（様式５）

	主	副	副
指導教員 承 認 印	㊞	㊞	㊞

学 位 （ 博 士 ） 論 文 要 旨

論文提出者	生物システム応用科学府　生物システム応用科学専攻 博士後期課程　　生物システム応用科学　　専修 平成　24　年度入学 氏名　　諾恩達古拉　　㊞				
主指導教員 氏　　名	梶田真也	副指導教員 氏　　名		副指導教員 氏　名	
論文題目	Studies of the gene for NADPH-dependent oxidoreductase involved in the biosynthesis of plant secondary metabolites				

論文要旨（和文要旨(2000 字程度)または英文要旨(500words)）

Lignans, including neolignans and norlignans, are phenylpropanoid-derived secondary metabolites found in a wide range of plant species with varied content and composition. It has been presumed that secondary metabolites such as these are involved in defense against biotic and abiotic stresses and physiological integrity of plant cells, but few roles and functions have been clarified. Lignan and neolignan have two C6-C3 units, the former linked by an 8–8′-bond and the latter linked by carbon-carbon bond(s) at other positions. Studies showing advantageous effects of the natural compounds on human health have attracted much attention to lignan biosynthesis processes as a focus of recent research.

Phenylcoumaran benzylic ether reductase (PCBER), a member of the isoflavone reductase (IFR) family, is thought to be an enzyme crucial in the biosynthesis of 8–5′-linked neolignans. It has been isolated and characterized in several plant species, as the enzyme responsible for conversion of dehydrodiconiferyl alcohol (DDC) and isodihydrodehydrodiconiferyl alcohol (DDDC) to isodihydrodehydrodiconiferyl alcohol (IDDDC) and tetrahydrodehydrodiconiferyl alcohol (TDDC), respectively. PCBER has a close phylogenetic relationship to pinoresinol-lariciresinol reductase (PLR), which catalyzes the two reduction steps of typical lignans pinoresinol (PR) and lariciresinol (LR).

Although genes that encode polypeptides with sequences similar to identified PCBERs are found in the genome of *Arabidopsis thaliana*, no functional characterization studies have been reported thus far in this species. In this study, we characterized a putative PCBER (At4g39230, designated *AtPCBER1*) in *A. thaliana*. We cloned cDNA and the promoter region of *AtPCBER1* from *A. thaliana*. At the amino acid level, AtPCBER1 shows high sequence identity (64–71 %) with PCBERs identified from other plant species. Expression analyses of *AtPCBER1* by reverse transcriptase-polymerase chain reaction and histochemical analysis of transgenic plants harboring the promoter-GUS construct indicate that expression is induced by wounding and is expressed in most tissues, including flower, stem, leaf, and root. Catalytic analysis of recombinant AtPCBER1 with neolignan and lignans in the presence of NADPH suggests that the protein can reduce not only the 8–5′-linked neolignan, DDC, but also 8–8′ linked lignans, PR and LR, with lower activities. To investigate further, we performed metabolomic analyses of transgenic plants in which the target gene was up- or down- regulated. Our results indicate no significant effects of *AtPCBER1* gene regulation on plant growth and development; however, levels of some secondary metabolites, including lignans, flavonoids, and glucosinolates, differ between wild-type and transgenic plants. Taken together, our findings indicate that *AtPCBER1* encodes a polypeptide with PCBER activity and has a critical role in the biosynthesis of secondary metabolites in *A. thaliana*.