論 文 の 内 容 の 要 旨

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Transcriptome analysis of the parasitic plant *Phtheirospermum japonicum*

（寄生植物 *Phtheirospermum japonicum* のトランスクリプトーム解析）

Angiosperms have acquired a parasitic lifestyle at least 12 times during evolution. A common feature of all parasitic plant is the presence of haustorium, a root feeding structure responsible for connecting the parasite to the host. Parasitic plants in Orobanchaceae cause serious agricultural problems in worldwide. *Phtheirospermum japonicum*, a member of Orobanchaceae, is a facultative parasite native in East Asia which infects a broad range of hosts. *P. japonicum* represented a good model for parasitism studies since it is suitable for genetic and reverse-genetic analyses, such as crossing and transformation.

In this study I established a large-scale transcriptome platform of *P. japonicum* to identify the genes involved in haustorium development. First I used next-generation sequencing technologies to *de novo* assemble the transcriptome of *P. japonicum* with enrichment of transcripts expressed in the haustoria tissue. The results were discussed in Chapter 2. In this experiment the whole root transcriptome of *P. japonicum* was newly assembled using two platforms, Roche 454 with longer reads (~300 bp) and Illumina Hi-Seq with short reads (~90 bp). Combined sequencing strategies helped increasing the quality of *de novo* assembly. At the end, 58,137 sequences were assembled, showing an average size of 811 bp. Out of them, 45,323 (78%) had predicted coding proteins and 29,767 (51.2%) blast hits against *Arabidopsis* genome. Evaluation of the coverage rate showed that *P. japonicum* assembly covered all the 357 highly conserved single-copy orthologs found in eukaryotic genomes, showing that the assembly presented a reasonable coverage of *P. japonicum* transcriptome. Gene Ontology analysis revealed that genes in the category of “structural molecules activity” and “ribosome” cell compartment were overrepresented in the haustoria tissues, suggesting extensive protein synthesis for parasitism. In addition, my results showed

that the expression profile of quinone reductase 1 (*QR1*), a gene described as necessary for haustorium development in the facultative parasitic plant *Triphysaria vesicolor*, was unaltered by the contact of host or haustorium-inducing chemical. Instead, the related quinone reductase 2 (*QR2*) was up-regulated. Similar results were found in *S. hermonthica*, where *QR2* expression was higher in haustoria tissues compared with *QR1*, which was more expressed in reproductive structures.

Interestingly, among the transcripts enriched from the parasitic stage, many of them were annotated to encode subtilases (SBT). Thus I decided to further investigate the role of these enzymes in plant parasitism and the results are discussed in Chapter 3. The subtilase family comprehended one of largest protein families in plants. It has been suggested that *SBT* appeared in the genome of land plants through a single event of horizontal gene transfer from a bacterial, followed by rapid duplication events. SBT is composed of a signal peptide, prodomain, catalytic, PA and Fn-III domains. It is translated as pre-pro-protein with a signal peptide at the N-terminal end targeting to the apoplastic region. To be translocated to outside of plant cell, a maturation step, in which the prodomain is cleaved, is required. I monitored the *SBT* expression pattern by qRT-PCR during the interaction of the parasite *P. japonicum* with a host (*O. sativa*) or a nonhost (*L. japonicus*) plant for 1, 2, 3 and 7 days of infection. The expression of five *SBT* genes (*PjSBT2,-4,-7,-8 and -11*) were detected only at 7 days after the interaction with the host, while no expression of these genes was detected in contact with nonhost *L. japonicus*. Morphological studies with of a haustorium stained by *Safranin-O* revealed that the formation of a vascular bridge, which connects xylem vessels between a host and a parasite, occurs after 7 days of interaction. Promoter analysis of a *PjSBT* gene showed that its expression was localized at the interacting site in haustoria with the host at penetrating stage. The parasitism-induced *PjSBTs* are the homologs of *Arabidopsis AtSBT* group 5, including symbiosis-induced *L. japonicus SbtS,* and *Arabidopsis AIR3* (At2g04160) involved with lateral root formation, indicating a possible shared mechanism in haustorium, nodulation and lateral root formation. In addition, the parasitism-induced *ShSBT1*, an *S. hermonthica* gene phylogenetically distant from *PjSBTs* of group 5 suggested that this gene may have had acquired a distinct function during the development of terminal haustoria.

In order to understand early molecular events associated with host perception on the first 48 h of haustorium development. I designed a custom microarray based on the assembled sequences. The gene expression was analyzed in 8 different time points upon the treatment with DMBQ (2, 6-dimethoxy-p-benzoquinone), a natural compound which induces haustorium *in vitro*. Comparison of gene expression profiles between DMBQ-treated and non-treated roots

identified 1577 differentially expressed genes divided into three clusters according to Self-Organizing Maps algorithm. Cluster 1 contains 706 genes negatively modulated along the time course. Cluster 2 has 396 positively regulated genes with a peak of expression before 3 h of treatment, designated as early responsive genes. Cluster 3 contains 475 positively regulated genes with a peak of expression after 3 h of treatment, designated as late responsive genes. Based on their best BLAST-hit annotations, the gene ontology (GO) terms were assigned for each gene, aiming to investigate functional gene populations in each cluster. Blast alignment indicated that large amount of parasite sequences did not have any similar sequence in available database, indicating that haustorium development is a rich material for discovery and further characterization of novel gene function.

Cluster 2 has the GO term “transcription factor activity” overrepresented compared with all the entities in the microarray, indicating that massive transcriptional reprograming occurs in the first 3 h of haustorium development. Based on this analysis I found that members of WRKY family, which are often involved in disease resistance in plants, were up-regulated suggesting that plant immunity system is activated on the first hours of haustorium development. In contrast, Cluster 1 showed higher percentage of sequences assigned as “other molecular functions”, 139 sequences out of 701. This cluster includes downregulated of genes encoding proteins responsible for lignification and strengthening of cell wall. Similarly, Cluster 3 is composed of late responsive up-regulated genes involved in cell-wall-modification, indicating that from 3 h to 48 h of induction of haustorium formation the parasite cells undergo intense restructuring, mirroring the morphological changes observed in the organ.

Transcription of plant hormone-related enzymes such as YUCCA, CKX3 and GA2ox8 was induced during the haustorium development. To further understand the involvement of auxin in plant parasitism I focused on the homologs of *Arabidopsis YUCCA* genes which encode key auxin biosynthesis enzymes. Four YUCCA homologs were identified from the *P. japonicum* transcriptome but only *PjYUC3,* which belongs to Cluster 3, was specifically up-regulated after the haustorium-inducing treatments. *P. japonicum* hairy roots transformed with the *PjYUC3* overexpression construct resulted in a typical auxin-overproducing phenotype, indicating that *PjYUC3* encodes a functional YUCCA enzyme. Promoter analysis showed that *PjYUC3* expression occurs in two different locations, at the root apical meristem tip and at the haustorium initiation site. *PjYUC3* is responsible for accumulation of newly-synthesized auxin specifically in epidermal and cortical cell of the emerging haustorium. Silencing *PjYUC3* provoked reduction of haustorium numbers, emphasizing its relevance for the haustorium

development. These results suggested that the *de novo* auxin biosynthesis by *PjYUC3* at the root tip and/or haustorium initiation region is essential for the haustorium development.

Finally, in the chapter 6 I described the transcriptome analysis that I have done in the tissues of the parasitic plant *P. japonicum* interacting with its host rice. I identified regulated genes in the parasitic as well as in the host tissues after 1 or 7 days of the host-parasite interaction. At the first step I generated Illumina Hi-Seq sequences and *de novo* assembled the *P. japonicum* transcriptome. The transcript levels were determined by mapping those reads against the assembled transcriptome. The rice transcripts were mapped against available rice cDNAs and expression levels were accessed. Finally, statistical analysis identified 2917 parasite and 1155 rice genes regulated during parasitism. These genes were divided into nine clusters according to their expression profiles. Co-expression analysis identified the hub genes which are highly co-expressed with other genes. Among them, a hub gene encoding the transcription factor CXX RXX FXXX (CRF) was highly up-regulated. CRF is a member of AP2/ERF transcriptional factor family. In both parasitic and host tissues, up-regulation of cell wall-modifying enzymes were observed, inferring intense modification of cell shape during the establishment of plant parasitism. To identify the rice genes similarly regulated during its interaction with *P. japonicum* and *S. hermonthica*, I compared the RNA-Seq data described in this chapter with published microarray data from *S. hermonthica*-interacting rice roots. This analysis revealed that the rice homeobox-leucine zipper transcription factor, Oshox16, is up-regulated after interaction with both parasitic plants. Taken together, I identified host and parasite genes relevant for host-parasite infections, which includes two transcription factor encoding genes.

In summary, I have established the whole genome-scale transcriptome of the model parasitic plant *P. japonicum*. Expression analyses using custom microarray and RNA-seq technologies revealed important genes involved in the haustorium development. Thus this work has enabled to obtain the first insight of molecular mechanism underlying the infection strategy by a parasitic Orobanchaceae plant. Future studies using this data set will help to combat against the other Orobanchaceae plants such as *Striga* and *Orobanche* that cause devastating agriculture losses in the world.