LONG NON-CODING RNAS FROM PLANT-PARASITIC NEMATODE *BURSAPHELENCHUS XYLOPHILUS* ARE POTENTIALLY INVOLVED DURING THE INTERACTION WITH THE HOST

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INTRODUCTION

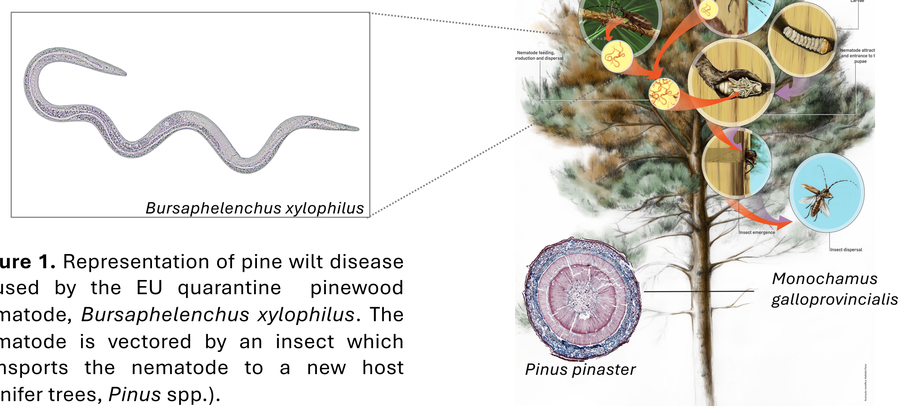

Figure 1. Representation of pine wilt disease caused by the EU quarantine pinewood nematode, *Bursaphelenchus xylophilus*. The nematode is vectored by an insect which transports the nematode to a new host (conifer trees, *Pinus* spp.).

Pinewood nematode (PWN), *Burspahelenchus xylophilus*, is **a migratory endoparasite** that causes major forestry losses worldwide. Non-coding RNAs (ncRNAs) play critical roles in cellular processes, including gene expression regulation, RNA processing, and modification, as well as the maintenance of chromosome stability. Due to their function as key regulators of different biological processes, long non-coding RNAs (lncRNA) can be involved in nematode virulence regulation, adaptation and pathogenesis. In this study, we explored the preditive regulatory role of lncRNAs in **pine wilt disease** (PWD) pathogenesis by PWN (Fig. 1).

METHODOLOGY

To characterize PWN lncRNAs that may mediate host-pathogen interactions we based our study in the sequencing of PWN-lncRNA libraries from nematodes exposed to pine extracts from a susceptible host, maritime pine (*Pinus pinaster,* PP), and a tolerant host - radiata pine (*Pinus radiata,* PR) (Fig. 2).

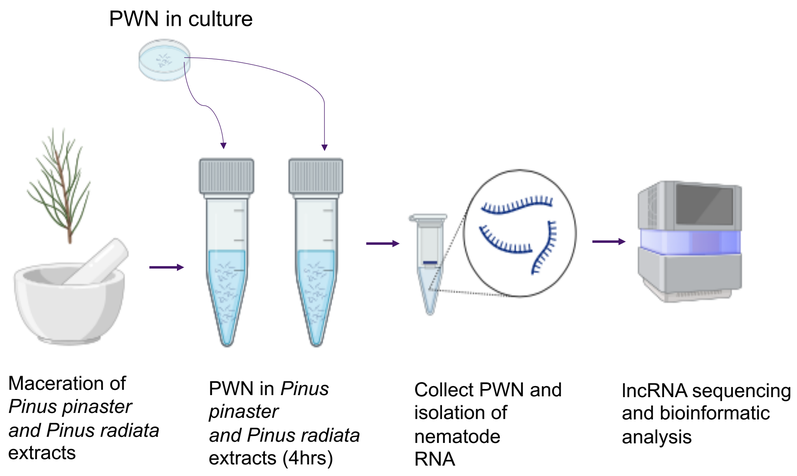

Figure 2. Cultured PWN exposed to two pine extracts (*Pinus pinaster* and *Pinus radiata*). PWN was collected and RNA was extracted with RNAeasy mini kit (Qiagen) and sequenced by HiSeq 2000 Illumina (Novogene, UK).

The **bioinformatic analysis** pipeline used in lncRNA-seq data: mapping to reference genome was performed using HISAT2. Transcript assembly, quantification and identification (novel_lncRNA) was performed using StringTie software. Prediction and classification of new lncRNAs was performed using FilterStat. A **positive correlation between a lncRNA and a mRNA** was defined as a **Pearson correlation greater than 0.70** (*p-value*<0.05). Functional enrichment analysis (GO) was performed on target genes of differential lncRNAs to predict their main functions.

RESULTS

Predicted PWN-lncRNAs

- Identification **of 925 novel PWN-lncRNAs**, from which, 300 are being differentially expressed during the interaction with the host (fig. 3).

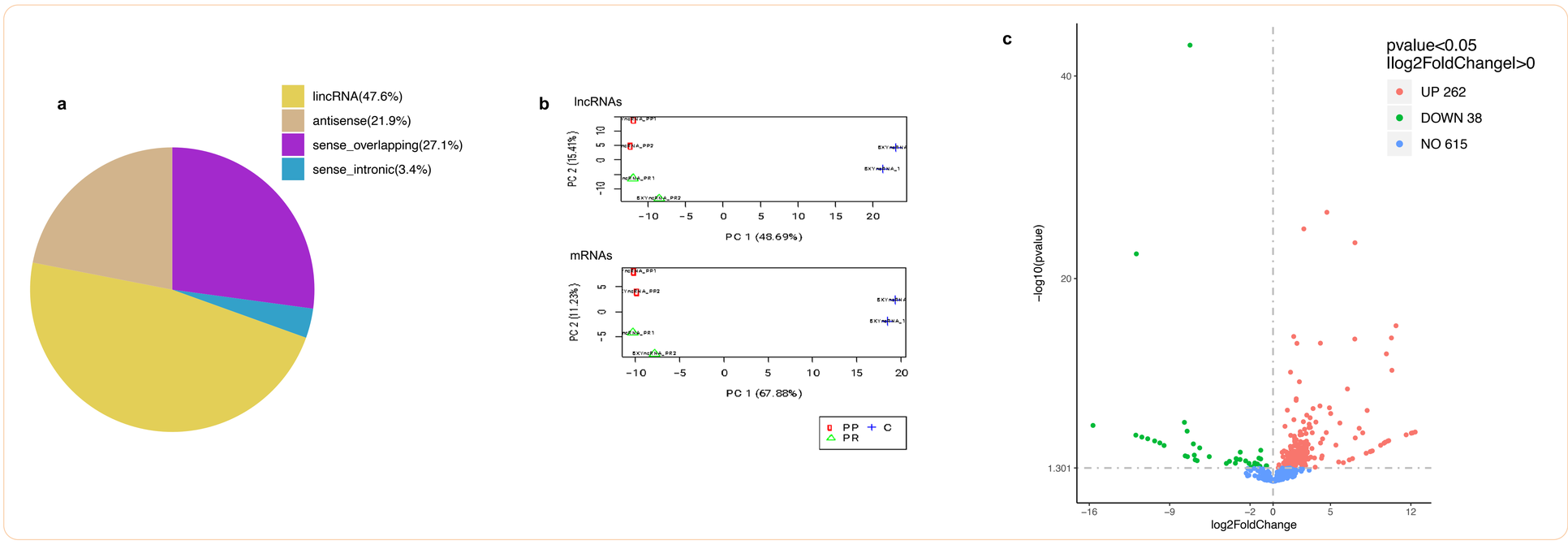

Figure 3. Classification of novel PWN-lncRNA. **(a)** Pie chart showing lncRNA distribution were divided into 4 types: (1) Antisense: overlap with one or more exons of protein-coding genes with opposite chains, or there is published evidence of reverse regulation of coding region genes; (2) lincRNA: non-coding RNA located in intergene region; (3) Sense overlapping: overlapped by one or more exons of the same protein coding gene; (4) Sense intronic: the intronic region of a coding gene that does not intersect with any exons on the same chain; **(b)** Principal component analysis showing the correlation between PWN-lncRNAs and mRNAs exposed to two pine extracts (PP, PR) compared to non-parasitic stage (C, control); **(c)** differentially expressed lncRNAs in PWN during exposure to the pine extracts of *P. pinaster* (PP) compared to the control (C). The threshold is *pvalue* < 0.05.

lncRNAs - mRNA co-expression network

- lncRNAs may have a potential co-expression between lncRNA and mRNAs which refers to the **transcriptional action on distant target genes** involved in iron ion binding, heme binding, oxireductase activity and peptidase activity (Fig. 4).

- Some of these genes are important during the interaction with the susceptible host and are highly abundant in the gland cells .

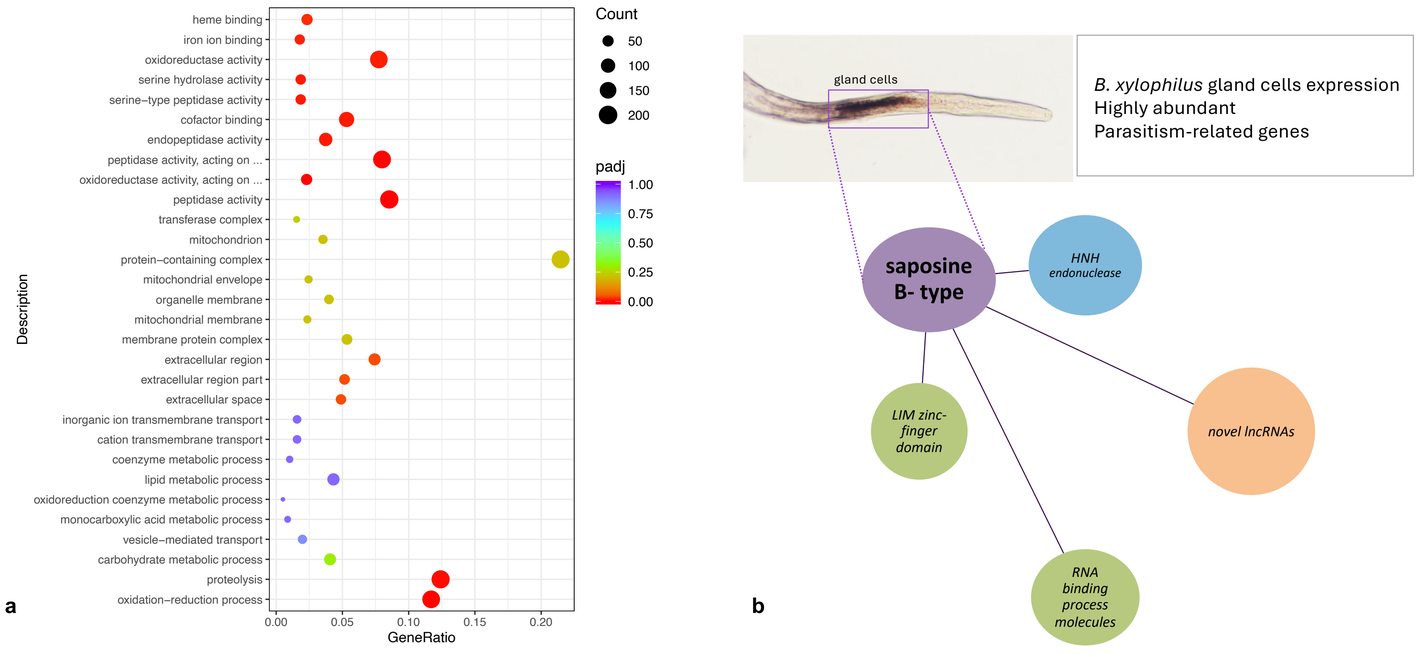

Figure 4. The PWN-lncRNA-coding genes co-expression **(a)** network in PWD (PP vs. C). GO functional enrichment analysis was performed in target genes of differential lncRNAs to predict their main functions. **(b)** Potential lncRNAs co-expression regulated the expression of *B. xylophilus* parasitism-related genes (like saposin type B) (Pearson correlation > 0.96; *p-value*<0.0005).

lncRNAs are emerging players in host-pathogen interactions. Differentially expressed PWN lncRNAs are correlated with protein coding genes involved in biological and celular pathways. By exploring the regulatory roles of PWN-lncRNAs in PWD pathogenesis, will open potential avenues for future research on how non-coding RNAs interact with the parasitism-related genes.

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