

Phylogeny of some Botryosphaeriaceae spp. and genetic diversity of dominant *Botryosphaeria dothidea* in China

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Abstract: The species-rich family, Botryosphaeriaceae (Ascomycota, Dothideomycetes), has a cosmopolitan distribution and causes canker and die-back on branches and stems of various important economically trees including poplar and fruit trees in China. The pathogen are commonly nominated according to the disease and host plant for long, which brings the same original with different names or different originals with the same name, and troubles of nomination and taxonomy of Botryosphaeriaceae. These problems hindered the effective control and management of disease. The type *Botryosphaeria dothidea* is one of the most common species in this family. This species had been found in many climate regions, and its occurring frequency is the highest of Botryosphaeriaceae. It is significant to find out genetic diversity and difference of *B. dothidea* populations for detecting and managing disease by this species.

This study was aim to investigate the pathogens of canker and die-back and the diversity of Botryosphaeriaceae in China, especially the botryosphaeriaceous fungi on poplar and rosaceous fruit trees. The morphology and phylogenetic analyses showed that the 58 isolates on poplar and 37 isolates on rosaceous fruit trees from five typical climate regions in China were considered as 8 or 9 species of Botryosphaeriaceae afflicting to five genera. *Botryosphaeria dothidea* populations had better genetic diversity (Nei's genetic diversity index $H= 0.6777$).

B. dothidea, *Neofusicoccum parvum*, *Diplodia seriata*, *D. mutila*, *Dothiorella viticola* and an undescribed new species, *Fusicoccum* sp.1 on poplar were identified based on morphology combined comparisons of DNA sequences for the ITS, β -tubulin and EF1- α regions. Fifty-three isolates were regarded as *B. dothidea*, the other four species have one isolates each. *B. dothidea*, *Lasiodiplodia pseudotheobromae*, *Neof. parvum/Neof. ribis* complex and another undescribed new species, *Fusicoccum* sp.2 on fruit trees were identified. There were 15 isolates were reported as *B. dothidea*, 3 isolates as *L. pseudotheobromae*, *Neof. parvum/Neof. ribis* complex each, and the other 6 isolates as *Fusicoccum* sp.2. *D. seriata* was firstly reported on poplar in China, and

Dothiorella viticola was observed on from *Cyclobalanopsis glaucoides* in this study as well as on poplar in a previous study. There was reason to suspect the corresponding relation between *B. rhodina* and *L. theobromae* according to the phylogenetic results based on β -tubulin sequence, and consider that the anamorphic *B. rhodina* is *L. pseudotheobromae*. These results showed that distribution of most isolates had no direct correlation with climate regions, excepting that *L. pseudotheobromae* was only found in South Asian tropical in China.

Comparison of cultural characteristics and phylogenetic relationships based on LSU region and ITS/EF1- α combined sequences supported the phylogenetic position of *Fusicoccum* sp.1 and *Fusicoccum* sp.2, belonging to *Botryosphaeria* complex. *Fusicoccum* sp.1 was closest to *Fusicoccum fabicercianum*. *Fusicoccum* sp.1 and *Fusicoccum* sp.2 appeared obviously different from the other species in *Botryosphaeria* complex based on cultural characteristics.

Botryosphaeria dothidea, the dominant species on both poplar and fruit trees, was located from mid-temperate to south semitropical zone, which indicated the differentiation potency adapting to different host and geography. To find out genetic differences and genetic diversity of *B. dothidea* population, ten polymorphic (Simple Sequence Repeat) SSR loci were selected for analyzing genetic diversity and genetic structure of 77 isolates from 5 climate regions, namely mid-temperate sub-humid climate region (12B), warm-temperate sub-humid climate region (13B), north-subtropical humid climate region (21A), mid-subtropical sub-humid climate region (22A) and south-subtropical humid climate region (23A). In this study on the 77 isolates, 5 populations were found to have a better gene diversity ($H = 0.6777$) and a low genetic variation ($F_{st}=0.0758$). Most of genetic variation was from inner population, while those between populations were low. Except 12B, the other 4 populations had unique alleles, and the number was 21, 2, 2 and 3. The reason for low genetic differentiation and high gene flow in 5 populations were probably that the locations of 77 isolates were the places with frequent human activities, and were directly assisted by movement with their host plant, especially poplar. Meanwhile, the unique alleles were found in different populations, including 407bp and 425bp of 21A, 424bp and 429bp of 22A, 234bp, 408bp and 416bp of 23A, and 313bp, 333bp, etc. of 13B. These unique alleles were very significant for detecting *B. dothidea* when latent in tissues.

Key words: Botryosphaeriaceae; *Fusicoccum*; SSR; genetic diversity; genetic differentiation