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(167) Analysis of Transcriptional Expression Variation of Pine Wood Nematode (*Bursaphelenchus xylophilus*) using EST-based Simple Sequence Repeats

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ABSTRACT

EST-SSR marker was used to study the transcription variation of Pine Wood Nematode (PWN) *Bursaphelenchus xylophilus*. The results imply that there is very low transcriptional expression variation level of *B. xylophilus* in China, and Chinese *B. xylophilus* were more likely to be introduced from Japan.

INTRODUCTION

Pine wilt disease was first detected in Nanjing in 1982 and then it has spread to 176 counties of 15 provinces (SFA 2013) in China. The EST-SSR marker could reveal the population genetics of individual species based on exon sequence. Thus, EST-SSR was used to identify the differential expressed genes of *B. xylophilus* from different areas aiming at addressing the relationship between various genetic population and spread route in *B. xylophilus*.

MATERIALS AND METHODS

138 *B. xylophilus* DNA samples assorted randomly and mixed equally to 3 DNA pooling samples, which from 59 infected areas in 12 provinces of China. And 2 DNA pooling samples consist of 6 DNA samples from Japan and 3 from America respectively and one *B. mucronatus* DNA pooling sample including 63 DNA samples from different areas of China. ESTs of *B. xylophilus* which 13357 from NCBI and 884 from our laboratory were preprocessed by EST-trimmer, removed vector by cross-match, spliced by CAP 3, and searched SSR loci by MISA (Hu 2005). All the EST-SSRs were designed primers by Primer 5.0, and pre-screened with one Chinese *B. xylophilus* DNA pooling for PCR, after

that, re-screened with 6 DNA poolings. The result of products detected by QIAxcel automatic gel electrophoresis analysis system (Wang 2009), and NTSYS-pc 2.10e was used for UPGMA cluster.

DISCUSSION

A total of 14241 ESTs of *B. xylophilus* were spliced, and 6945 Unigenes (non-redundant ESTs) were obtained. 237 Unigenes contained 265 SSR loci, comprising 76 types of repeat motif, which account for 3.41% of all Unigenes. The average distance between SSRs was 12.14 kb. In the SSRs, the tri-nucleotide repeat motifs were the most abundant (64.53%), followed by tetra-nucleotide repeat nucleotide motifs (20.38%). Further, 189 primers were designed and synthesized based on the above motif types, then verified with 5 DNA pooling samples of *B. xylophilus* and one *B. mucronatus* DNA pooling sample for PCR. The result of products showed that the products of 130 primers are clear and effective in *B. xylophilus*, and 120 primers are versatile in *B. mucronatus*. All of EST-SSRs showed no polymorphism in *B. xylophilus* from China, including 101 homozygous loci and 29 heterozygous loci, which indicate the SSRs developed from the exons have no obvious differentiation. However, there are 8 pleomorphic EST-SSRs developed in *B. xylophilus* from America and Japan, and *B. mucronatus* from China respectively, which showed higher polymorphic content in *B. mucronatus*. Above all, transcriptional expression variation level of *B. xylophilus* is very low, after invading China 30 years. The result of UPGMA dendrogram supports the view that *B. xylophilus* was introduced into China from Japan.

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