

## 梨黑斑病及抗病育种研究进展

杨晓平, 陈启亮, 张靖国, 范净, 何秀娟, 胡红菊\*

(湖北省农业科学院果树茶叶研究所, 武汉 430064)

**摘要:** 梨黑斑病是梨树重要的病害之一, 主要危害梨树叶片、新梢和果实, 引起梨树早期落叶和返青返花, 降低梨果品质和产量。笔者描述了梨黑斑病危害梨树叶片、新梢和果实的症状, 综述了侵染我国梨树的梨黑斑病病原菌的种类和田间发生规律; 重点在细胞壁降解酶和AK毒素研究方面阐述了梨黑斑病致病机制; 简述了梨种质资源抗黑斑病鉴定评价、抗病育种和梨抗病分子生物学等方面的研究进展。分析了国内外梨黑斑病病原研究及抗病育种研究方面存在的不足, 提出了下一步梨黑斑病病原研究及抗病育种研究的方向, 旨在为梨黑斑病的深入研究和综合防治提供参考。

**关键词:** 梨; 黑斑病; 病原学; 鉴定评价; 抗病育种

中图分类号: S661.2

文献标志码: A

文章编号: 1009-9980(2017)10-1340-09

### Research progress of pear black spot and breeding for disease resistance

YANG Xiaoping, CHEN Qiliang, ZHANG Jingguo, FAN Jing, HE Xiujuan, HU Hongju\*

(Research Institute of Fruit and Tea, Hubei Academy of Agricultural Science, Wuhan 430064, Hubei, China)

**Abstract:** Pear is one of the most important fruit crops in China, pear plantation areas were 1 124 040 hm<sup>2</sup>, and production was 18 698 560 t in 2015 according to Ministry of Agriculture statistics. Areas and production of the pear were the first in the world. However, pear diseases have become to a major constraint to the pear industry worldwide. Pear black spot disease, caused by *Alternaria* sp. is one of the most serious diseases in pears. Pear black spot is a widespread disease in world, which was serious occurrence in Japan, Korea and South Chinese pear producing areas. Pear black spot was first reported in Japan in 1933, and spread to the rest of the world. Pear black spot disease could cause necrosis on pear leaves, twigs, fruits and early falling leaves, reduce the productivity and quality of fruit. In high temperatures and high humidity areas, pear black spot disease could cause pear yield loss up. This disease is primarily controlled by application of synthetic fungicides. However, large-scale use of fungicides has potential toxic effects on humans and wildlife, and lead to environment pollution. Moreover, it is becoming increasingly difficult and costly due to heavy use of fungicides to ensure good quality fruit. Pear black spot pathogen was reported by many research scholars. *A. gaisen* Nagano was considered to be the pathogen of pear black spot in 1920 by Nagano. However, *A. kikuchiana* Tanaka was considered to be the pathogen of pear black spot in 1933, and many scholars used *A. kikuchiana* as pear black spot pathogen in a long time. Simmon used *A. gaisen* Nagano as pear black spot pathogen according to the nomenclature, and the *A. kikuchiana* as a junior synonym. *A. alternata* (Fr.) keissl could also cause pear black spot was reported in 1937, Nishimura suggested that *A. kikuchiana* might be pathotype of *A. alternata* according to its morphological studies. In 1993, Simmons thought that *A. kikuchiana* and *A. alternata* were two distinct species ac-

收稿日期: 2017-05-24 接受日期: 2017-06-30

基金项目: 国家自然科学基金(31601721); 现代农业产业技术体系(CARS-29-34); 湖北农业科技创新专项资金(2016-620-000-001-029); 农业部华中作物有害生物综合治理重点实验室·农作物重大病虫害防控湖北省重点实验室开放基金(2015ZTSJJ2)

作者简介: 杨晓平, 男, 副研究员, 博士, 主要从事砂梨种质资源鉴定评价、砂梨栽培育种和砂梨病虫害等方面的研究。Tel: 027-87770812, E-mail: yangxiaoping1981@163.com

\*通信作者 Author for correspondence. E-mail: hongju@sina.com

According to the morphological and toxin evidence of hundreds of strains of Japanese pear. At present, there are 9 species of *Alternaria* isolated from pear fruit, and 6 species were reported in China, they are *A. alternata* (Fr.) Keissler, *A. gaisen* K. Nagan, *A. tenuissima*, *A. yaliinficiens* R. G. Roberts, *A. infectoria* and *A. ventricosa* R. G. Roberts. Spores and mycelium of disease shoots, leaves and fruit were used by pear black spot pathogen to live through the winter. Overwintering spores were spread by rain in the spring of second year, and fall into the leaves of pear, germinated germ tube, grown along the pear leaf epidermis with suitable temperature and humidity conditions. The top of the germ tube expands to form an attachment to invade the pear leaf by stoma or wound. Under the stoma, the infected hypha was released into the nearby cells to absorb nutrients and water from the leaves of pear, and completed the primary infection. Host specific toxin AK-toxin was isolated from *A. kikuchiana* by Nakashima in 1982, and analyzed the composition and structure of AK toxin. AK toxin can cause physiological damage to the plasma membrane of host cells, and AK toxin is one of the main reasons pear black spot to infect the pear. In recent years, the pear black spot disease resistance of pear cultivars has been evaluated by the many domestic scholars. Different pear cultivars resistance to pear black spot disease were significantly different, however, the resistance to black spot disease of sand pear is relatively stable by different investigation methods. So we could find the resistance gene from sand pear cultivar. Plants often were invaded by various kinds of pathogens in growth and development, plants could recognize pathogen, and release substances, established defense system against external disease, gene encoding plant disease resistance protein is called resistance genes. Because of the long period of the pear tree, the genetic background is complex, and most of the varieties are self incompatible, the traditional breeding for disease resistance is of high cost and low efficiency, and the use of molecular breeding can shorten the breeding period and improve the breeding efficiency. In recent years, the research on the identification of disease resistance genes in pear has been carried out by using map based cloning, homologous cloning, and transcriptome sequencing. 5 213 differently expressed genes related to pear black spot resistance were obtained, 34 microsatellites were detected in these genes, 28 genes were found to be closely related to pear black spot resistance by transcriptome sequencing, which were reported in 2015. Pear black spot is one of the most important diseases of pear, many researches on pathogen and pear resistance to pear black spot disease were reported, but the overall research related to pear black spot was still lagging behind. The researches of pear resistant mechanism to the black spot disease, drug resistance mechanism of pathogen and pear black spot disease mechanism were still not thorough. Practical molecular marker assisted breeding of pear disease resistant and comprehensive prevention and control measures to pear black spot were still relatively few studied. This paper expounds pear black spot disease symptoms, pathogen, identification and evaluation of resistance to black spot of pear germplasm resources, molecular biology of pear resistance and others. This review provides a reference for the further research and integrated control of pear black spot.

**Key words:** Pear; Black spot; Pathogen; Identification and evaluation; Breeding for disease resistance

梨是我国主要的栽培果树,2015年中国种植业网统计,中国梨种植面积112.4万 $\text{hm}^2$ ,梨果产量1 869.9万t,面积和产量均占世界第一<sup>[1]</sup>。梨树病害严重制约了梨产业发展<sup>[2]</sup>,由链格孢真菌(*Alternaria* sp.)感染引起的梨黑斑病是梨树主要的病害之一。

梨黑斑病是一种广泛发生的世界性病害,在亚

洲的日本、韩国和中国南部砂梨产区发生严重。Tanaka<sup>[3]</sup>在1933年首次报道梨黑斑病发生;1999年在法国,Baudry等<sup>[4]</sup>报道了梨黑斑病发生;1935年在中国发现梨黑斑病<sup>[5]</sup>。梨黑斑病主要危害梨树叶片、新梢和果实,导致树体衰弱,结果年限缩短,给梨农造成严重的经济损失<sup>[6]</sup>;黑斑病还可以造成果实贮藏

期腐烂,在梨的进出口贸易中受到进口国的严密关注。进入21世纪后,随着‘翠冠’‘园黄’和‘黄冠’等优良梨品种推广,梨黑斑病的发生日益严重。目前防治梨黑斑病的方法是使用苯醚甲环唑、甲基托布津和代森锰锌等化学药剂,长期使用这些化学药剂,如果使用不当必然会导致病原菌的抗药性、污染环境和危害人体健康等问题<sup>[7-8]</sup>。为此,笔者从梨黑斑病危害症状、病原学、梨种质资源抗黑斑病鉴定评价及梨抗病分子生物学等方面,简述了梨黑斑病相关研究进展,以期为梨黑斑病的深入研究和综合防治提供参考。

## 1 梨黑斑病危害症状

梨黑斑病主要危害梨树的叶片、新梢和果实。

危害叶片,幼叶容易发病、形成褐色至黑褐色圆形斑点,随着时间逐渐扩大,形成近圆形或不规则形病斑,病叶开始焦枯、畸形,形成早期落叶。田间湿度较大时,病斑表面出现黑色霉层,为病菌的分生孢子梗和分生孢子(图1)。果实受到危害,果面出现1至数个黑色斑点,逐渐扩大,颜色变浅,形成浅褐色至灰褐色圆形病斑,略凹陷。发病后期病果龟裂、畸形,裂缝可深达果心,果面和裂缝内产生黑霉,引起落果。果实在成熟时染病,前期表现与幼果基本一致,但病斑较大,黑褐色,后期果肉软腐脱落。新梢受危害时,病斑初期为黑色,椭圆形,稍有凹陷,后期形成长椭圆形或不规则形、明显凹陷的黑色病斑,在病健交界处产生裂缝,病梢容易折断或枯死<sup>[9-10]</sup>。



A. 幼叶发病症状; B. 成熟叶片发病症状; C. 幼果发病症状; D. 成熟果实发病症状。

A. The symptoms of young leaves; B. The symptoms of mature leaves; C. The early symptoms of young fruit; D. The symptoms of mature fruit.

图 1 梨黑斑病田间侵染梨树叶片和果实症状

Fig. 1 Symptoms on leaves and fruit of sand pear caused by pear black spot in the fields

## 2 梨黑斑病病原学研究

### 2.1 病原菌

梨黑斑病是一种分布广泛的世界性病害。早期学者<sup>[11]</sup>认为梨黑斑病的学名为 *A. gaisen* Nagano, 后来各国学者采用 *A. kikuchiana* 作为梨黑斑病病原菌的学名。在中国真菌总汇中,戴芳澜<sup>[12]</sup>提到 *A. gaien*

种名,把它作为 *A. kikuchiana* 的异名,在真菌鉴定手册中,魏景超<sup>[13]</sup>提到 *A. gaisen*, 但是误将它作为与 *A. kikuchiana* 不同的种。 *A. alternata* (Fr.) Keissl 也可以引起梨黑斑病,是世界分布广泛的种,在进出口贸易中没有被列为危险性病虫害,而 *A. gaisen* 对梨危害比较大,该病原主要分布在日本、韩国和我国<sup>[14]</sup>, 1993年在法国报道此病原菌引起梨黑斑病危害<sup>[15]</sup>。



目前,从梨果实上已分离到链格孢有9个种<sup>[16]</sup>。在我国已有报道的有6个种,分别是链格孢[*A. alternata* (Fr.) Keissler]、梨黑斑链格孢(*A. gaisen* K. Nagan)、细极链格孢(*A. tenuissima*)、鸭梨侵染链格孢(*A. yaliinficiens* R. G. Roberts)、侵染链格孢(*A. infectoria*)和*A. ventricosa* R. G. Roberts。2015年Woundenberg等<sup>[17]</sup>利用全基因组和转录组数据对15个链格孢属的CBS标准菌株进行系统进化树构建,链格孢[*A. alternata* (Fr.)Keissler]与细极链格孢(*A. tenuissima*)亲缘关系最近,其次为梨黑斑链格孢(*A. gaisen* K. Nagan),侵染链格孢(*A. infectoria*)的亲缘关系最远。2009年刘新伟等<sup>[18]</sup>基于形态学、ITS序列和AK毒素基因研究,发现链格孢[*A. alternata* (Fr.) Keissler]、细极链格孢(*A. tenuissima*)和梨黑斑链格孢(*A. gaisen* K. Nagan)构成了一个稳定的分枝,亲缘关系较近。*A. gaisen*既可以侵染梨叶片也可以侵染梨果实,能够产生寄主专化性毒素-AK毒素,主要侵染日本梨品种;*A. alternata*,*A. infectoria*和*A. tenuissima*是广适性链格孢种,存在于植物的枯死部分或是衰弱组织上<sup>[19]</sup>。*A. yaliinficiens*和*A. ventricosa*是美国从中国出口的‘鸭梨’果实上分离并命名的2个新种<sup>[20]</sup>。2003年,张志铭等<sup>[10]</sup>鉴定引起河北‘鸭梨’黑斑病的病原为*A. alternata* (Fr.) Keissler;2008年常有宏等<sup>[21]</sup>在江苏省农业科学院园艺研究所梨园采集感病的梨树叶片进行梨黑斑病分离,鉴定引起黑斑病的病原为日本梨致病型*A. gaisen*;2006年李永才等<sup>[22]</sup>报道引起‘苹果梨’贮藏期梨黑斑病发生的病原菌为链格孢*A. alternata*;2013年王凤军等<sup>[23]</sup>利用实时荧光PCR检测‘库尔勒香梨’的梨黑斑病病原菌为*A. alternata*,2016年宋博等<sup>[24]</sup>利用ITS、GPD、EF-1 $\alpha$ 保守基因确定‘库尔勒香梨’果萼黑斑病病原为链格孢属链格孢(*A. alternata*)。

## 2.2 梨黑斑病的发生规律与致病机制

### 2.2.1 梨黑斑发生规律

(1)病原菌的侵染时期及侵染途径。梨黑斑病病菌以分生孢子和菌丝体在病梢、病叶和病果等病残体上越冬。翌年春季产生的分生孢子借风雨传播,落到梨叶片上,遇合适的温、湿度条件即萌发长出芽管,沿着梨叶片表皮生长。遇到气孔或伤口后,芽管顶端膨大形成附着胞,然后从附着胞下方伸出一条管状的侵入丝,钻入气孔或伤口内。在气孔下长出侵染菌丝,伸入附近细胞内,用以从梨叶片组织中吸取养料和水分,至此,梨黑斑

病孢子萌发侵入寄主的过程完成。而后以发病植株为中心在田间引起再侵染。一般4月下旬开始发病,嫩叶极易受到危害,6—7月如遇阴天多雨,空气湿度较大时更易流行。地势低洼、偏施化肥,土壤贫瘠,梨园密闭,树势衰弱以及梨瘿蚊、梨木虱、梨网蝽和蚜虫猖獗危害等不利因素均可加重梨黑斑病的流行危害。

Prusky等<sup>[25-26]</sup>报道了链格孢菌可以在果实发育中通过果皮组织侵染,在不同种类果实上侵染部位和时期均不同。呼丽萍等<sup>[27]</sup>研究了花柱的开放程度与黑斑病侵染率的关系,随着花瓣的逐渐开放,黑斑病侵染率也在相应增高。李永才等<sup>[22]</sup>研究了黑斑病在‘苹果梨’中潜伏性侵染途径,发现链格孢(*A. alternata*)在花期和果实发育期均可侵染‘苹果梨’。梨黑斑病菌是在‘苹果梨’花朵的开放时侵入花柱,随着花瓣的逐渐开放,黑斑病侵染率增高;梨黑斑病可以在‘苹果梨’果实发育不同阶段侵入果皮组织,侵染初期主要集中在侵染萼端,果梗端带菌率最低,采收时期梗端果皮的带菌率急剧增高,高于萼端和中部,这可能与初期果实萼端朝上后期果实增重萼端下垂,不易黏附露水有关<sup>[22]</sup>。

(2)流行规律。同一地区不同年份之间,梨黑斑病的严重程度不同,降雨量对梨黑斑病发生程度的影响最为显著,降雨量与梨树的黑斑病发生呈正相关。根据多年观察,南方梨产区降雨多,连日多雨的年份梨黑斑病发生严重。

不同树体结构和土壤、肥料和水份管理水平不同的梨园,梨黑斑病的发生程度也有明显差异。合理的树体结构不仅保证梨树高产、稳产,生产高品质梨果,同时也有利于阻止梨黑斑病的发生和流行。梨园密闭程度与梨黑斑病的发生呈正相关,因此梨园良好的树体结构和通风透光条件,可避免梨黑斑病发病适宜环境的产生,降低梨黑斑病发生的概率。梨黑斑病的发生和流行与树势有关,树体生长势强时,梨黑斑病侵染不易扩展,或者侵染后不表现发病症状,一般引起潜伏侵染;树体生长势较弱时,容易引起梨黑斑病病原菌侵染,一旦病原菌侵染成功,病斑就会迅速扩大,表现出梨黑斑病发生症状。因此培养健壮树体是一种防治梨黑斑病标本兼治的方法,维持健壮树势的主要途径是避免梨树超负载结果,保持梨园土壤通透性及进行科学的施肥和灌水。在梨树施肥过程中,要尽量多施有机肥,并注重

磷钾肥和微肥的施用,避免偏施氮肥,因为氮肥水平过高会造成树体旺长、枝条发育不结实,引发冻伤后,容易招受梨黑斑病的侵染。

**2.2.2 梨黑斑致病机制** 了解和揭示病原菌的致病机制是合理防治病菌的关键。病原菌侵入健康植物的组织和细胞后,破坏寄主植物细胞的正常生理功能,病原菌除了夺取寄主的水分和营养物质外,还可以对植物施加机械压力,以及产生危害寄主的正常生理活动的代谢产物,如毒素、酶、生长调节物质等<sup>[28]</sup>,诱发一系列病变,使植株表现出组织坏死和萎蔫症状,产生病害特有的症状。针对植物细胞壁中的每一种糖类,植物病原菌都有相应的细胞壁降解酶,主要包括纤维素酶(Cx)、多聚半乳糖醛酸酶(PG)、多聚半乳糖醛酸反式消除酶(PGTE)、果胶甲基半乳糖醛酸酶(PMG)和果胶甲基反式消除酶(PMTE)等细胞壁降解酶。关于病原菌产生细胞壁降解酶的相关研究报道较多,1998年,陈捷等<sup>[29]</sup>报道了玉米茎腐病菌产生的细胞壁降解酶的致病作用;2000年,高增贵等<sup>[30]</sup>研究了玉米茎腐病菌产生的细胞壁降解酶种类及其活性分析;2000年,李宝聚等<sup>[31]</sup>研究了黄瓜黑星病菌细胞壁降解酶在致病中的作用。关于对梨黑斑病能否产生细胞壁降解酶及其致病机制的研究还未见报道。因此研究梨黑斑病细胞壁降解酶对于探索梨黑斑病致病机制有着重要意义。

真菌毒素(mycotoxin)是由植物病原真菌产生的、对寄主植物有毒性且能够使寄主产生典型症状的一类物质。它既不属于激素也不属于酶类,且在浓度很低的情况下仍表现很强的生理活性。根据对寄主植物的种或栽培品种是否具有高度专化性作用位点和特异生理活性,致病毒素分为寄主选择性毒素(HST)和非寄主选择性毒素(NHST)。Nakashima等<sup>[32-33]</sup>1982年从梨黑斑病菌菊池链格孢(*A. kikuchiana*)中分离出寄主专化性毒素(AK-toxin),分析了AK毒素的组成成分和结构,从此展开了梨黑斑病AK毒素的研究;Aiko等<sup>[34]</sup>2000年报道了控制AK毒素合成基因的结构和功能。

目前,研究已经证明,AK毒素能够导致寄主细胞质膜的生理和超微结构的损害<sup>[35-39]</sup>。当AK毒素进入梨细胞后,先从胞间连丝的作用位点侵入,使细胞质膜发生凹陷,增大膜对K<sup>+</sup>,Na<sup>+</sup>的渗透性,降低膜电势;随后毒素作用于线粒体、核仁和高尔基体等细

胞器,产生胞饮作用,同时诱导细胞产生大量糖类,增加细胞的胞外分泌和内吞作用。Shinogi等<sup>[40]</sup>采用显微检测O<sub>2</sub><sup>-</sup>、二氨基联苯胺(DAB)法显微检测H<sub>2</sub>O<sub>2</sub>,硝基蓝四唑(NBT)法和铈氯化物法超微结构检测(H<sub>2</sub>O<sub>2</sub>)3种方法检测到*A. alternata*致病型与寄主植物交互作用中产生活性氧(ROS);使用AK毒素处理感病的梨树叶片,也产生大量活性氧,说明真菌和植物细胞在相互作用时产生活性氧,可能与梨黑斑病的感病表达有关。

### 3 梨种质资源抗黑斑病鉴定评价

近年来,国内梨研究学者关于砂梨品种抗黑斑病的研究报道较多。刘永生等<sup>[41]</sup>对主要推广的砂梨品种进行黑斑病抗性调查,发现‘金水2号’‘今村秋’‘江岛’‘德胜香’‘黄花’‘长十郎’‘湘南’和‘蒲瓜’表现为抗病,‘柠檬黄’‘二宫白’‘金花’和‘金水1号’表现为中抗,‘土佐锦’‘安农1号’和‘青云’表现为感病;李国元<sup>[42]</sup>对‘金水1号’‘晚三吉’‘金水2号’和‘黄花’的黑斑病抗性调查发现,‘黄花’对黑斑病抗性较强,‘金水1号’‘金水2号’和‘晚三吉’对黑斑病表现为中抗;胡红菊等<sup>[43]</sup>对368份梨种质资源进行梨黑斑病抗性评价,提出杜梨抗性最强,其次为砂梨和豆梨,白梨居中,西洋梨最弱,并筛选出高抗黑斑病品种‘德胜香’,抗黑斑病品种‘云绿’‘松岛’‘回溪梨’‘短把早’‘柳城凤山梨’‘金水1号’‘安农1号’和‘杭青’;张玉萍<sup>[44]</sup>报道了‘真寿’抗黑斑病;盛宝龙等<sup>[45]</sup>对80个梨品种进行了梨黑斑病田间抗性调查,发现砂梨对黑斑病的田间抗性强于白梨,我国一些传统的梨品种如‘苍溪梨’‘富源黄’等较感黑斑病,而我国近年培育的梨新品种如‘华酥’‘黄花’和‘中翠’等对黑斑病有较强的抗性;蔺经等<sup>[46]</sup>对引进的85份砂梨种质资源进行抗黑斑病鉴定,筛选出高抗黑斑病品种‘金二十世纪’和‘奥萨二十世纪’,鉴定出抗病品种‘华酥’‘黄花’‘德胜香’‘早美酥’‘丰水’‘喜水’‘寿新水’‘新世纪’‘秋荣’‘黄金’‘圆黄’‘秋黄’和‘华山’13个品种;刘仁道等<sup>[47]</sup>对17个梨栽培品种的黑斑病田间抗性进行了调查,提出砂梨系统中早熟品种多为抗或中抗品种,对黑斑病的抗性相对强于中熟和晚熟品种,白梨系统品种的抗性与成熟期的关系相反,即早熟白梨品种对黑斑病抗性弱,而晚熟白梨品种对黑斑病的抗性相对较强;刘卮洲等<sup>[48]</sup>采用田间自然发病和人工接种鉴定2种方法对



16个梨品种进行黑斑病抗性鉴定,筛选出4个高抗黑斑病的梨品种‘华酥’‘早美酥’‘黄花’和‘丰水’。国内外的研究发现,不同梨品种间黑斑病抗性有显著差异;不同的研究者使用不同调查方法鉴定梨品种抗黑斑病的结果不同,总体来说砂梨品种对黑斑病的抗性比较稳定。但是由于前期研究者对于砂梨抗黑斑病抗性研究时,待鉴定梨品种的数量较少,一般采用田间自然调查的方法,田间病菌选择压力较小,不能系统反映砂梨品种对梨黑斑病抗性。

#### 4 梨抗病分子生物学研究进展

植物在生长发育过程中,经常受到各种病原物的侵袭,植物可以特异性地识别病原物释放的物质,建立防御系统抵御外界病害,植物控制识别病原物效应因子,编码植物抗病蛋白的基因被称为R基因(resistant genes)。R基因的多态性介导了植物与病原物不同生态型间的抗性差异。迄今为止,一大批的R基因在水稻<sup>[49]</sup>、小麦<sup>[50]</sup>、大豆<sup>[51]</sup>、苹果<sup>[52]</sup>和葡萄<sup>[53]</sup>等植物中被分离出来,目前已经分离鉴定的R基因超过100种<sup>[54]</sup>。

由于梨树的童期长,遗传背景复杂,大部分品种自交不亲和等特性,传统的抗病育种成本高、效率较低,而采用分子育种可以缩短育种年限,提高育种效率。向现有栽培品种引入抗病基因的同时,不会形成基因的大规模重组。近年来研究者利用同源克隆和图位克隆的方法开展了梨抗病基因鉴定方面的研究。Dondini等<sup>[55]</sup>利用SSRs、MFLPs、AFLPs、RGAs和AFLP-RGAs等分子标记对抗火疫病梨品种和感火疫病梨品种进行遗传图谱构建,在遗传图谱上鉴定了4个假定的抗火疫病QTLs。Terakami等<sup>[56]</sup>利用SSRs和AFLPs等分子标记对抗梨疮痂病梨品种和感梨疮痂病梨品种进行遗传图谱构建,在遗传图谱上定位了抗梨疮痂病基因Vnk的连锁区域。Faize等<sup>[57]</sup>利用RT-PCR和RACE技术鉴定出砂梨中可能抗梨疮痂病的LRPK基因。2015年Yang等<sup>[58]</sup>以砂梨高抗黑斑病品种‘金晶梨’和高感黑斑病品种‘红粉梨’为试材,利用双末端RNA-seq技术筛选出与抗黑斑病相关的5 213个差异表达基因,检测到34个微卫星序列和107 525可信的SNPs位点。

#### 5 展望

梨黑斑病是梨树重要的病害之一,随着国内外

研究者对梨黑斑病的研究日渐重视,关于梨黑斑病病原菌和梨抗黑斑病等方面的研究日渐增多,但是总体来说梨黑斑病相关的研究仍然滞后,梨黑斑病抗药机制、梨黑斑病致病机制和梨抗黑斑病机制等不少问题还远未研究透彻,可辅助梨抗黑斑病育种的实用分子标记和可应用综合防控梨黑斑病的技术措施仍然较少。未来需要从以下几个方面着重研究:

##### 5.1 梨黑斑病抗药机制研究

目前防治梨黑斑病的方法主要是使用甲基托布津、多菌灵、代森锰锌和苯醚甲环唑等化学药剂,长期使用这些化学药剂过程中,由于梨农为了提高防治效果盲目提高杀菌剂浓度,致使这些杀菌剂的防治效果降低,出现部分菌株对多菌灵和苯醚甲环唑敏感性降低的现象。2016年朱红艳等<sup>[59]</sup>测定了苯醚甲环唑对43株梨黑斑病菌株菌丝生长的 $EC_{50}$ ,发现部分梨黑斑病菌株对苯醚甲环唑的敏感性降低。因此应加强梨黑斑病抗药机制研究,开发新型梨黑斑病杀菌剂。

##### 5.2 梨黑斑病致病机制研究

关于梨黑斑病致病机制的研究主要集中在梨黑斑病毒素,但对梨黑斑病能否产生细胞壁降解酶及其致病机制的研究还未见报道。因此研究梨黑斑病细胞壁降解酶对探索梨黑斑病致病机制有着重要意义。

##### 5.3 梨抗黑斑病机制研究

目前已通过转录组测序和基因组测序筛选出梨抗黑斑病相关的基因,但还不能确定这些基因的抗病功能。因此应加强抗病基因连锁分析、抗病基因精细定位与基因表达、功能验证相结合的综合研究,筛选梨抗黑斑病关键基因,为梨抗黑斑病育种提供理论支撑。

#### 参考文献 References:

- [1] 中国种植业信息网.水果数据查询[R/OL][2017-05-24]. <http://www.zzys.moa>.  
China planting information network. Data research of fruit [R/OL][2017-05-24]. <http://www.zzys.moa>.
- [2] ZHANG H, WANG Y, ZHANG C, WANG X, LI H, XU W. Isolation, characterization and expression analysis of resistance gene candidates in pear (*Pyrus* spp.)[J]. *Scientia Horticulturae*, 2011, 127(3): 282-289.
- [3] TANAKA S. Studies of black spot diseases of the Japanese pear (*Pyrus serotina* Rehd.)[M]//Memoirs of the College of Agriculture,

- Kyoto Imperial University No. 28 (Phytopathology Series No. 6), 1933: 31.
- [4] BAUDRY A, MORZIERES J P, LARUE P. First report of Japanese pear black spot caused by *Alternaria kikuchiana* in France [J]. Plant Disease, 1993, 77(4): 428.
- [5] 李云飞, 陈雪娇, 张爱芳, 杨雪, 陈雨, 姚剑. 砀山梨黑斑病分子检测技术研究[J]. 中国农学通报, 2016, 32(4): 150-154.  
LI Yunfei, CHEN Xuejiao, ZHANG Aifang, YANG Xue, CHEN Yu, YAO Jian. Molecular detection of *Alternaria alternata* causing black spot of Dangshan pear[J]. Chinese Agricultural Science Bulletin, 2016, 32(4): 150-154.
- [6] 杨晓平, 胡红菊, 王友平, 田瑞, 陈启亮, 张靖国. 梨黑斑病原菌的生物学特性及其致病性观察[J]. 华中农业大学学报, 2009, 28(6): 680-684.  
YANG Xiaoping, HU Hongju, WANG Youping, TIAN Rui, CHEN Qiliang, ZHANG Jingguo. Biological characteristics and pathogenicity of pear black spot by *Alternaria alternata* (Fr.) Keissl[J]. Journal of Huazhong Agricultural University, 2009, 28(6): 680-684.
- [7] KNIGHT S C, ANTHONY V M, BRADY A M, GREENLAND A J, HEANYE S P, MURRAY D C, YOULE A D. Rationale and perspectives on the development of fungicides [J]. Annual Review of Phytopathology, 1997, 35(1): 349-372.
- [8] NGUYEN V N, SEO D J, PARK R D, JUNG W J. Antimycotic activities of cinnamon-derived compounds against *Rhizoctonia solani* in vitro [J]. BioControl Science, 2009, 54(5): 697-707.
- [9] 杨晓平. 梨黑斑病菌 *Alternaria* sp 鉴定及砂梨抗黑斑病分子机制初探[D]. 武汉: 武汉大学, 2015.  
YANG Xiaping. Identification of pear black spot *Alternaria* sp. and study on molecular mechanism of sand pear disease resistance to pear black spot[D]. Wuhan: Wuhan University, 2015.
- [10] 张志铭, 宋福, 孙淑贞, 赵文胜. 河北鸭梨黑斑病病原菌的鉴定[J]. 植物检疫, 2003, 17(4): 212-214.  
ZHANG Zhiming, SONG Fu, SUN Shuzhen, ZHAO Wensheng. The identification of pathogen causing Ya pears black spot[J]. Plant Quarantine, 2003, 17(4): 212-214.
- [11] NAGONO K. Ping sport disease of pear [J]. Journal of the Japanese Horticultural Society, 1920, 32(3): 16-19.
- [12] 戴芳澜. 中国真菌总汇[M]. 北京: 科学出版社, 1979: 819-828.  
DAI Fanglan. China fungi confluence[M]. Beijing: Science Press, 1979: 819-828.
- [13] 魏景超. 真菌鉴定手册[M]. 上海: 上海科学技术出版社, 1979: 56-57.  
WEI Jingchao. Handbook of fungal identification[M]. Shanghai: Shanghai Science and Technology Press, 1979: 56-57.
- [14] 曹若彬, 冷怀琼, 张志铭. 果树病理学[M]. 北京: 中国农业出版社, 1997: 80-84.  
CAO Ruobin, LENG Huaiqiong, ZHANG Zhiming. Fruit tree pathology[M]. Beijing: China Agriculture Press, 1997: 80-84.
- [15] SIMMONS, EMORY G. *Alternaria* themes and variations [J]. Mycotaxon, 1993, 48: 109-140.
- [16] ROBERTS R G. *Alternaria yaliinfriciens* sp. nov. on Yali pear fruit: from interception to identification [J]. Plant Disease, 2005, 89(2): 134-145.
- [17] WOUNDENBERG J H C, SEIDL M F, GROENEWALD J Z, DE VRIES M, STIELOW J B, THOMMA B P H J, CROUS P W. *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes? [J]. Studies in Mycology, 2015, 82: 1-21.
- [18] 刘新伟, 陈岩, 宋福, 赵文胜, 吴品珊, 章桂明, 沈崇尧, 严进. 我国梨和部分国外梨果实上链格孢菌的鉴定研究[J]. 植物检疫, 2009, 23(5): 1-5.  
LIU Xinwei, CHEN Yan, SONG Fu, ZHAO Wensheng, WU Pinshan, ZHANG Guiming, SHEN Chongyao, YAN Jin. Identification of *Alternaria* isolates from some Chinese and foreign pear fruits[J]. Plant Quarantine, 2009, 23(5): 1-5.
- [19] 付余波. 我国四省区梨主要病害的病原鉴定、分子检测与药剂筛选研究[D]. 南京: 南京农业大学, 2010.  
FU Yubo. Identification, molecular detection and fungicides screening of pear major diseases pathogens in four Chinese province[D]. Nanjing: Nanjing Agriculture University, 2010.
- [20] 孙霞. 链格孢属真菌现代分类方法研究[D]. 泰安: 山东农业大学, 2006.  
SUN Xia. The methodological study on taxonomy of the genus *Alternaria* Nees[D]. Tai'an: Shandong Agriculture University, 2006.
- [21] 常有宏, 刘永锋, 王宏, 刘邮洲, 陈志谊. 梨黑斑病病菌的致病条件[J]. 江苏农业学报, 2008, 24(3): 316-320.  
CHANG Youhong, LIU Yongfeng, WANG Hong, LIU Youzhou, CHEN Zhiyi. Infection conditions of *Alternaria geisen* to pear[J]. Jiangsu Journal of Agricultural Sciences, 2008, 24(3): 316-320.
- [22] 李永才, 毕阳. 苹果梨黑斑病的发生及侵染过程[J]. 植物保护学报, 2006, 33(2): 131-135.  
LI Yongcai, BI Yang. Occurrence and infection of *Alternaria* rot of Pingguolipear, *Pyrus pyrifolia*[J]. Journal of Plant Protection, 2006, 33(2): 131-135.
- [23] 王凤军, 张祥林, 马海波, 冯新忠, 冯俊丽, 华鹏. 实时荧光 PCR 检测香梨黑斑病菌[J]. 食品科学, 2013, 34(20): 170-173.  
WANG Fengjun, ZHANG Xianglin, MA Haibo, FENG Xinzhong, FENG Junli, HUA Peng. Real-time fluorescence PCR identification of *Alternaria alternata* causing black spot disease of fragrant pear[J]. Food Science, 2013, 34(20): 170-173.
- [24] 宋博, 朱晓锋, 徐兵强, 阿布都克尤木·卡德尔, 杨森. 库尔勒香梨果萼黑斑病原菌鉴定及其 ITS、GPD 和 EF-1 $\alpha$  序列分析[J]. 园艺学报, 2016, 43(2): 329-336.  
SONG Bo, ZHU Xiaofeng, XU Bingqiang, ABUDUKEYOUMU·Kadeer, YANG Sen. Identification of Kolra Fragrant pear calyx-end black spot pathogen and its sequence analysis of ITS, GPD and EF-1 $\alpha$ [J]. Acta Horticulturae Sinica, 2016, 43(2): 329-336.
- [25] PRUSKY D, FUCHS Y, YANKO U. Assessment of latent infec-

- tions as a basis for control of postharvest disease of mango [J]. *Plant Disease*, 1983, 67(7): 816-818.
- [26] PRUSKY D, BENAIRE R, REICHS G. Etiology and histology of *Alternaria* rot of persimmon fruits [J]. *Phytopathology*, 1981, 71(11): 77-79.
- [27] 呼丽萍, 马春红, 张健, 谭维军, 杨光明, 张领耘. 苹果霉心病菌的侵染过程[J]. *植物病理学报*, 1995, 25(4): 351-356.  
HU Liping, MA Chunhong, ZHANG Jian, TAN Weijun, YANG Guangming, ZHANG Lingyun. Infection process of pathogenic fungi contributing to mouldy core and core rot of starting apple fruits[J]. *Journal of Plant Pathology*, 1995, 25(4): 351-356.
- [28] 许志刚. 普通植物病理学[M]. 北京: 中国农业出版社, 2004: 292-301.  
XU Zhigang. *General plant pathology*[M]. Beijing: China Agriculture Press, 2004: 292-301.
- [29] 陈捷, 高洪敏, 纪明山. 玉米茎腐病菌产生的细胞壁降解酶的致病作用[J]. *植物病理学报*, 1998, 28(3): 221-226.  
CHEN Jie, GAO Hongmin, JI Mingshan. Pathogenicity of cell wall degrading enzymes produced by corn stalk rot pathogen[J]. *Journal of Plant Pathology*, 1998, 28(3): 221-226.
- [30] 高增贵, 陈捷, 高洪敏. 玉米茎腐病菌产生的细胞壁降解酶种类及其活性分析[J]. *植物病理学报*, 2000, 30(2): 148-152.  
GAO Zenggui, CHEN Jie, GAO Hongmin. Analysis of cell wall degrading enzymes and their activity in *Maize stalk rot* pathogen[J]. *Journal of Plant Pathology*, 2000, 30(2): 148-152.
- [31] 李宝聚, 周长力, 赵奎华. 黄瓜黑星病菌致病机理的研究 II 细胞壁降解酶及其在致病中的作用[J]. *植物病理学报*, 2000, 30(1): 13-18.  
LI Baoju, ZHOU Changli, ZHAO Kuihua. Pathogenic mechanism of scab of cucumber caused by *Cladosporium cucumerinum* II the cell wall degrading enzymes and its pathogenic action[J]. *Journal of Plant Pathology*, 2000, 30(1): 13-18.
- [32] NAKASHIMA T, UENO T, FUKAMI H. Structure elucidation of AK-toxins, host-specific phytotoxic metabolites produced by *Alternaria kikuchiana* Tanaka[J]. *Tetrahedron Letters*, 1982, 23(43): 44-69.
- [33] NAKASHIMA T, UENO T, FUKAMI T. Isolation and structure of AK-toxin I and II, host-specific phytotoxic metabolites produced by *Alternaria alternata* Japanese pear pathotype[J]. *Agricultural and Biological Chemistry*, 1985, 49(3): 807-816.
- [34] AIKO T, TAKASHI T. Structural and functional complexity of the genomic region controlling AK-Toxin biosynthesis and pathogenicity in the Japanese pear pathotype of *Alternaria alternata* [J]. *The American Phytopathological Society*, 2000, 13(9): 975-986.
- [35] PARK P. Origin of inclusive materials between cell walls and invaginated plasma membranes in cells of susceptible leaves of Japanese pear treated with a host-specific toxin from *Alternaria kikuchiana* Tanaka[J]. *Physiological Plant Pathology*, 1977, 11(1): 39-42.
- [36] PARK P, OHNO T, NISHIMURA S, KOHMOTO K, OTANI H. Leakage of sodium ions from plasma membrane modification associated with permeability change, in host cells treated with a host-specific toxin from a Japanese pear pathotype of *Alternaria alternata*[J]. *Canadian Journal of Botany*, 1987, 65(2): 330-339.
- [37] PARK P, OHNO T, NISHIMURA S, KOHMOTO K, OTANI H. Golgi host cells treated with a host-specific toxin vesicles fused with invaginated plasma membranes in from a Japanese pear pathotype of *Alternaria alternata*[J]. *Japanese Journal of Phytopathology*, 1988, 54(4): 493-502.
- [38] PARK P, OHNO T, NISHIMURA S, KOHMOTO K, OTANI H. Activation of carbohydrate-containing golgi vesicles in susceptible leaf cells of Japanese pear responding with AK-toxin [J]. *Japanese Journal of Phytopathology*, 1989, 55(3): 290-295.
- [39] PARK P, OHNO T, NISHIMURA S, KOHMOTO K, OTANI H. Artifact in plant tissues fixed with chemical fixatives pathological estimation of membranous fragments appearing at AK toxin-induced plasma membrane modification in susceptible Japanese pear leaves[J]. *Japanese Journal of Phytopathology*, 2009, 60(4): 431-440.
- [40] SHINOGI T, SUZUKI T, NARUSAKA Y, PYOYUN P A R K. Ultrastructural localization of hydrogen peroxide in hostleaves treated with AK-toxin I produced by *Alternaria alternata* Japanese pear pathotype[J]. *Journal of General Plant Pathology*, 2002, 68(1): 38-45.
- [41] 刘永生, 吴四宝, 周忠良, 钟守艳. 砂梨主要推广品种对黑斑病、黑星病和轮纹病的抗性调查[J]. *湖北植保*, 1995(5): 26-27.  
LIU Yongsheng, WU Sibao, ZHOU Zhonggen, ZHONG Shouyan. Investigation of resistance of main varieties of pear black spot, scab and ring rot disease[J]. *Hubei Plant Protection*, 1995(5): 26-27.
- [42] 李国元. 不同梨树品种对梨轮纹病、锈病和黑斑病的抗性调查[J]. *孝感师专学报(自然科学版)*, 1998, 18(4): 67-69.  
LI Guoyuan. Resistant investigation of different pear varieties on *Physalospora piricola* Rust and *Alternaria kikuehiana*[J]. *Journal of Xiaogan Teachers College (Natural Sciences)*, 1998, 18(4): 67-69.
- [43] 胡红菊, 王友平, 甘宗义, 田瑞, 陈启亮. 梨种质资源对黑斑病的抗性评价[J]. *湖北农业科学*, 2002(5): 113-115.  
HU Hongju, WANG Youping, GAN Zongyi, TIAN Rui, CHEN Qiliang. Evaluation of resistance to black spot disease of pear germplasm resources[J]. *Hubei Agricultural Sciences*, 2002(5): 113-115.
- [44] 张玉萍. 日本抗病早熟梨新品种——真寿[J]. *西北园艺*, 2003(1): 27.  
ZHANG Yuping. A new variety of resistance to early ripening pear Zhen Shou in Japan[J]. *Northwest Horticulture*, 2003(1): 27.



- [45] 盛宝龙, 李晓刚, 蒯经, 颜志梅, 杨青松. 不同梨品种对黑斑病的田间抗性调查[J]. 中国南方果树, 2004, 33(6): 76-77.  
SHENG Baolong, LI Xiaogang, LIN Jing, YAN Zhimei, YANG Qingsong. Field investigation on resistance of different pear cultivars to black spot disease[J]. South China Fruits, 2004, 33(6): 76-77.
- [46] 蒯经, 杨青松, 李小刚, 颜志梅, 盛宝龙. 砂梨品种对黑斑病的抗性鉴定和评价[J]. 金陵科技学院学报, 2006, 22(2): 81-85.  
LIN Jing, YANG Qingsong, LI Xiaogang, YAN Zhimei, SHENG Baolong. Identification and evaluation of resistance of sand pear to black spot disease[J]. Journal of Jinling Institute of Technology, 2006, 22(2): 81-85.
- [47] 刘仁道, 邓国涛, 刘勇, 魏荣洲, 刘娟, 范理璋, 张猛, 范理璋, 张猛. 不同梨品种对梨黑斑病抗性差异研究[J]. 北方园艺, 2008(3): 6-8.  
LIU Rendao, DENG Guotao, LIU Yong, WEI Rongzhou, LIU Juan, FAN Lizhang, ZHANG Meng. Resistance of pear varieties to pear black spot (*Alternaria alternata*) [J]. Northern Horticulture, 2008(3): 6-8.
- [48] 刘卹洲, 常有宏, 陈志谊, 王宏, 刘永峰, 罗楚平, 聂亚峰. 不同梨品种对黑斑病抗性鉴定[J]. 江苏农业科学, 2009(3): 125-127.  
LIU Youzhou, CHANG Youhong, CHEN Zhiyi, WANG Hong, LIU Yongfeng, LUO Chuping, NIE Yafeng. Identification of disease resistance of different pear varieties to black spot[J]. Jiangsu Agricultural Sciences, 2009(3): 125-127.
- [49] LEI C, HAO K, YANG Y, MA J, WANG S, WANG J, WANG C. Identification and fine mapping of two blast resistance genes in rice cultivar 93-11[J]. The Crop Journal, 2013, 1(1): 2-14.
- [50] HOU L, MA D F, HU M L, HE M M, LU Y, JING J X. Genetic analysis and molecular mapping of an all-stage stripe rust resistance gene in *Triticum aestivum*-*Haynaldia villosa* translocation line V3[J]. Journal of Integrative Agriculture, 2013, 12(12): 2197-2208.
- [51] DEMIRBAS A, RECTOR B G, LOHNES D G, FIORITTO R J, GRAEF G L, CREGAN P B, SPECHT J E. Simple sequence repeat markers linked to the soybean genes for *Phytophthora* resistance[J]. Crop Science, 2001, 41(4): 1220-1227.
- [52] SANZANI S M, SCHENA L, de GIROLAMO A, IPPOLITO A, GONZALEZ-CANDELAS L. Characterization of genes associated with induced resistance against *Penicillium expansum* in apple fruit treated with quercetin[J]. Postharvest Biology and Technology, 2010, 56(1): 1-11.
- [53] KATULA-DEBRECENI D, LENCSES A K, SZOKE A, VERES A, HOFFMANN S, KOZMA P, KISS E. Marker-assisted selection for two dominant powdery mildew resistance genes introgressed into a hybrid grape population[J]. Scientia Horticulturae, 2010, 126(4): 448-453.
- [54] GUPTA S K, RAI A K, KANWAR S S, SHARMA T R. Comparative analysis of zinc finger proteins involved in plant disease resistance[J]. PloS One, 2012, 7(8): e42578.
- [55] DONDINI L, PIERANTONI L, GAIOTTI F, CHIODINI R, TARTARINI S, BAZZI C, SANSAVINI S. Identifying QTLs for fire-blight resistance via a European pear (*Pyrus communis* L.) genetic linkage map[J]. Molecular Breeding, 2005, 14(4): 407-418.
- [56] TERAKAMI S, SHODA M, ADACHI Y, GONAI T, KASUMI M, SAWAMURA Y, HAYASHI T. Genetic mapping of the pear scab resistance gene Vnk of Japanese pear cultivar Kinchaku[J]. Theoretical and Applied Genetics, 2006, 113(4): 743-752.
- [57] FAIZE M, FAIZE L, ISHII H. Characterization of a leucine-rich repeat receptor-like protein kinase (LRPK) gene from Japanese pear and its possible involvement in scab resistance[J]. Journal of General Plant Pathology, 2007, 73(2): 104-112.
- [58] YANG X P, HU H J, YU D Z, SUN Z H, HE X J, ZHANG J G, CHEN Q L, TIAN R, FAN J. Candidate resistant genes of sand pear (*Pyrus pyrifolia* Nakai) to *Alternaria alternata* revealed by transcriptome sequencing[J]. PloS One, 2015, 10(8): e0135046.
- [59] 朱红艳, 蒋小芬, 李先明, 伍涛, 杨夫臣, 涂俊凡, 刘政, 秦仲麒. 梨黑斑病菌对苯醚甲环唑的敏感性分析[J]. 湖北农业科学, 2016, 55(24): 6448-6450.  
ZHU Hongyan, JIANG Xiaofen, LI Xianming, WU Tao, YANG Fuchen, TU Junfan, LIU Zheng, QIN Zhongqi. Sensitivity analysis on *Alternaria alternata* to difenoconazole[J]. Hubei Agricultural Sciences, 2016, 55(24): 6448-6450.