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Review

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Recent Advances in the Metabolic Engineering of Lignan Biosynthesis Pathways for the Production of Transgenic Plant-Based Foods and Supplements

Honoo Satake,*[,†] Eiichiro Ono,[‡] and Jun Murata[†]

[†]Bioorganic Research Institute, Suntory Foundation for Life Sciences, 1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka 618-8503, Japan

[‡]Institute for Plant Science, Suntory Business Expert Ltd., 1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka 618-8503, Japan

ABSTRACT: Plant physiological, epidemiological, and food science studies have shed light on lignans as healthy diets for the reduction of the risk of lifestyle-related noncommunicable diseases and, thus, the demand for lignans has been rapidly increasing. However, the low efficiency and instability of lignan production via extraction from plant resources remain to be resolved, indicating the requirement for the development of new procedures for lignan production. The metabolic engineering of lignan-biosynthesizing plants is expected to be most promising for efficient, sustainable, and stable lignan production. This is supported by the recent verification of biosynthetic pathways of major dietary lignans and the exploration of lignan production via metabolic engineering using transiently gene-transfected or transgenic plants. The aim of this review is to present an overview of the biosynthetic pathways, biological activities, and metabolic engineering of lignans and also perspectives in metabolic engineering-based lignan production using transgenic plants for practical application.

KEYWORDS: *lignan, metabolic engineering, biosynthesis, transgenic plants*

■ INTRODUCTION

According to the World Health Organization, there are currently 650 million people aged 60 years and older worldwide, and it is predicted that there will be almost 2 billion people aged 60 years or older, including 450 million people 80 years of age or older, by 2050 (http://www.who.int/ageing/en/). Such a tremendous increase in the number of elderly people has clearly been accompanied by a rapid and significant increase in medical care expenses, which may eventually lead to a serious disruption of essential medical care systems and a national financial burden. To address these issues, of particular importance is that extensive efforts are made to increase healthy life expectancy, prevent lifestyle-related diseases, and make progress in medical treatments (http://whqlibdoc.who.int/hq/2012/WHO_DCO_WHD_2012.2_eng.pdf). The consistent and appropriate intake of healthy foods and dietary supplements is one of the most promising and effective ways to achieve these goals. Indeed, their consumption has been rapidly increasing in developed countries, suggesting that there will be an even higher demand for sources of healthy diets and supplements in the near future.

A large part of healthy diets and dietary supplements is derived from "secondary metabolites" (also called "specialized metabolites") of plants, including alkaloids, flavonoids, isoflavonoids, and lignans, which have received dietary and medicinal attention over the past several decades. Plant resources of lignans are frequently limited because of the high cost of plant hunting and collection, poor cultivation systems, long growth phase, and their low lignan content. For instance, the acquisition of sesamin, a multifunctional sesame seed lignan, depends upon its extraction from sesame seed oil, and at most, sesamin comprises 0.4−0.6% (w/w) of sesame seed oil, which is known to produce the most abundant sesamin of all plants. Moreover, sesame seeds are cultivated only once a year. Likewise, podophyllotoxin, a lignan lead compound for an anti-breast cancer drug, is isolated from the roots and rhizomes of *Podophyllum hexandrum*, which is distributed in very limited regions and is now endangered due to overharvesting and environmental disruption.[1] In addition, organic synthesis is not practical for the mass supply of dietary lignans in light of its high cost and noncompliance with the Food Sanitation Law, particularly in Japan. These disadvantages in the current procedures for lignan acquisition indicate that the establishment of an efficient, stable, and sustainable production system for lignans is of keen interest. There has recently been a growing body of studies that have elucidated the enzymes involved in the biosynthesis of dietary lignans as well as their biological activities in mammals. These outcomes have allowed us to attempt the metabolic engineering of lignan-producing plants or cultured plant cells. In this review, we provide essential and current knowledge of the chemical structures, biosynthesis, and biological activities of lignans and the current status and perspectives in lignan production via metabolic engineering.

■ CHEMICAL STRUCTURES OF MAJOR LIGNANS

The term lignan was introduced by R. D. Haworth in 1936 and refers to naturally occurring phenylpropanoid dimers (C6−C3 unit; e.g., coniferyl alcohol) in which the phenylpropane units are bound by the C8 central carbons of the propyl side chains.[2] To date, a wide range of lignans with various chemical structures and

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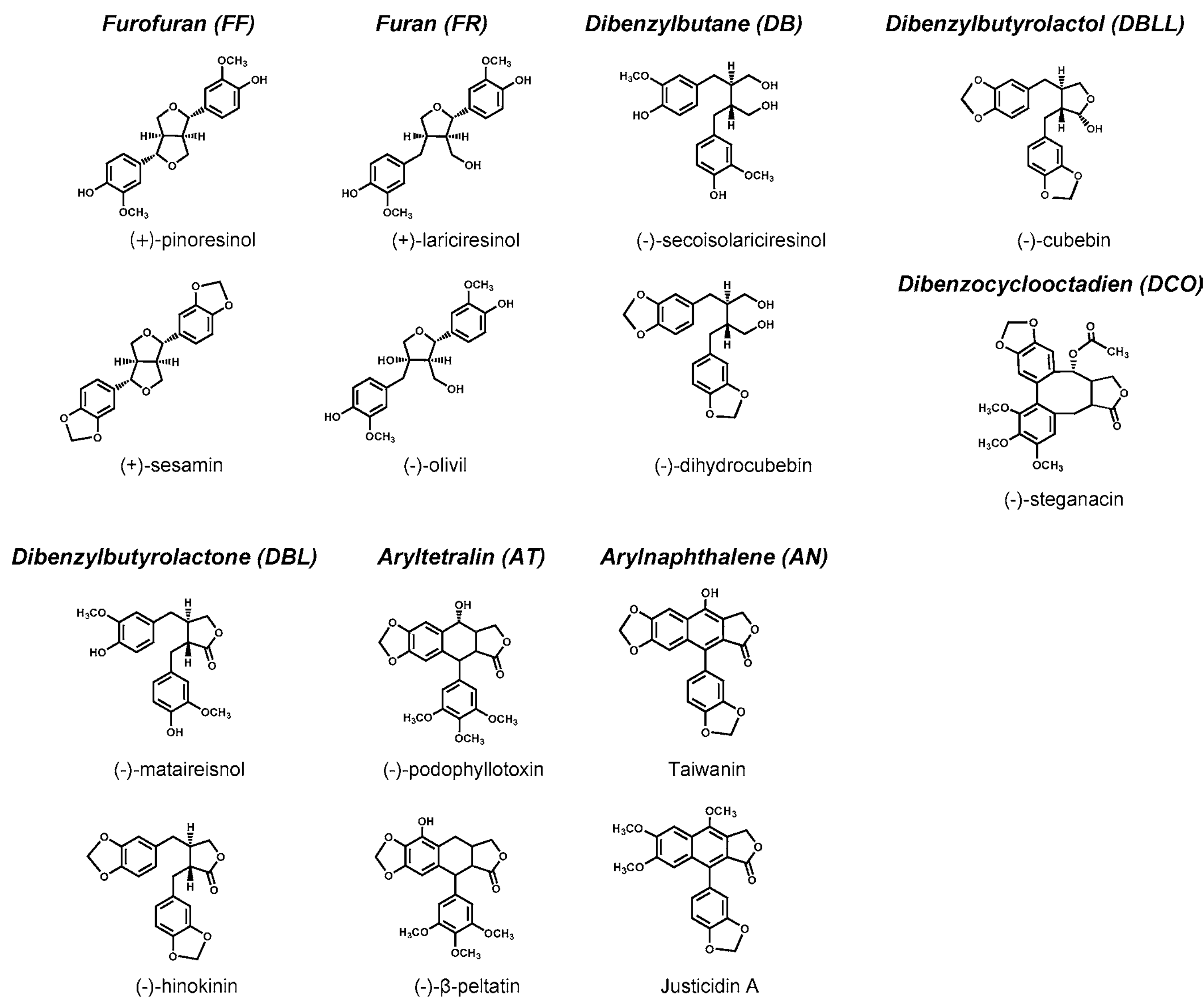

Figure 1. Chemical structures of typical lignans in dietary and medicinal sources.

biological activities have been characterized from diverse plants. As shown in Figure 1, lignans are classified into eight groups on the basis of their structural patterns, including their carbon skeletons, the way in which oxygen is incorporated into the skeletons, and the cyclization pattern: furofuran, furan, dibenzylbutane, dibenzylbutyrolactone, aryltetralin, arylnaphthalene, dibenzocyclooctadiene, and dibenzylbutyrolactol.[2,3] In addition, lignans can also occur as mixtures of enantiomers, which are closely associated with their biosynthetic processes and biological activities.[2,3]

The lignan components in a wide variety of foods, medicinal sources, and model plants have thus far been investigated,[2−12] revealing that lignans are widespread, but not ubiquitous, in plant species and that sesame seeds and flax seeds contain the highest amounts of lignans thus far reported. In the context of dietary and medicinal lignan components, pinoresinol, lariciresinol, secoisolariciresinol, matairesinol, sesamin, and podophyllotoxin (Figure 1) have been extensively investigated. Therefore, we focus on the phytochemical, biological, and biotechnological aspects of these lignans in the subsequent sections.

BIOLOGICAL ACTIONS OF DIETARY LIGNANS

The major biological actions of lignans are classified into three types: bioactivities on plants, insects, or mammals. Pinoresinol, hinokinin, and podophyllotoxin exhibit pyrethrum-synergistic or antifeedant effects on insects, although their precise molecular mechanisms remain largely unknown.[13] In most cases, these lignans manifested the aforementioned activities at high concentrations, raising the question of whether their biological actions in insects are significant in nature. Nevertheless, a unique ecological role for lignans was proposed by Schroeder and colleagues, who revealed their biological roles as defensive factors against predators in insects.[14] When caterpillars of the cabbage butterfly, *Pieris rapae*, were fed cabbage containing (+)-pinoresinol, they secreted pinoresinol in the effluent. Moreover, pinoresinol-treated fruitflies were less frequently targeted by ants, their main predator. These data suggest that cabbage-derived pinoresinol, taken up by and secreted from *Pieris* caterpillars, serves as a defensive compound in caterpillars against ants via its antifeedant activity. Intriguingly, such feedant deterrence is highly likely to be specific to pinoresinol.[14] These data point to the specific and complicated physiological and ecological roles of lignans.

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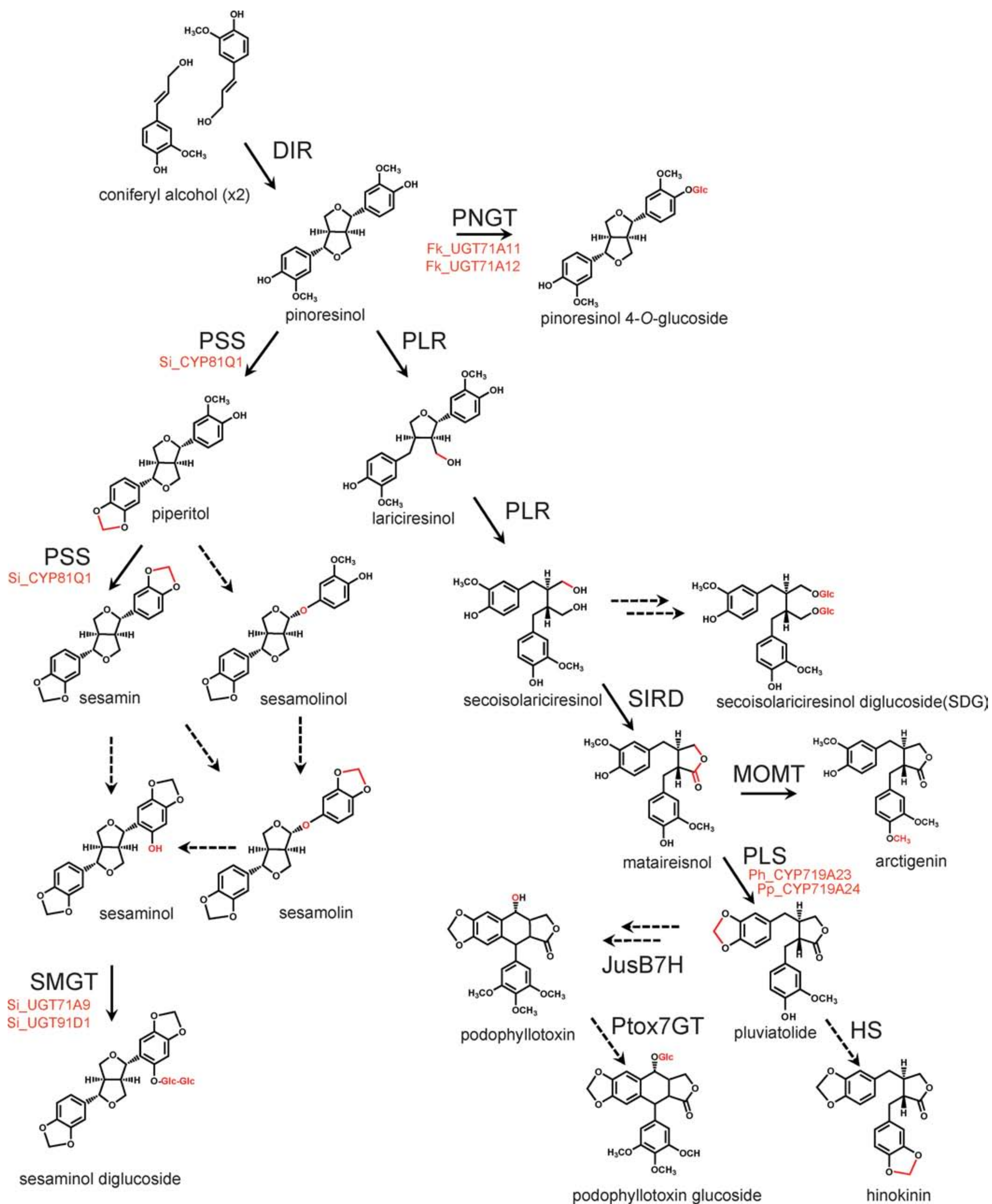

Figure 2. Biosynthesis pathways of major lignans. Chemical conversions at each step are indicated in red. Solid and broken lines represent identified and unidentified enzyme-catalyzed reactions, respectively.

Some studies have shown that lignans can regulate plant growth and germination. For example, (−)-lariciresinol and its related lignans potently suppressed lettuce germination.[15,16] Moreover, (−)-lariciresinol stereoselectively inhibited the root growth of Italian ryegrass to 51−55% compared to the untreated control.[15] These activities of lariciresinol are presumed to be

functionally correlated with a reduction in extractable glutamate synthase activity via alterations in carbon and nitrogen metabolism.[17] Together, these findings suggest that lignans may be involved in optimization of the environmental density of plants via inhibition of plant growth or germination.

Lignans and their glycosides, including pinoresiniol, sesamin, lariciresinol, secoisolariciresinol, and matairesinol, are metabolized by intestinal microflora into two major compounds, enterodiol and enterolactone, which are well-known as "enterolignans" or "mammalian lignans".[18−21] These lignans, combined with those of isoflavones and coumestans, are also included in "phytoestrogens" because of their modest estrogen-like activity in mammals.[22,23] Notably, low levels of intact lignans were also detected in the serum of mammals fed lignan-containing diets, suggesting that lignans exert their biological functions without being metabolized in vivo.[7,21] Some studies have demonstrated the binding of enterolignans to the mammalian estrogen receptors (ER), ERα or ERβ,[24,25] whereas ER-independent activities, such as tumor growth suppression, angiogenesis inhibition, production of sex hormone-binding proteins, and apoptosis induction, have also been revealed.[26−31] Consistent with these bioactivities, the greatest attention has been paid to the reduction of cancer risk by dietary lignans. In particular, a wide range of epidemiological analyses have indicated that the consistent intake of enterolignan-rich foods correlates with a reduction in breast cancer risk and an improved prognosis in the breast cancer-specific survival of postmenopausal women.[29,32−37] Moreover, serum enterolactone levels were also found to positively correlate with the prognosis of women with postmenopausal breast cancer.[38] (+)-Lariciresinol administration via food intake suppresses tumor growth and angiogenesis in human MCF-7 breast cancer xenografts in athymic mice and carcinogen-induced rat tumor.[30] In the former, induction of apoptosis and up-regulation of ERβ expression were also observed.[30] Furthermore, dietary (−)-secoisolariciresinol diglucosides, as well as a flaxseed lignan mixture, inhibited cell proliferation and induced the apoptosis of breast cancer cells via down-regulation of ER- and growth factor-mediated gene expression in athymic mouse.[39] Both similar and specific antitumor effects of sesamin were also detected; for instance, only sesamin reduced signaling downstream of mitogen-activated protein kinase.[40] In addition, sesamin is likely to elicit a more potent reduction of breast tumor growth, compared to secoisolariciresinol diglucosides.[40] Collectively, these findings demonstrated that lignans are promising dietary compounds for the prevention of breast cancer and exhibit both common and specific activities.

Recently, lignans have also been shown to exhibit positive effects on other lifestyle-related diseases. The 3 month administration of flaxseed lignan complexes resulted in a decrease of plasma glucose and type 2 diabetes markers in elderly patients with the disease.[41] (+)-Sesamin and its digests were shown to exert antihypertensive and antioxidant effects.[42−44] Moreover, the antioxidative property of sesamin is also believed to play a role in protecting the liver from oxidation by alcohol, lipids, and oxygen radicals.[42,45−47] (+)-Pinoresinol was found to reduce the production of inflammatory factors, such as interleukin-6 and prostaglandin E2, following the up-regulation of an inducible prostaglandin synthase, Cox-2, in human intestinal Caco-2 cells.[21] These findings prove that (+)-pinoresinol possesses potent anti-inflammatory effects.[21] In contrast, (−)-matairesinol increased levels of Cox-2-derived prostaglandin E2.[21] In combination, these epidemiological and physiological studies demonstrate that respective lignans possess diverse, but specific, bioactivities and reinforce their potential as dietary compounds for the prevention of lifestyle-related diseases, such as cancer, diabetes, hepatocirrhosis, and hypertension. Notably, each lignan was found to exhibit both similar and differential bioactivities in mammals, which underscores the dietary and medicinal significance of the efficient and specific production of a targeted lignan, instead of lignan mixtures. To date, mammalian proteins targeted by lignans or their metabolites (proteins other than mammalian metabolizing enzymes) have not yet been fully identified. The molecular and functional characterization of such target proteins will facilitate the elucidation of more precise molecular mechanisms underlying the prevention of diseases.

MAJOR LIGNAN BIOSYNTHETIC PATHWAYS

Lignan biosynthesis is initiated by the generation of pinoresinol, a basal lignan (Figure 2). Although a pinoresinol synthase has never been characterized, a dirigent protein (DIR) was shown to participate in the stereospecific coupling of achiral *E*-coniferyl alcohol.[48] In *Sesamum* plants, (+)-pinoresinol is metabolized into (+)-piperitol, followed by further conversion into (+)-sesamin via formation of two methylenedioxy bridges by sesame cytochrome P450 (CYP) Q1, the first P450 enzyme responsible for lignan biosynthesis.[49] CYP81Q1 homologues were also detected in the diverse *Sesamum* genus, including *S. indicium*, *S. radiatum*, *S. shinzianum*, and *S. latifolium*.[49] CYP81Q1 gene expression is predominantly detected in sesame seeds, which is in accordance with the high levels of sesamin production in sesame seeds.[49] As shown in Figure 2, sesamin is presumed to be metabolized into sesaminol and sesamolin, although the relevant biosynthetic enzymes have yet to be identified.[3,48] In many plant species, pinoresinol-lariciresinol reductase (PLR), a pinoresinol-lariciresinol/isoflavone/phenylcoumaran benzylic ether reductase (PIP) family enzyme, converts pinoresinol to secoisolariciresinol via lariciresinol in an enantiomer-specific manner (Figure 2).[50−54] In *Arabadopsis thaliana*, AtPrR1 and 2, which merely reduce (−)-pinoresinol to (−)-lariciresinol, have been characterized.[54] Subsequently, secoisolariciresinol dehydrogenase (SIRD) was found to convert secoisolariciresinol into matairesinol (Figure 2).[55] These findings reveal that the early biosynthetic pathways, from pinoresinol to matairesinol, are shared by diverse plant species, including *Forsythia*, *Linum*, and *Podophyllum*. In *Forsythia*, (−)-matairesinol is metabolized into (−)-arctigenin via methylation of a phenolic hydroxyl group (Figure 2). Quite recently, arctigenin synthase, namely, matairesinol *O*-methytransferase (MOMT), was identified in *Carthamus tinctorius*.[56] In *Linum*, *Anthriscus*, and *Podophyllum*, matairesinol is further converted into hinokinin or podophyllotoxin.[2,3] However, few enzymes responsible for these biosynthetic pathways have been identified. An *O*-methyltransferase (OMT) isolated from *Anthriscus sylvestris* was shown to specifically methylate the 5-hydroxyl group of thujaplicatin, which is an intermediate of podophyllotoxin-related lignans.[57] More recently, the genome sequence of *Linum usitatissimum* was determined,[58] followed by extensive genomic, transcriptomic, and bioinformatic analyses, such as the in silico characterization and classification of CYP and UGT genes in *L. usitatissimum*.[59,60] These findings are expected to remarkably enhance the molecular and functional characterization of lignan biosynthetic enzymes.

A next-generation sequencer-based transcriptomic analysis of *Podophyllum hexandrum* and *Podophyllum peltatum* led to the

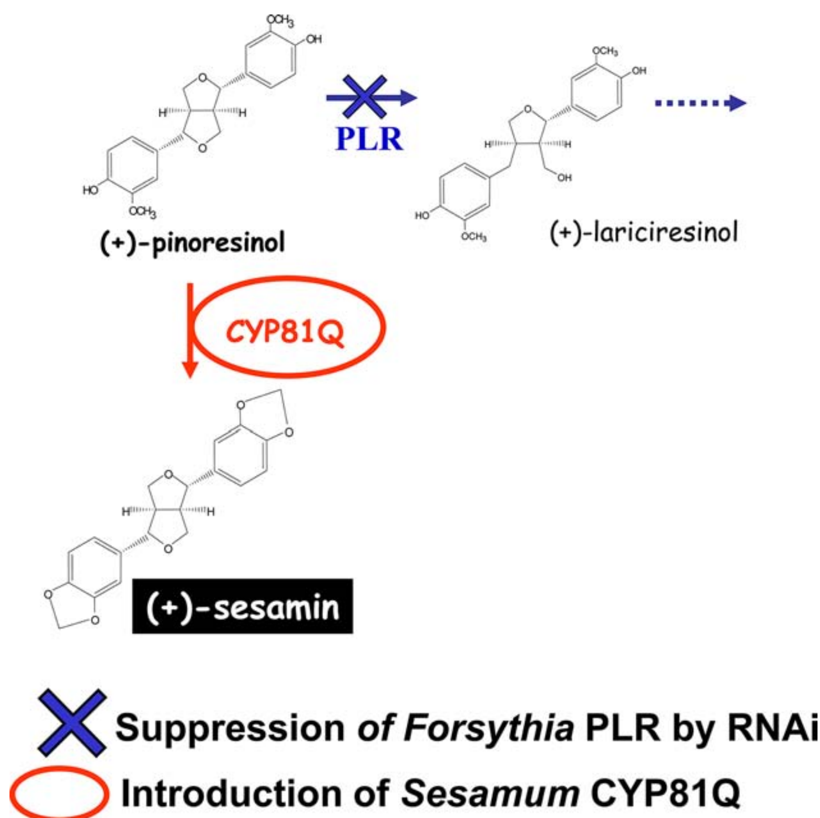

Figure 3. Metabolic engineering of *Forsythia* suspension cell cultures. Endogenous metabolism of pinoresinol into lariciresinol was suppressed by RNAi of PLR gene expression, and exogenous metabolism of pinoresinol to sesamin was introduced by stable transfection of an exogenous (*Sesamum*) CYP81Q1 gene. The resultant transgenic *Forsythia* suspension cell culture, CPi-Fk cells, acquired the ability to produce sesamin, whereas native *Forsythia* did not biosynthesize sesamin.

molecular and biochemical characterization of CYP719A23 (from *P. hexandrum*) and CYP719A24 (from *P. peltatum*) as novel lignan biosynthetic enzymes; these homologous enzymes have been shown to participate in the conversion of (−)-matairesinol into (−)-pluviatolide, an intermediate of (−)-podophyllotoxin, via catalysis of methylenedioxy bridge formation.[61] Intriguingly, a phylogenetic tree analysis revealed that these two enzymes belong to the CYP719A clade of enzymes,[61] which catalyze the methylenedioxy bridge formation of alkaloids, and do not belong to the CYP81Q clade of enzymes, which catalyze the methylenedioxy bridge formation of furofuran lignans.[3,49] Such a complicated molecular and functional correlation reinforces the existence of unprecedented molecular diversity in the biosynthesis of plant specialized metabolites. Thus, next-generation sequencing is also a promising approach for the molecular and functional characterization of lignan biosynthetic enzymes in nonmodel plants (of which the whole genome has yet to be explored), including *Sesamum*, *Podophyllum*, and *Forsythia* species.

In general, large parts of lignans are stored in their glycosylated forms.[2,3,62,63] Accordingly, the molecular and functional characterization of lignan-glycosylating enzymes is also indispensable for the elucidation of total lignan biosynthetic pathways and the biological significance of lignans. However, no lignan-glycosylating enzymes were identified until 2008. The first lignan-glucosylating enzyme reported was UGT71A9, which was identified in *S. indicium*. This enzyme was found to transfer one glucose to the 2-hydroxyl group of sesaminol using UDP-glucose as a glucose donor.[64] Moreover, UGT71A8 and UGT71A10, homologues of UGT71A9 in *S. radiatum* and *S. alatum*, respectively, were also shown to share the sesaminol glucosylation activity.[64] Furthermore, UGT94D1, another UDP-glucose-dependent glucosyltransferase, was shown to specifically participate in the biosynthesis of sesaminol diglucoside via glucosylation of sesaminol 2-*O*-monoglucoside at the 6-position of the conjugated glucose.[64] Recently, UGT71A18, identified in *Forsythia koreana*, was found to be responsible for glucosylation of pinoresinol in a UDP-dependent manner and, at least in part, for the accumulation of pinoresinol glucosides in *Forsythia*.[65] Collectively, these findings suggest that lignan-glucosylating enzymes belong to the UDP-glucose-dependent glucosyltransferase family. Therefore, the comprehensive molecular and functional transcriptomic analyses of lignan-producing plants will lead to the efficient identification of lignan-glycosylating enzymes.

■ METABOLIC ENGINEERING OF *FORSYTHIA* SPP.

Forsythia (Oleaceae) is a perennial plant commonly known as the golden bell flower. *Forsythia* spp. produce large amounts of various lignans in leaves, stems, fruits, and flowers, which are used for a variety of Chinese medicines and health diets.[2,3,6,8] The major lignans of *Forsythia* are pinoresinol (furofuran), phillygenin (furofuran), secoisolariciresinol (dibenzylbutane), matairesinol (dibenzylbutyrolactone), and arctigenin (dibenzylbutyrolactone) (Figure 1). Furthermore, a large portion of these lignans accumulates as *O*-glucosides.[2,62,63] Such a large amount of lignans and the identification of lignan biosynthetic enzymes (Figure 2) suggest the prominent potential for the platform of lignan production. In 2009, the metabolic engineering of lignan using *Forsythia* was originally reported. A transgenic *Forsythia*

suspension culture of cells was stably transfected with PLR-RNA interference (RNAi) construct (*PLR*-RNAi), resulting in the complete loss of matairesinol and an approximately 20-fold accumulation of pinoresinol in its glucoside form in comparison to untransfected cells.[63] Moreover, *Forsythia* transgenic cells coexpressing *PLR*-RNAi and the sesamin-producing enzyme, *CYP81Q1* (designated CPi-Fk cells), produced (+)-sesamin (0.1 mg/g dry weight of the cell, DW) (Figure 3), which is not biosynthesized by native *Forsythia*.[63] This is the first report of the production of an exogenous lignan using transgenic plant cells. More recently, CPi-Fk cells were found to possess the ability to increase both endogenous and exogenous lignan production. Specifically, CPi-Fk cells elicited a 2.3-, 2.7-, or 1.6-fold increase in sesamin production after irradiation for 2 weeks with white fluorescent, blue LED, or red LED light, respectively, compared to sesamin levels obtained in the dark.[66] Likewise, CPi-Fk cells showed an approximately 1.5−3.0-fold increase in pinoresinol (aglycone and glucosides) production.[66] Furthermore, expression of the endogenous pinoresinol-glucosylating enzyme UGT71A18 was suppressed in CPi-Fk cells under blue or red light.[66] However, the underlying molecular mechanism remains to be investigated. This is also the first report of the elevation of lignan biosynthesis by light. Although the current amount of sesamin by CPi-Fk cells is 10−20-fold lower than that by sesame seeds, CPi-Fk systems possess special advantages as lignan producers. First, CPi-Fk cells proliferate 10-fold in 2 weeks in standard culture medium[63] and can be cultivated anytime and anywhere, whereas sesame seeds are cultivated in limited regions only once a year. Second, the conditions (e.g., temperature, light wavelength and intensity, medium components) for growth of CPi-Fk cells and sesamin production can be readily altered and are free from climate risk. In addition, the RNAi-based suppression of UGT71A18 (pinoresinol-glucosylating enzyme) may lead to the dramatic improvement of sesamin production in CPi-Fk cells, given that pinoresinol glucoside cannot be utilized by CYP81Q as its substrate[49] and 90% of pinoresinol is glucosylated in *Forsythia* cells.[63] Thus, the *Forsythia* cell culture system is an efficient and promising platform for producing both endogenous and exogenous lignans by transgenic metabolic engineering.

These studies also suggest the potential for lignan production via metabolic engineering using *Forsythia* transgenic plants, due to the much greater biomass and higher amount of lignans in these plants compared to suspension cell cultures. Furthermore, *Forsythia* rapidly grows and propagates from a small cut explant without the requirement of flowering or seed formation. Several studies on the construction of *Forsythia* transgenic plants indicate the possibility of generating lignan-producing *Forsythia* transgenic plants,[67−69] although it should be noted that the transformation efficiency of *Forsythia* plants is markedly low and varies among *Forsythia* species.[67−69] Improvements in the transgenic methods of *Forsythia* species, combined with the aforementioned phytochemical and biological characteristics, will lead to the establishment of on-demand lignan production systems using *Forsythia* transgenic plants.

■ METABOLIC ENGINEERING OF *LINUM* SPP.

The acquisition of podophyllotoxin mainly depends on extraction from the roots and rhizomes of *Podophyllum* plants. Consequently, several natural podophyllum spp. (e.g., *P. hexandrum*) have become endangered due to overharvesting. Such limitations suggest the need to develop the sustainable and efficient production of (−)-podophyllotoxin using other plants. For example, podophyllotoxin is contained at 1.5 mg/g in immature and mature (but not juvenile) leaves of the Eastern Red-cedar, *Juniperus virginiana*.[70] In addition, large amounts of podophyllotoxin have been found in the leaves and rhizomes of the American mayapple, *Podophyllum peltatum*.[71] It should be noted that leaves are renewable organs, suggesting that the *P. peltatum* leaves are promising sustainable platforms for podophyllotoxin production. Currently, domestication, micropropagation, and in vitro and in situ conservation of germplasms of this genus have been established. Moreover, the *P. peltatum* geodatabase provides information regarding phenotypes, genotypes, lignan contents, and environmental factors.[71] Combined with verification of a total biosynthesis pathway for podophyllotoxin, these data should prompt the development of either transgenic or nontransgenic podophyllotoxin production.

Linum spp. (flax, Linaceae) are annual flowering plants comprising approximately 200 species. This genus has received pharmaceutical and medicinal attention due to the presence of various lignans, including (−)-podophyllotoxin and its related compounds, which are practically applied for the semisynthesis of antitumor drugs for breast and testicular cancers. Because *Linum* is also known to biosynthesize (−)-podophyllotoxin and its derivatives, and the procedures for tissue and cell culture are well established, optimal conditions and stimulating factors for production of various lignans, including (−)-podophyllotoxin, by *Linum* calli, suspension cell cultures, and roots have been extensively investigated.[72,73] For instance, suspension cell cultures of *L. linearifolium* were shown to yield 5.38 mg/g DW (−)-podophyllotoxin after 30 days in culture, which is higher than (−)-podophyllotoxin production by suspension cell cultures of *L. album*, *L. nodiflorum*, and *L. tauricum*.[73] Recently, (−)-podophyllotoxin production in *Linum* was also found to be affected by light, as seen in (+)-pinoresinol and (+)-sesamin production by suspension cell cultures of *Forsythia*.[66] A suspension of *L. album* cells produced 2-fold more (−)-podophyllotoxin under red light compared to a suspension of cells cultured under no light,[74] although the underlying molecular mechanisms remain to be understood. Moreover, a recent study verified the functional implication of plant hormones in lignan biosynthesis. Treatment of developing *L. usitatissimum* with abscisic acid resulted in 3-fold more accumulation of (+)-secoisolariciresinol diglucoside and up-regulation of PLR gene expression, compared to untreated plants.[75] In contrast, both (+)-secoisolariciresinol diglucoside accumulation and PLR gene expression were considerably suppressed in the presence of fluoridone, an inhibitor of abscisic acid synthesis.[75] Such abscisic acid-regulated secoisolariciresinol diglucoside biosynthesis is compatible with the fact that the promoter region of the *L. usitatissimum* PLR gene encompasses abscisic acid-responsive elements and binds to nuclear proteins in response to abscisic acid.[76] These findings support the notion that the addition of plant hormone is an effective tool for enhancing lignan biosynthesis engineering.

Metabolic engineering of *Linum* was also attempted for another endogenous lignan, hinokinin (Figure 2). The transient transfection of cultured *L. corymbulosum* hairy roots with PLR-RNAi resulted in a marked reduction of (−)-hinokinin.[53] As shown in Figure 2, PLR converts pinoresinol into secoisolariciresinol, which is in turn dehydrogenated into matairesinol by SIRD. Together, these findings provide evidence that (−)-hinokinin is mainly biosynthesized from matairesinol yielded by SIRD via PLR metabolism of pinoresinol. In other words, PLR-directed conversion of pinoresinol into secoisolar-

iciresinol is concluded to be a rate-limiting step in hinokinin biosynthesis, at least in the hairy roots of *L. corymbulosum*. The identification and genetic manipulation of hinokinin synthase would contribute a great deal to the elucidation of the biological roles of hinokinin and to the establishment of procedures for the direct metabolic engineering of hinokinin. In addition, the 7-*O*-glucosylating and 6-*O*-methylating activities in podophyllotoxin-related lignans were observed in cell suspension cultures of *Linum nodiforum*.[76,77] The draft genome of *L. usitatissimum* will accelerate the identification of the enzymes involved in the biosynthesis of *Linum* lignans.[58]

Solanum sogarandinum glycosyltransderase 1 (SsUGT1) is responsible for the 7-*O*-glycosylation of flavonoids and anthocyanidins.[78] Stable transformation of *L. usitatissimum* with SsUGT1 produced more lignans and proanthocyanins in the seeds compared to the wild type.[79] This finding supports the view that glycosylation is the limiting step for lignan production in planta, probably via sequestration to vacuoles, as in the case of flavonoids.[79] Also of interest is the fact that transgenic *L. usitatissimum* is conferred with higher resistance to infection by pathogenic fungi, *Fusarium culmorum* and *Fusarium oxyporum*.[79] Although the molecular and functional correlation between increased plant metabolites and resistance to pathogens has yet to be investigated, these findings suggest the unexpected benefits by metabolic engineering via introduction of an exogenous gene.

In vitro cell/tissue culture systems enable the simple manipulation of biosynthetic enzymes and optimization of cultivation parameters. Moreover, they possess the special advantage of productivity due to their high growth rate and short proliferation cycle under optimized conditions, although, in general, cells produce considerably less lignans than plants. Plants contain 10-fold more lignans per unit of dry weight than suspension cell cultures and also have a much larger biomass. Thus, future studies are needed to determine if both cell/tissue cultures and plants can be effectively employed as lignan-producing platforms. Furthermore, in both systems, efficient procedures for generating transgenic plants are needed, given that no such technologies are currently available.

CONCLUSION

Recent studies on lignans have made the following extensive and fruitful advances: (i) elucidation of biosynthesis pathways of dietary and medicinal lignans; (ii) elucidation of the biological actions of lignans in mammals and, in part, the underlying molecular mechanisms; (iii) identification of positive factors for production of lignans by cultured cells or plants; (iv) emergence of metabolic engineering of lignans via transient or stable transfections of lignan biosynthetic genes into cultured cells or tissues of *Forsythia* and *Linum*; (v) demonstration of metabolic engineering using transgenic plant cells as markedly effective for producing both endogenous and exogenous lignans of interest. Moreover, the genome database of lignan-producing plants and genomic and transcriptomic analyses using next-generation sequencers are expected to facilitate the molecular and functional characterization of enzymes responsible for unidentified lignan biosynthetic pathways, which in turn has expanded the variety of lignans produced via transgenic plant-based metabolic engineering. Taken together, these recent advances in lignan studies have paved the way for the drastic conversion of "agricultural lignan production" into "industrial lignan production" using transgenic plants. No lignans produced by transgenic plants have ever been commercially available. Furthermore, the production of supplement lignans via transgenic plants may raise concerns with regard to "transgenic products". Nevertheless, lignans produced by transgenic plants or cells are chemically identical to natural ones and free from any recombinant genes or proteins. Thus, their public acceptance is expected to be more easily garnered than that of transgenic foods. Accordingly, the establishment of efficient procedures for the generation of transgenic lignan-rich plants will surely enhance the industrialization of efficient, sustainable, and reproducible lignan production in a plant factory using transgenic plants.

AUTHOR INFORMATION

Corresponding Author

*(H.S.) E-mail: satake@sunbor.or.jp. Phone: +81-75-962-6092. Fax: +81-75-962-2115.

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ABBREVIATIONS USED

CYP, cytochrome P450; DW, dry weight of cells; PIP, pinoresinol-lariciresinol/isoflavone/phenylcoumaran benzylic ether reductase; PLR, pinoresinol/lariciresinol reductase; RNAi, RNA interference; SIRD, secoisolariciresinol dehydrogenase; Ss, *Solanum sogarandinum*; UGT, UDP-sugar-dependent glycosyltransferase

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