北京杨树炭疽病菌群体遗传多样性分析

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摘 要：北京地区杨树炭疽病发生严重，杨树行道树及散生林均受到不同程度侵染。已报到的杨树炭疽病病原为 *Colletotrichum gloeosporioides*。但近年来研究表明‘*gloeosporioides* complex’中包含多个炭疽菌新种，此外 *C. gloeosporioides* 菌株间在形态特征上、致病性等方面存在较大差异，常在鉴定上与相近种混淆，增加了防治的难度。为明确杨树炭疽病病原，本研究对 2009 年和 2010 年采自北京 4 个地区杨树病叶标本进行了采集，对病原菌进行了分离及纯化，并依据培养性状、形态学特征观察、多基因片段系统发育分析对病原菌进行了综合鉴定。利用离体叶片和活体植株作为寄主，通过喷雾接种了解炭疽菌菌株间致病性以及不同杨树（品）种间的抗病性差异。利用 SRAP 分子标记法研究 4 组群炭疽菌的遗传结构及其多样性，有利于对病害防范及抗病育种的进一步开展。本研究结果如下：

1. 从北京昌平、延庆、密云和石景山 4 地区的北京杨、青杨、黑杨及钻天杨的杨树炭疽病病叶标本中分离纯化得到 54 株炭疽病菌。通过形态及培养特征观察结果，将供试菌株分为两类，该分类结果与分子系统发育分析结果一致。鉴定结果为病原Ⅰ（50 个菌株）：*Colletotrichum gleosporidoies* (Penz.) Penz, & Sacc.,，该病原培养特征多变，附着胞圆形或卵圆形；病原Ⅱ（4 个菌株）：炭疽菌新种命名为 *Colletotrichum populi* C.M.Tian&Zheng Li sp. nov.，采自石景山地区，以钻天杨为寄主，新种培养形态单一，在 PDA 上长有白色羊毛状气生菌丝，分生孢子堆不明显，其附着胞呈不规则形状，瓣状。
2. 致病性测试结果表明，*C.gloeosporioides* 对杨树离体叶片、活体植株的致病性均强于 *C.populi*。

   从寄主抗病性角度评价离体叶片结果，表明四（品）种离体杨树叶片的致病性结果感病性程度为北京杨>黑杨>加拿大杨>毛白杨。
3. 利用 SRAP（sequence-related amplified polymorphism）分子标记技术对北京地区 4 个胶胞炭疽菌种群的遗传多样性和遗传分化进行了研究。4 对 SRAP 引物对 50 株胶胞炭疽菌进行了 PCR 扩增，共扩增出 63 条带，其中多态性带 61 条，占 96.83%。延庆 2 种群的遗传多样性最丰富，Nei 多样性指数（*h*）为 0.1825，Shannon 指数（*I*）为 0.2799。
4. 种群间遗传分化系数（*Gs*）为 0.2223,基因流（*Nm*）为 1.7497，说明种群间存在基因迁

移现象。种群间遗传相似性范围在 0.8960~0.9820，种群间遗传相似性高，亲缘关系近。利用非加权成组配对法（UPGMA）对 4 个群体进行聚类分析，种群基本以地理采集地聚类，个别菌株自聚一组，且与杨树寄主种无明显相关性。

关键词：杨树；炭疽病；系统发育分析；群体遗传；SRAP

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Identification, characteristics and population genetics analysis of pathogens causing poplar anthracnose in the Beijing region

ABSTRACT：Poplar anthracnose occurred seriously in Beijing region where poplars as street or scattered trees were infected extensively. *Colletotrichum gloeosporioides* has been reported as the pathogen to poplar. But recent researches revealed that on the one hand,'*gloeosporioides* complex' included several new *Colletotrichum* species, on the other hand, isolates of *C. gloeosporidoies* in morphology and virulence were in great difference which increases the difficulty of identification among similar species as well as disease control.To identify the pathogen(s), samples of infected poplar leaves in four districts of Beijing during 2009 and 2010 were collected, and the strains were isolated and purified for examining in morphological, cultural characteristics, multi-gene phylogenetic analysis and inoculation test. Detached poplar leaves and live saplings were selected as the host of treatment by spray inoculation to determine the differentiation of pathogenicity/resistant of isolates and poplar specie s respectively. In addition, genetic diversity and genetic differentiation of 4 *C. gloeosporioides* populations causing poplar anthracnose in Beijing region were analyzed by sequence-related amplified polymorphism (SRAP) for further development on disease control and poplar breeding. The main results are as follows:

1. Fifty four isolates of *Colletotrichum* were obtained from those samples from Chang Ping, Yan Qing, Mi Yun and Shi Jingshan, and the poplar samples were *Populus*×*beijingensis*, *Populus nigra*, *Populus cathayana* and *Populus nigra* var. *italica*. All the tested isolates were separated in two groups in term of morphological and culture features in conformity with

phylogenetic analysis .The identification results revealed two colletotrichum species were responsible to anthracnose including *Colletotrichum gleosporidoies* (Penz.) Penz, & Sacc.,, (50 isolates) with unconstant cultures and round or oval appressoria and *Colletotrichum populi* C.M.Tian&Zheng Li sp. nov.from Shi Jingshan district, host of *Populus nigra* var. *italica*, recognized as a new species with constant cultures (cottony aerial hyphae and indistintive conidia mass) producing irregular claved appressoria.

2. Inoculation test illustrated *C.populi* had weaker pathgenicity to poplar leaves compared with *C.gloeosporioides* in both leaves and saplings treatments. Disease susceptible species of poplar in descending order were *Populus×beijingensis*>*Populus Canadensis*>*Populus nigra*>*Populus tomentosa.*
3. Four pair primers screened from 36 pair-primers were applied in the polymerase chain reaction (PCR) of 50 individuals of *C. gloeosporioides* .Sixty-one polymorphic loci were observed from total 63 loci, accounting for 96.83%.The population from Yan qing 2 possessed the highest genetic diversity compared with the other populations, given that Nei's gene diversity (*h*) and Shannon's information index (*I*) were 0.1825 and 0.2799 respectively.
4. The genetic differentiation coefficient (*Gst*) among populations was 0.2223, and the gene flow (*Nm*) was 1.7497, implying the migration phenomenon. The range of genetic identity of 4 populations was from 0.8960 to 0.9820, indicating a high level of genetic similarity and a close relationship among populations. The analysis of unweighted pair group method with arithmetic mean (UPGMA) showed that the isolates were clustered on the basis of geographic origin, few of which ran into a separate group.

Key words: poplar; anthracnose; phylogenetic analysis; population genetics; SRAP