氏　　　　名	SOBHY SALAMA HUSSEIN ABDELSALAM
授与した学位	博　士
専攻分野の名称	農　学
学位授与番号	博甲第　　　６２７８　　　号
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学位授与の要件	環境生命科学研究科　　　農生命科学専攻 （学位規則第４条第１項該当）
学位論文の題目	Identification and characterization of effector candidate protein genes of *Rhizoctonia solani*, a causal agent of sheath blight disease using *Brachypodium distachyon* （ミナトカモジグサを用いた紋枯病菌のエフェクター候補遺伝子の同定と機能解析）
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学位論文内容の要旨

　　Rice sheath blight disease caused by a necrotrophic fungus *R. solani* inflicts losses of up to 50% yield. No rice cultivars with durable resistance to *R. solani* exist. To develop crop protection technologies, understanding of infection mechanism of *R. solani* is mandatory. For this aim, employment of model plants is useful. *Brachypodium distachyon* is an experimental cereal plant which was shown to be infected by *R. solani*. Unlike rice, two *B. distachyon* accessions were resistant to *R. solani* by inducing a disease resistance response which mainly acts against biotrophs, suggesting that *R. solani* may pass through a short biotrophic phase in which small secreted effector proteins are used to suppress host immunity before killing host plants. To verify this hypothesis, this study aimed to identify the potential secreted effector proteins in *R. solani* using the model plant *B. distachyon*.

　　Identifications of effector protein genes of *R. solani* on rice during 10–72 hours post inoculation (hpi) have been reported previously using RNA-sequencing technique, however, the number of the early expressed effectors was thought to be limited due to scarcity of the fungal RNAs in plant tissues. To overcome this issue, an improved inoculation method was developed in this study. Firstly, the number of inocula on a single detached leaf of *B. distachyon* Bd2 (a susceptible accession) was increased from 1 to 3. Secondly, a sheet was inserted between the inocula and leaves to increase synchronicity of the infection hyphae. Then the inoculated leaves were harvested at 4, 6, 10, 16, 24, and 32 hpi and subsequently used for RNA extraction for cDNA synthesis and qRT-PCR. The fungal RNA was traced over the time course using 18S rRNA and it could be reproducibly detected from 6 hpi. Hence, this infection method enabled to study early expressed genes in *R. solani* with higher sensitivity compared with the previous approach.

　　The potential effector protein genes of *R. solani* were then predicted using the genome sequence and bioinformatics tools and 88 *R. solani secretory effector-like protein genes* (*RsSEPGs*) were identified. The gene models of *RsSEPGs* were calibrated with RNA-sequencing data and most of their predictions needed to be corrected. This result suggested the difficulty of prediction of gene model for small secreted proteins. In the end, 68 *RsSEPGs* were identified and 52 genes out of them were successfully detected with variable and phase-specific expression patterns during the time course. In this study, the expression of 22 *RsSEPGs* were newly characterized. These *RsSEPGs* were classified into 6 clusters with their expression patterns. Clusters 1–3 comprise 23 *RsSEPGs* whose expression levels mainly peaked before 24 hpi, and the remaining clusters 4–6 contain 29 *RsSEPGs* whose highest expression levels were at 24 or 32 hpi. Because disease symptom was observed from 24 hpi in the developed infection system, *RsSEPGs* in the clusters 1–3 and 4–6 were expected to be involved in biotrophic and necrotrophic interactions, respectively. These results opened new avenues to study if *R. solani* employs effector proteins in its infection.

論文審査結果の要旨

　本研究は，イネ・トウモロコシ・サトウキビなどの単子葉作物の重要病害として知られる紋枯病の原因菌である*Rhizoctonia solani*の病原性機構を明らかにする目的で，特に本菌が宿主免疫を抑制するために用いる分泌タンパク質（エフェクター）に着目し，その同定を行った。イネ紋枯病の研究においても感染過程で発現する分泌タンパク質が注目され，遺伝子発現を指標にした同定が行われてきた。しかし，エフェクターが重要な機能を果たす感染初期については，サンプル中の菌由来RNA量が少ないためにその同定が困難であった。そこで本研究では，モデル単子葉植物ミナトカモジグサを利用し，接種源の数を増やすこと（菌由来RNAの増量）および接種源と葉の間にシートを挟むこと（感染ステージの同調性向上）により，従前より早い接種6時間後での発現検出を実現した。一方，紋枯病菌の全予測遺伝子から生物情報学的手法でエフェクター候補を88個抽出した。しかし，それらの機械構造予測の不正確性を見出したため，これをRNA-seqデータで補正し，最終的に68個のエフェクター候補を特定した。そしてこれらのうち52個の遺伝子において，感染過程における発現を検出した。このうち22個は新規に発現が示された遺伝子であった。発現パターンからこれらは6グループにクラスタリングされ，病斑形成の前と後に発現ピークを示す23個と29個に分類された。これにより，各々の植物免疫抑制と病斑形成への関与が推定された。感染後期に発現する13個がベンサミアナタバコでの一過的発現法に供され，そのうち3個が病斑誘導能を持つことが示された。

　本研究は紋枯病エフェクター候補遺伝子を新規に同定し，その病原性機構を分子レベルで解明する道筋を拓くもので，一定の学術的成果が認められる。学位審査発表会とその後の質疑応答においても，その内容の理解度が十分に確認されたことから，博士の学位に値するものと判断した。