Comprehensive profiling and characterization of Arachis stenosperma (peanut) and Meloidogyne arenaria (plant-root nematode) small-RNAs identified during the course of the infection

Priscila Grynberg, Larissa A. Guimarães, Marcos M. C. Costa, Roberto C. Togawa, Ana C. M. Brasileiro, Patrícia M. Guimarães

Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Brasília-DF

Plant-parasitic nematodes have a worldwide distribution. They are virtually able to infest any human-cultivated plant. Annual losses caused by nematodes on life-sustaining crops are estimated to exceed 14% of the production (approximately 65 billion € of loss worldwide). Previously studies were responsible for major advances in the identification of genes and mechanisms responsible for plants response to the Meloidogyne, the root-knot nematode. Meloidogyne spp. are obligate endoparasites that maintain a biotrophic relationship with their hosts. During the infection root cells are differentiated into specialized giant feeding cells through the releasing of effector proteins. However, despite the continuing efforts to identify new effectors and plant resistance mechanisms, studies have shown that the repertoire of both systems is limited. Recently, researchers published strong evidence that small RNAs from a phytopathogenic fungus act as effectors. These small RNAs hijack the host RNA interference (RNAi) machinery by binding to Arabidopsis Argonaute 1 (AGO1) and selectively silencing host immunity genes. These findings gave new insights on nematode-plant interaction as well as for the development of new control strategies through biotechnological methods. The goal of this work is to verify the possible role of Meloidogyne arenaria small RNAs (sRNA) as effectors by identifying, in Arachis stenosperma (peanut), downregulated target genes during the infection. A. stenosperma plants were infected with approximately 5,000 M. arenaria larvae in triplicate. Control and infected A. stenosperma roots were collected 3, 6 and 9 days post-infection. The infected samples were pooled. SIx samples (3 controls, 3 infected) and two M. arenaria J2 small-RNA libraries were sequenced with technical replicates using Illumina HiSeq 2500 system. After adaptor removal, reads were submitted to Infernal 1.1. This program uses Rfam as database. The plant samples output was used as input to test two different plant microRNA prediction tools. For A. stenosperma control samples, 325 (65 known) and 1255 (56 known) miRNAs were predicted by miRDP and miR-PREFeR respectively. 34 known and 177 unkown miRNAs were predicted by both tools. For peanut infected samples, 275 (34 known) and 1187 (32 known) miRNAs were predicted by miRDP and miR-PREFeR respectively. 21 known and 157 unkown miRNAs were predicted my both tools. Next steps include: 1) to perform small-RNA prediction at the nematode samples; 2) to classify and validate the plant and nematode small RNAs; 3) to search for nematode small RNA targets in plant; 4) assessment of microRNA differential expression between controls and infected plant samples.