

学 位 論 文 要 旨	
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題 目	Functional analysis of <i>Arabidopsis</i> LBP/BPI related-1 and -2 in lipopolysaccharide-induced plant defense responses (LPS に対する防御応答におけるシロイヌナズナ LBP/BPI 関連タンパク質-1 と-2 の機能解析)
<p>Lipopolysaccharide (LPS), a major constituent of the outer membrane of Gram-negative bacteria, acts as a pathogen-associated molecular pattern for both animals and plants. LPS-binding protein (LBP) and bactericidal/permeability-increasing protein (BPI), which bind to LPS and play important roles in immunity of mammals, have been well studied. However, the molecule contributing to LPS binding in plants is mostly unknown. The <i>Arabidopsis</i> genome carries two genes encoding LBP/BPI-related proteins which we designated as AtLBP/BPI related-1 (AtLBR-1) and AtLBP/BPI related-2 (AtLBR-2). I found that their N-terminal domains were co-purified with cell wall derived LPS when expressed in <i>E. coli</i>. Since this finding implied the direct binding of AtLBRs to LPS, I also confirmed binding by using LPS-free AtLBRs and purified LPS. AtLBRs directly bind to both rough and smooth types of LPS. I also demonstrated that LPS-treated <i>atlbr</i> mutant <i>Arabidopsis</i> exhibit a significant delay of induction of defence-related gene <i>pathogenesis-related 1</i> (<i>PR1</i>) but no other <i>PR</i> genes. Furthermore, LPS-treated <i>atlbr</i> mutants showed defects in reactive oxygen species (ROS) generation. These results demonstrate that, as well as LBP and BPI of mammals, AtLBRs also play an important role in the LPS-induced immune response of plants.</p> <p>AtLBR-2, in particular, located in apoplastic region, which is an important place for plant-pathogen interactions, and exhibited a high LPS-binding affinity. I investigated the role of AtLBR-2 in more detail by comprehensive transcriptomic analysis using mRNA sequencing (RNA-Seq) technology. RNA-Seq data analysis revealed that LPS treatment significantly altered the expression of 2,139 genes, with 605 up-regulated and 1,534 down-regulated genes in WT. Comparative analysis of differentially expressed genes between WT and <i>atlbr-2</i> mutant revealed that 65 genes were identified as a AtLBR-2-dependent up-regulated genes. Gene ontology (GO) analysis demonstrated the importance of these 65 genes for the enrichment of some defense-related GO terms, including responses to bacterium, wounding, drug, abscisic acid stimulus, and salicylic acid (SA) stimulus. In fact, among of these 65 genes, 14 genes, including <i>PR1</i>, are known to be induced by SA. Therefore, I investigated whether LPS-induced SA accumulation levels were altered in <i>atlbr-2</i> mutants. I found that the accumulation levels of SA glucoside (SAG) between WT and the <i>atlbr-2</i> showed significant differences at 8 h after LPS treatment. Furthermore, I observed the up-regulation of <i>PR1</i> in the SA-treated <i>atlbr-2</i> to be at the same level, as or more, than that of WT plants. These results suggested the existence of an SA (SAG)-mediated LPS signaling system via AtLBR-2. AtLBR-2 might be a key molecule that is indispensable for the up-regulation of defense-related genes and for SA signaling pathway. This study is the first to demonstrate the importance of AtLBRs in LPS-induced plant defense responses.</p>	