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(131) Analysis of genetic diversity of *Bursaphelenchus mucronatus* and *B.xylophilus* isolates based on ISSR markers

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ABSTRACT

Nine of 130 primers were collected by using PCR screening. The nine primers were used to amplify the genomic DNA of 11 *Bursaphelenchus mucronatus* parasitic groups from six provinces and of 10 *B.xylophilus* groups from 10 provinces. A total of 1111 clear strips were amplified with 1069 polymorphic strips, which reached a percentage as high as 96.2%. Primer PTY1424 amplified the maximum number of bands, whereas primer PTY888 amplified the least bands. The result showed that the genetic similarity coefficient range of the 21 nematodes was from 0.4014085 to 0.9436620. The DNA molecular dendrogram of these 21 groups were established through the un-weighted pair group method with arithmetic average (UPGMA) cluster analysis. The 11 *B.mucronatus* groups were gathered as one category when the similarity coefficient value was 0.715, whereas the 10 *B.xylophilus* groups were gathered as one when the coefficient was 0.755. However, the 21 groups divided into two species when the similarity coefficient was 0.52. No obvious difference was observed in the geographic relationship among the clustering results. The results of the inter simple sequence repeat (ISSR) markers can be used to effectively distinguish the genetic relationship between *B.mucronatus* and *B.xylophilus* groups. Based on the result of PCR, ISSR produced higher polymorphism on *B.mucronatus*as compared with *B.xylophilus* groups. The reason may be that compared with *B.xylophilus*, *B.mucronatus* are native species that have undergone geographical isolation for a long time. Further study is required to explain whether the genetic diversity of *B.mucronatus* is related to their pathogenic differentiation.

Key words: *Bursaphelenchus mucronatus*, *B.xylophilus*, genetic diversity, ISSR

INTRUDUCTION

Recent research supported that *B.mucronatus* has pathogenicity to pine trees, especially to those with adversity stress(Chen *et al* 2010; Zhang *et al* 2002, 2004). In this study, ISSR

was used to explore the genetic diversity between different *B.mucronatus* and *B.xylophilus* groups.

MATERIALS AND METHODS

Nematode sources: 11 *B.mucronatus* groups from 6 provinces and of 10 *B.xylophilus* groups from 10 infected areas of China.

PCR amplification primer: 100 primers (UBC800-UBC900) and 30 PTY primers.

Detection of PCR products: QIAxcel was used to detect PCR products.

CONCLUSION AND DISCUSSION

11 *B.mucronatus* groups and 10 *B.xylophilus* groups were analyzed by using the nine primers of ISSR. The results obtained 1111 amplified DNA fragments with the size ranging from 200 to 2000 bp (Fig.1). The band spectrum of these primers could clearly distinguish the geographical population of the 21 nematodes. Compared with *B.xylophilus* groups, *B.mucronatus* groups have more varieties. Considering the origin of these two kinds of nematode, *B.mucronatus* are widely distributed in Europe and Asia, whereas *B.xylophilus* originated in North America. *B.xylophilus* began to spread in Japan in the early 20th century, and to China in the 1980s. In China, *B.mucronatus* are native species, which have undergone geographical isolation for a long time. Such long isolation may be proven by their larger genetic variation. By contrast, *B.xylophilus* have spread to China for a short time, leading to higher genetic similarity between groups.

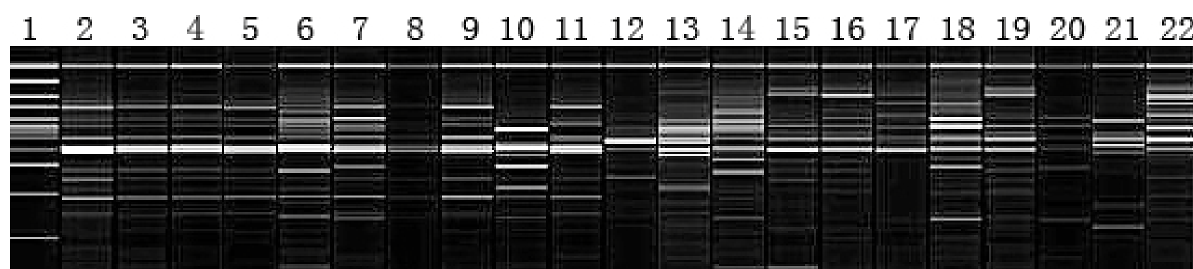

Fig.1 PCR-amplifiedfragments of 21isolated nematodes with primer PTY1425
1 : marker ; 2-11:*B.xylophilus* groups, 12-22: *B. mucronatus* groups

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