Resumos do 58º Congresso Brasileiro de Genética • 11 a 14 de setembro de 2012
Rafain Palace Hotel e Convention Center • Foz do Iguaçu • PR • Brasil
www.sbg.org.br - ISBN 978-85-89109-06-2

Development of microsatellite-enriched genomic library for *Deladenus siricidicola*

Derami, MS[1,2]; Mantello, CC[1]; Souza, AP[1]; Sousa, VA[2]; Kestring, DR[2]; Aguiar, AV[2]; Penteado, SRC[2]
[1]Centro de Biologia Molecular e Engenharia Genética, UNICAMP, Campinas, SP; [2]Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Colombo, PR.
mariana.esd@gmail.com

Keywords: *Deladenus siricidola*, accessions, genomic-enriched library, microsatellite markers, clones.

Deladenus (Beddingia) siricidicola Bedding (Nematoda: Neotylenchidae) is a biological control of pine-killing woodwasp (*Sirex noctilio*) pest which attacks *Pinus*, causing loss of productivity and economic damage in activity forestry. *D. siricidicola* has two morphologically different forms: (1) a fungal feeding form; (2) a parasitic form. The fungal feeding form lives in the pine tree eating the symbiotic fungus (*Amylostereum areolatum*) injected into the tree by an adult *S. noctilio* female. The nematode can reproduce for many generations in the absence of the woodwasp. In the presence of *S. noctilio* larvae, *D. siricidicola* develop into parasitic adults. These infective adults penetrate into *S. noctilio* larvae and produce offspring which migrate as juveniles into the reproductive organs of the host causing a "sterilization of the female reproductive system". Currently, it has been observed slow growth of the isolates in the laboratory and also variation in the efficiency of field isolates, interfering in the control of the pine-killing woodwasp. The aim of this study was to develop molecular markers from a microsatellite-enriched library to characterize different accessions of *D. siridicola* and characterize the Brazilian population of the nematode. A genomic-enriched library with repeats of $(CT)_8$ and $(GT)_8$ was constructed from genomic DNA digested with *AFAI.* The amplification of fragments was performed by polymerase chain reaction (PCR) followed by insertion into a vector pGEM T-easy. Transformation with competent *E. coli* XL1-Blue cells were performed by electroporation. The positive clones were selected using *the* β-*galactosidase* gene and further were sequenced in both directions using the T7 and SP6 primers. The obtained sequences were edited using Chromas and Seqman (DNAStar). The microsatellite (SSR) regions were identified using the Simple Sequence Repeat Identification Tool (SSIT) and primer pairs were designed using Primer Select software (DNAStar) and Primer 3 Plus. A total of 48 positive clones were sequenced which 42 (87%) contained microsatellite sequences. It was identified 73 SSRs which dinucleotide motifs was the most abundant with 61 (84%) followed by 10 (13%) tetranucleotide and 2 (3%) trinucleotide. Most of the SSRs found were perfect 44 (69%) and the imperfect repeats were 20 (31%). Until now a total of 27 primer pair were designed with an efficiency of 56% starting from the 48 clones sequenced. These primer pairs will be characterized in different Brazilian accessions of *D. siridicola* and used for conservation and population genetic studies. Financial Support: Embrapa.