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In silico detection of putative effectors of the DnaJ family in *Meloidogyne incognita*

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Graphical Abstract

Abstract.

Eukaryotic pathogens have developed the ability to colonize a variety of hosts, including animals and plants. In that sense, these organisms have developed proteins called effectors, which have the ability to manipulate innate immunity and host homeostasis (Whisson et al., 2007). For example, the Hopl1 effector of Pseudomonas syringae promotes bacterial virulence in hosts by suppressing plant defenses. One of the main characteristics of Hopl1 is the presence of a J domain (Jelenska et al., 2007). This domain characterizes the group of proteins called DnaJ (also called Hsp40). Members of this family are a heterogeneous group of multidomain proteins defined by the highly conserved J domain. They function as co-chaperones of the Hsp70 proteins and are involved in several essential cellular processes that include folding, degradation and protein refolding (Ito et al., 2016).

Although DnaJ effectors have been identified in phytopathogens of bacterial origin, so far this type of protein has not been reported in nematodes (one of the most important groups of plant parasites). For this reason, the present work aimed to explore the presence of DnaJ effectors in the parasitic nematode proteome of M. incognita plants through in silico analysis. In the first instance, a search was

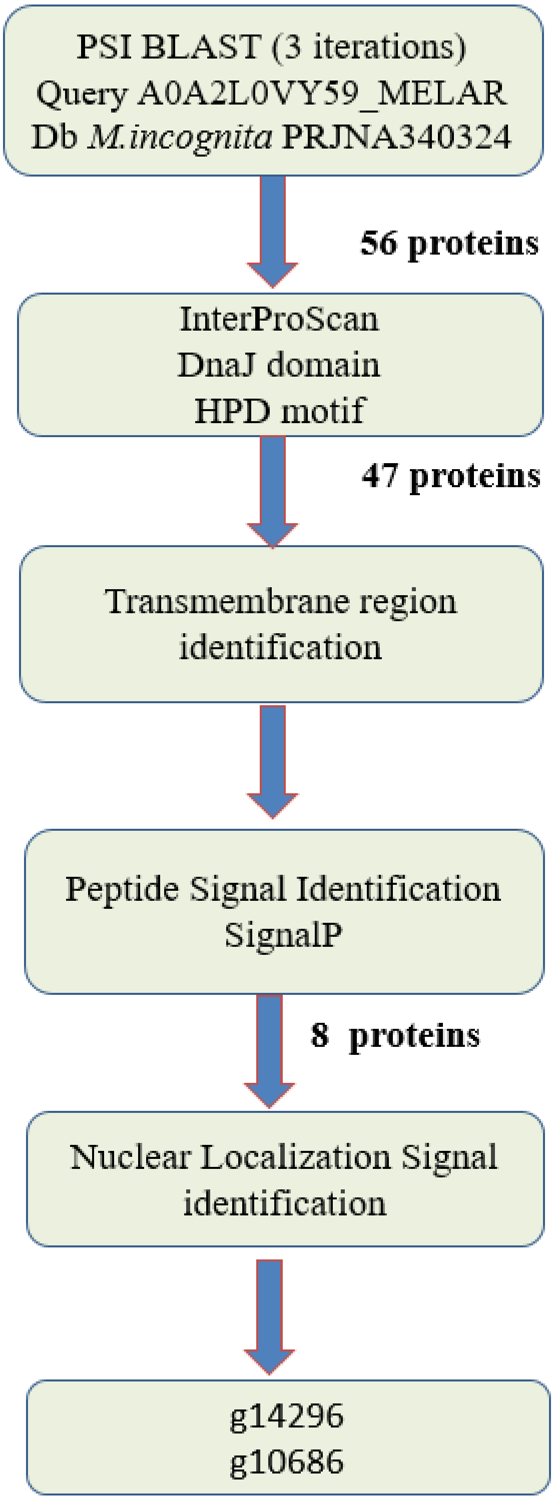

Figure 1 Flowchart of *in silico* detection of putative DnaJ effectors in *M.incognita*.

performed with PSI-BLAST (3 iterations), using the M. incognita proteome (PRJNA340324) as a database and the A0A2L0VY59_MELAR protein (annotated as DnaJ in the InterPro database) as a query (Figure 1). We identified 56 DNAj proteins but only 47 with a J domain with the conserved HPD motif. We searched within this group of proteins for signal peptide containing sequences, using SignalP program. Additionally, the transmembrane regions were detected using the TMHMM program. Eight putative extracellular proteins were identified with DnaJ annotation, signal peptide and without transmembrane regions. Finally, nuclear localization signals were predicted using NLS mapper software.. Two of the eight proteins obtained high prediction values for nuclear localization sites (g14296 and g10686).

Previous studies have identified effectors with similar characteristics to the candidates found in this work (Figure 2). The MiISE6 effector of Meloidogyne incognita has a signal peptide, an OGFr_N domain, and two NLS motifs to target the nucleus and facilitate parasitism in Arabidopsis (Shi et al., 2018). White, Potnis, Jones, & Koebnik (2009) determined that the conserved C-terminal portion of the TAL effectors of the genus Xanthomonas contains a nuclear localization signal (NLS) motifs which are essential for pathogen virulence and effects associated with the symptoms of the disease in the host. Likewise, Mueller et al. (2008) reported in Ustilago maydis secreted effector proteins, which also have nuclear localization signals (NLS). The functional analysis of the effector PsCRN63 from Phytophthora sojae showed that this is an inducer of cell death in the host which is secreted and has nuclear localization signals (Liu et al., 2011). These examples suggest that the presence of secretion signal and NLS characterize important effectors in different plant parasites.

In conclusion, 2 M. incognita proteins with characteristics frequently observed in effectors were identified. These proteins could be secreted from the nematode during infection and then transported to the host nucleus to alter their cellular processes and facilitate the infection. It should be noted that the effectors of the DnaJ family have not been previously reported in a parasitic plant nematodes. This work constitutes a basis for future in-plant studies and provides possible targets for nematode control.

Figure 2 Scheme of the g14296 and g10686 putative effectors of *M.incognita.*

Keywords: M. incognita, effector, DnaJ, signal peptide, nuclear localization signal

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