

## Functional characterisation of a key transcription factor regulating parasitism-related genes in the plant-parasitic nematode, *Bursaphelenchus xylophilus*

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Plant-parasitic nematodes (PPN) are major economic and ecological threats to crops and forest species worldwide. The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, is a quarantine organism responsible for the pine wilt disease (PWD), which leads to the decline of conifer species in Europe and East Asia. The lack of effective control measures for PWD highlights the urgent need for innovative and sustainable strategies targeting the parasite, including a deeper understanding of the molecular mechanisms of PWN parasitism. Interactions between the PWN and the host are mediated by parasitism proteins, primarily produced in specialised cells, known as gland cells (GC), that play a crucial role in nematode migration, defence and host immunity response. Based on a nematode transcriptomic comparative analysis (Espada *et al.*, 2016, 2018), we were able to select seven predicted genes encoding for transcription factors (TF), that were putatively more expressed in the GC tissues, compared to the whole-nematode body. Therefore, we hypothesised that these molecules may be involved in the regulation of the expression in parasitism genes. All seven candidate genes are expressed in the GCs and were validated by *in situ* hybridisation (ISH). One of the candidate TF, *BXY\_079*, was successfully knocked down by RNA interference. Transcriptomic data from the silenced TF, revealed that several parasitism-related genes were also significantly downregulated. When analysed together with PWN life-stage transcriptomic data (Tanaka *et al.*, 2019), these genes were found to be highly expressed during parasitic juvenile stages of PWN and are predicted to encode lytic functions that are associated with nematode feeding and migration during host interaction. Moreover, yeast one-hybrid functional analysis showed that *BXY\_079* binds to the promoters of genes involved in both lytic functions and cell signalling regulation, proving the direct regulation of the predicted parasitism-related genes. We suggest that silencing this regulatory element could simultaneously disrupt the expression of several parasitism-related genes and potentially reduce PWN infection. Exploring regulatory molecules in PPN could have a major biotechnological impact on the forestry system, as these regulators represent promising targets for genetic editing to achieve effective nematode resistance in plants.