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(140) Gene silencing in *Bursaphelenchus xylophilus*: knock down of a calponin gene and its effect on nematodes movement

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INTRODUCTION

Post-transcriptional gene silencing by RNA interference (RNAi) was first described in *Caenorhabditis elegans* (Fire *et al.* 1998) and occurs when double-stranded RNA (dsRNA) is recognized by an organism as foreign, triggering a chain of processes in which both dsRNA and its mRNA homolog are degraded preventing the synthesis of the encoded protein. RNAi became an established experimental technique to investigate the function of different genes and its application and efficiency in the pinewood nematode, *Bursaphelenchus xylophilus*, function genomics has also been studied (Park *et al.* 2008; Cheng *et al.* 2010; Kang *et al.* 2011; Li *et al.* 2011; Ma *et al.* 2011; Kang *et al.* 2012; Wang *et al.* 2012). There are also some evidences that RNAi can be used to confer resistance to engineered host plants that express dsRNA to target and silence specific nematode genes (Lilley *et al.* 2012).

New ways for the management of *B. xylophilus* are needed and gene silencing by RNAi is a potential strategy. The calponin gene (*unc-87*), in *C. elegans*, is required to maintain the structure of myofilaments in muscle cells of the body wall (Kranewitter *et al.* 2001). In the present study, the knock down of the calponin homolog *Bx-unc-87* was performed by RNAi to evaluate the role of this gene in *B. xylophilus* and the applicability of *Bx-unc-87* silencing as a control strategy for this nematode.

MATERIALS AND METHODS

The knock down of the *B. xylophilus* calponin homolog gene was carried out by soaking the nematodes in a solution containing dsRNA of the *Bx-unc-87* gene during 24 h. Afterwards, the phenotype of the nematodes was estimated by mobility and nematodes reproduction. The relative *Bx-unc-87* transcript abundance, after dsRNA treatment, was assessed by RT-PCR with SybrGreen using the ABI PRISM 7900HT Fast System (Applied Biosystems) and the Comparative C_T ($\Delta\Delta C_T$) method.

RESULTS

The dsRNA treated nematodes revealed some paralysis and uncoordinated movement in contrast to the regular and sinusoidal movement of the non-treated nematodes and reproduction was lower in treated nematodes. The reduction in the *Bx-unc-87* transcript abundance confirmed the effectiveness of *Bx-unc-87* gene knock down. Further studies are being conducted in order to improve the efficiency of the silencing effect.

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