SmallRNAome characterization of a plant-fungal root symbiosis

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Motivation
Small RNAs (sRNAs) are short non-coding RNA molecules (20-30 nt) that regulate gene expression at transcriptional or post-transcriptional levels in many eukaryotic organisms, through a mechanism known as RNA interference (RNAi). Beside the well-established roles in development and stress responses, recent investigations have highlighted that sRNAs are also involved in interspecies, and even interkingdom, communication. In particular, concerning the interactions between plants and fungal pathogens or parasitic plants, recent data suggest that sRNAs can move across the contact surfaces, from one organism to the other. Once in the host cells, sRNAs can target the expression of specific mRNAs, sometimes triggering secondary sRNAs production and thus leading to a modulation of host metabolic pathways and defenses responses (Weiberg et al., 2013, Science; Shahid et al., 2018, Nature). Almost nothing is known about RNAi mechanism and sRNAs occurrence in Arbuscular Mycorrhizal (AM) fungi, a key component of the plant root microbiota, that can provide several benefits to host plants, as improved mineral nutrition and tolerance to biotic and abiotic stresses. In this work we aimed to characterize the smallRNAome of the R. irregularis-Medicago truncatula symbiotic association, with particular attention to the fungal component, and to understand whether a sRNAs-based communication system occurs between the symbiotic partners.
Methods
We sequenced with an Illumina platform 9 sRNAs libraries constructed from different biological materials: 3 from extra-radical mycelium (ERM; fungal structures outside the roots), 3 from mycorrhizal roots from which we removed the extra-radical mycelium (RM) and 3 from non-mycorrhizal roots (RC). Data analysis was performed integrating different bioinformatics approaches, summarized in Figure 1, that will allow a complete fungal smallRNAome characterization and bring to the identification of the hypothetical fungal sRNAs involved in host genes regulation. Briefly, after quality check and filtering, reads from ERM and RM libraries were mapped on fungal genome for sRNA-generating loci discovery and characterization (including microRNAs-like prediction), differential expression (DE) analysis and fungal targets prediction. For the identification of plant genes potentially targeted by fungal sRNAs, we exploited a recently proposed approach (Shahid et al.,

2018, Nature) that allows to cope with the high false positive rates of targets prediction tools. The method relays on two steps; first, the identification of up-regulated plant mRNAs in the symbiotic condition (RM vs RC), that can denote the triggering of secondary sRNAs production induced by fungal interaction. Second, a targets prediction performed using as queries the fungal intra-radical (RM) up-regulated sRNAs as well as all the microRNAs. The reliability of identified sRNAs directed cleavages was validated using published degradome data collected on similar experimental conditions (Devers et al., 2011, Plant Physiol).

Fig.1 – Flowchart of bioinformatics pipeline. Softwares and R-packages used at each step are underlined.

Results

The analyses are still ongoing but preliminary results indicate that the mycorrhizal fungus R. irregularis is provided with a functional sRNAs population enriched in the 22nt-long molecules and that about two third of fungal sRNAs are originated by gene-coding sequences. We identified thousands of fungal sRNA-generating loci; few of them were classified as microRNA-like, and hundreds were induced in intra-radical phase. In the plant host, we observed dozens of genes characterized by a significant increased number of mapped reads during the symbiotic association suggesting a fungal-induced secondary sRNAs production.