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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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