of two coniferyl alcohol units where central carbon (C8) atoms of the allyl moieties are linked to each other.

During plant development, lignins encase the cellulose microfibrils and confer mechanical strength to cell walls. Regardless of its importance during plant growth, lignins become problematic to post harvest, cellulose-based wood processing such as pulping and saccharification which leads eventually to bioethanol production. As a result, there has been long-standing incentive to develop healthy trees that accumulate less lignin and/or more removable lignin to facilitate pulping and saccharification, which depends largely on the knowledge of lignin biosynthetic mechanisms. On the other hand, lignans have a wide range of biological effects on various organisms, including humans, at molecular, enzymatic, physiological, pharmacological, and even clinical levels. Hence, lignans are considered to be involved in plant defense systems in living tissues, though less information is available on their function and role in the plants producing them. In addition, antimicrobial lignans which are biosynthesized in transition wood regions, i.e. the inner parts of sapwood, are deposited in significant amounts in heartwood and serve as preservatives to prevent heartwood rot. Because heartwood formation is specific to trees and does not occur in herbaceous plants, biosynthesis of lignans can be a clue to elucidating heartwood formation mechanisms.

The cinnamate/monolignol pathway supplies precursors for various phenylpropanoid compounds including lignins and lignans. Many studies of the cinnamate/monolignol pathway have been conducted in relation to lignin biosynthesis, except that a biosynthetic route for lignan biosynthesis via ferulic acid has been proposed recently. Maturing seeds of *Carthamus tinctorius* (safflower) accumulate both lignins and lignans, and their biosynthesis is initiated at particular points of maturation. This species therefore a good plant material for comparative studies of lignin and lignan biosyntheses.

In the present study, the author aimed to elucidate the difference of lignin and lignan biosynthetic mechanisms. The author cloned caffeoyl CoA *O*-methyltransferase (CtCoAOMT) and 5-hydroxyconiferaldehyde *O*-methyltransferase (CtCAldOMT) that are key enzymes of the cinnamate/monolignol pathway from maturing seeds of *C. tinctorius* and examined the participation of each OMT clone to lignin or lignan biosynthesis. In addition, the author cloned novel *O*-methyltransferase from maturing seeds of *C. tinctorius* and showed that the OMT, named 5-hydroxyconiferaldehyde/5-hydroxyconiferyl alcohol OMT (CtAAOMT), is involved in lignin

biosynthesis.

Umezawa in the author's laboratory cloned the novel gene encoding lignan *O*-methyltransferase (matairesinol *O*-methyltransferase, CtMROMT) from maturing seeds of *C. tinctorius*. In addition, another cDNA which shows a high sequence similarity to *CtMROMT*, named tentatively *CtMROMTlike*, was cloned. In the present study, the author examined its function and found that the *bona fide* function of CtMROMTlike is flavonoid OMT and referred to the OMT as *C. tinctorius* flavonoid OMT1 (CtFOMT1).

At present, a number of genes encoding enzymes involved in lignan biosynthesis have cloned from several plant species. For analyzing the functions of those genes in detail, it is necessary to produce the transformants where the expression of target genes is up- or down-regulated. However, it was very difficult because gene transformation and regeneration systems for plants which are known as lignan producers including *C. tinctorius* were very limited. On the other hand, *Arabidopsis thaliana* is the most widely used model plant in plant bioscience sectors. Thus, the genome sequence is available, and its transformation and regeneration are very easy. So far, there has been no report of isolation of lignans from *A. thaliana*. However, in the *A. thaliana* genome database, there are two genes that are annotated as pinoresinol/lariciresinol reductase (PLR) that is key enzyme of lignan biosynthesis. In this context, the author analyzed the methanol extracts of *A. thaliana*, and found the presence of lignan lariciresinol in the roots. This means *A. thaliana* can be exploited for lignan biosynthetic studies. Therefore, as a first step to elucidate the difference between lignin and lignan biosynthetic mechanisms in *A. thaliana*, the functions of two *A. thaliana* genes that are annotated as PLR were characterized. The author demonstrated that *Arabidopsis thaliana* PLRs showed strict substrate preference towards pinoresinol, but only weak or no activity towards lariciresinol, unlike conventional PLRs, indicating their *bona fide* function is *A. thaliana* pinoresinol reductase (AtPrR). It was also demonstrated that the enantiomeric composition of lariciresinol in *A. thaliana* can be controlled by the differential expression of two *AtPrR*s by using biochemical characterization of their recombinant enzymes and spaciotemporal expression analysis of the genes. Next, the function of *A. thaliana* gene, At5g54160 annotated as caffeic acid *O*-methyltransferase that is a key enzyme of cinnamate/monolignol pathway was characterized and revealed the participation of At5g54160 gene in lignin biosynthesis.