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Review

Curvularia lunata and Curvularia Leaf Spot of Maize in China

Shaoqing Wang, Zhixiang Lu, Bo Lang, Xinhua Wang, Yaqian Li, and Jie Chen*

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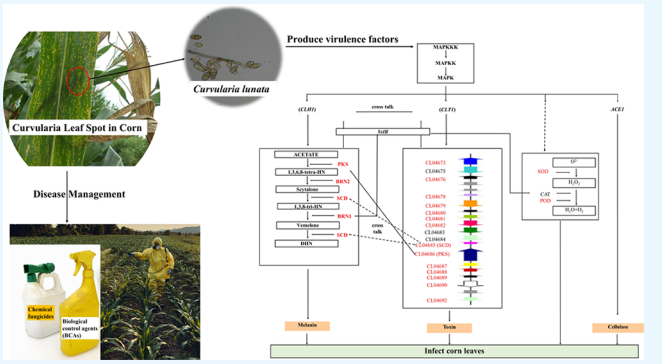
ABSTRACT: Curvularia leaf spot (CLS), primarily caused by *Curvularia lunata* (Wakker) Boedijn (*C. lunata*), is widely distributed in maize production regions in China. It occurs in all the developmental stages of maize and causes economic losses. The epidemic and yield loss estimation models have been constructed for the disease. *C. lunata* has obvious virulence differentiation and produces multiple virulence factors. CLS is managed by application of chemical and biological agents and by quantitative resistance conferred by 5 to 6 quantitative trait loci (QTL). This review summarizes research on the understanding of CLS biological characteristics, virulence factors of *C. lunata*, host resistance genetics, and disease management strategies in China.



INTRODUCTION

Curvularia leaf spot (CLS) is one of the common foliar fungal diseases of maize that is widely distributed worldwide.[1−7] In China, CLS was first observed in Shandong Province in the late 1970s, when it was considered an "unknown spot". In the mid-1980s, the disease was found on the maize inbred line Huangzao 4 in Henan Province of China. Later, the causative agent of CLS was identified as *Curvularia lunata* (Wakker) Boedijn.[8] Since 1980, the disease has been widely distributed throughout the maize-planting regions of China, including Liaoning, Hebei, Henan, Shanxi, Shandong, Beijing, Jilin, and Tianjin Provinces,[9,10] and it has become the third most serious leaf disease followed by northern leaf blight and southern leaf blight. In 1994, CLS affected 20% of the summer maize in the Beijing plains, which resulted in 60% grain loss.[11] Similarly, in 1996, the disease spread into southern Liaoning Province, and up to 192 000 ha were affected, with a complete loss of yield of up to 16 600 ha, causing a total loss of 250 000 tons.[12] After this outbreak, the cultivation of CLS-resistant varieties sprung up in various regions,[13,14] which decreased the disease prevalence and intensity, and as a result the CLS became not a serious problem in maize production. In the past decade (2010−2019), according to the reports by Zhang et al.,[15] and Chang et al.[16] and surveys by local base stations, some provinces in China, such as Anhui, Henan, Shandong, Hebei, and Liaoning, have higher disease severity indexes in maize that caused large leaf spots and wilting to occur in almost all maize growing areas of provinces, indicating a rerising threat of CLS occurrence to maize production. Although the severity of CLS varies significantly between provinces and among localities of the same province, it is still an important maize disease in China (Figure 1).

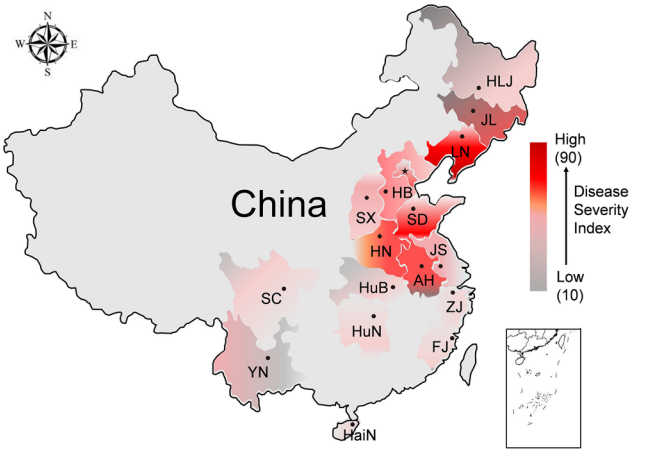


Figure 1. Curvularia leaf spot (CLS) distribution and disease index in maize-growing areas in China, 2010 to 2019. The abbreviations represent different provinces. HLJ: Heilongjiang; JL: Jilin; LN: Liaoning; HB: Hebei; SX: Shanxi; SD: Shandong; HN: Henan; AH: Anhui; ZJ: Zhejiang; FJ: Fujian; HuB: Hubei; HuN: Hunan; SC: Sichuan; YN: Yunnan; HaiN: Hainan. Disease severity index was according to the study by Zhang et al.[15] Disease severity index = $\Sigma(A \times B)/\Sigma(B \times 9) \times 100\%$, where A represents disease levels 1, 3, 5, 7, or 9 and B represents the number of plants at each disease level. (Note: Data based on Zhang et al. and Chang et al. 2020 and investigations from local base stations).

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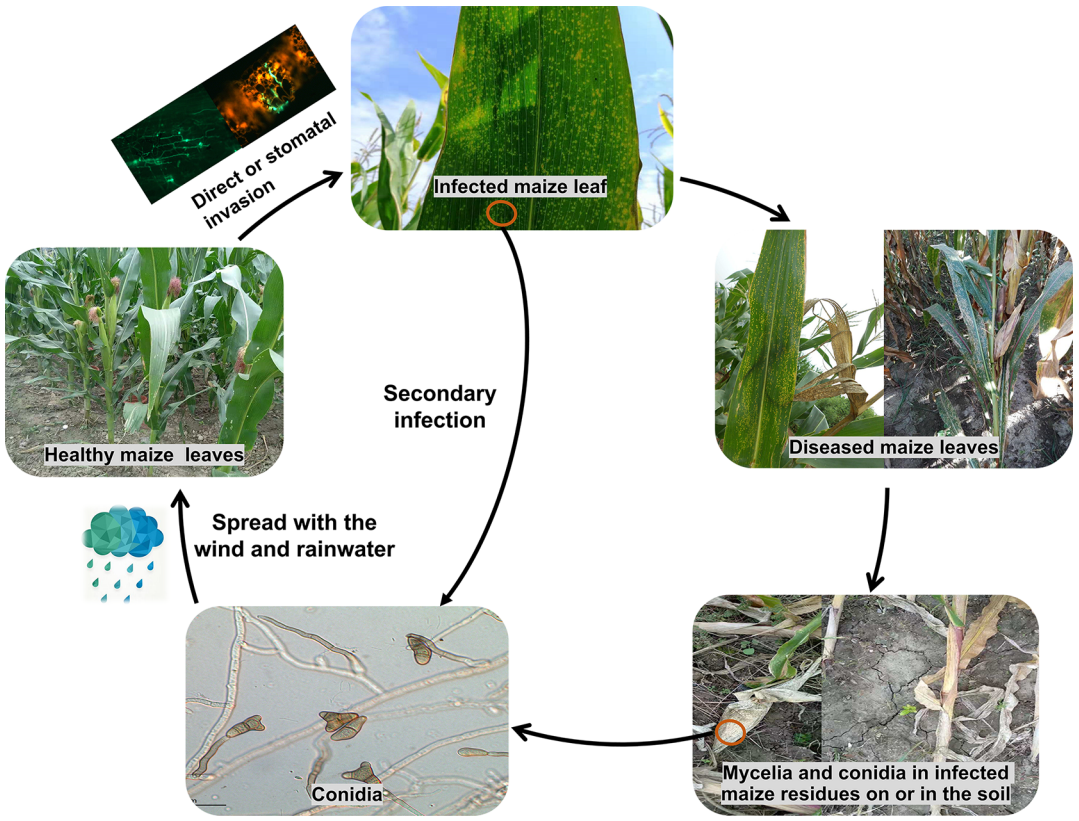

Figure 2. Curvularia leaf spot (CLS) disease cycle in maize fields. Conidia of the pathogen are released from infected maize leaf residue in the spring and spread to maize leaves by wind and rainwater, initiating primary infection by direct or stomatal invasion (the mycelia were tagged with GFP, and the maize cell showed a red signal under a fluorescence microscope). The conidia are subsequently formed in the resulting leaf lesions and spread to nearby plants or other fields via wind and rainwater, causing secondary infections.

SYMPTOMS AND EPIDEMIOLOGY

Symptoms. *C. lunata* mainly infects the leaves throughout the developmental stages of maize, particularly at the reproductive stage.[9,17,18] Typical CLS symptoms initiate as chlorotic small spots that gradually expand into round or oval chlorotic transparent lesions with a white or yellowish-brown center and a dark brown edge surrounded by a yellowish halo.[9] The symptoms of CLS vary greatly among different maize varieties. According to the size, shape, and color of CLS lesions on maize leaf, maize varieties are categorized into disease resistant (R), intermediate (M), and susceptible (S) types.[19] Susceptible type (S): large lesions, width of 1∼2 mm, length of 1∼4 mm, round or elliptical, strips or irregular shapes, pale or yellowish brown center, wide brown band at the edges, and wider translucent grass-yellow halo at the outermost periphery, and several lesions connect to form a necrotic area on the leaf. Intermediate type (M): small lesions, 1∼2 mm in size, round, elliptical, or irregular shapes, pale or light brown center, narrow or thin brown band at the edges, and distinct chlorotic halo at the outermost periphery. Resistant type (R): small lesions, 1∼2 mm in size, round, oval or irregular shape, pale or light brown center, thin brown band or no brown ring at the edges, and narrow translucent halo at the outermost periphery.

Epidemiology. CLS usually occurs from July to August in maize-planting regions of China, which are the months of the year with frequent heavy rains and high-temperature conditions.[9,20] The latent period of CLS infection is around 7−10 days for each infection cycle.[9] *C. lunata* persists as either mycelia or conidia in infected maize residues and soil throughout the year and infects the plants of the next growth cycle[20] (Figure 2). Leaf wetness encourages conidial germination and primary infections. Conidia from the primary lesions can be released and cause secondary infection through wind and rain on both adjacent plants and other fields.[9,21,22] There are many factors that can aggravate disease spread and severity, such as a high accumulation of this pathogen, high planting densities, continuous cropping, host susceptibility, excessive application of chemical fertilizers, and soil nutrient imbalance.[10,23,24] The disease was able to spread 250 m within one month through wind. A spread gradient model was constructed as $x_i = 159.33 \exp(-0.0103 d_i)$, in which x_i is the number of disease lesions per plant and d_i (m) is the distance from the epidemic center.[25]

A model to estimate yield loss due to CLS was established. The percentage of yield loss ranges between 1.7% and 36.9%, with an average of 15.4%.[26] Two types of yield loss estimation models have been constructed: (1) A model for the critical period of maize production (CPM): $L = 2.8219 + 0.7402X_1$ ($R = 0.879$, $N = 18$) and (2) a model for multiple periods of maize production (MPM): $L = 1.073 + 0.426X_1 + 0.170X_2$ ($R = 0.892$, $N = 18$); in these models, L is yield loss (%), X_1 the disease index at the pollination stage, and X_2 the disease index at the late grain filling stage, where the disease index = 100 × Σ (number of diseased leaves at different levels × representative value at different levels)/(total number of investigated leaves × highest representative value).[26] A significant linear correlation was observed between yield loss and disease index.

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VIRULENCE DIFFERENTIATION

The CLS causative agent, *C. lunata*, showed a virulence differentiation in China.[27−30] Differential host systems, such as Shen 135, Mo17, E28, 78599-1, Huangzao-4, C8605, 7922, and 477, have been used to establish the virulence assessment of *C. lunata* isolates.[27,30,31] The differences in virulence mainly involve lesion size, lesion margin color, and lesion distribution on the plants. The virulence identification method based on differential host systems allows monitoring of pathogen virulence and geographical distribution in the maize-planting regions to be easy.[27]

Biochemical and molecular identification techniques have also been utilized to differentiate the virulence of *C. lunata* isolates. Isozymes, random amplified polymorphic DNA (RAPD), universally primed PCR (UP-PCR), internal simple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP) methods have also been tested to analyze the genetic variability among *C. lunata* strains.[15,16,28,30,32−36] The classifications using one method or a combination of two or more methods were found to correlate with the classifications achieved using the differential host system method. Thus, biochemical and molecular identification methods have been used to assist the differential host system method in classifying the virulence of *C. lunata*.

The virulence variation in *C. lunata* is complex and controlled by several genes involved in metabolic pathways. Two-dimensional gel electrophoresis (2-DE), suppression-subtractive hybridization (SSH) cDNA library, and transcriptome analysis[37−39] have been used to identify some of the key genes and proteins involved in virulence differentiation. Proteins including superoxide dismutase (SOD), 1,3,8-triHN reductase (BRN1), scytalone dehydratase (SCD), polyketide synthase (PKS), and stress-tolerance-related proteins (HSP104 and HSP70) have been shown to be involved in the virulence differentiation. Genes such as SOD, SCD, and BRN1 have been used as markers to differentiate virulence, particularly with respect to high virulence and melanin production.[37,38,40]

VIRULENCE FACTORS OF *C. LUNATA*

Cutinase and Cell-Wall-Degrading Enzymes (CWDEs). Pathogenic fungi are challenged by barriers during infection of a host plant, including the cuticle layer and cell wall. Pathogens secrete cutinase (E.C. 3.1.1.74) and cell-wall-degrading enzymes (CWDEs) to degrade the cuticle layer and cell wall and invade the plant.[41,42] Thirteen cutinase genes in *C. lunata* have been found by genomic analysis.[43] The transcript level of *ClCUT7* was found to gradually increase during early pathogenesis (within 3 h post-inoculation), and the virulence of the *ClCUT7* knockout mutant was found to decrease in unwounded maize leaves but not wounded leaves. The invasion of *C. lunata* decreases the densities of cellulose, xylan, and pectin in the cell wall[44] by producing the CWDEs, polygalacturonase (PG), polymethylgalacturonase (PMG), and cellulase. These enzymes are produced sequentially during the infection process; PG and PMG are produced first, followed by cellulase. The activity of PG is higher than that of PMG.[45] Moreover, following the repeated inoculation of *C. lunata* on maize for six generations, a positive correlation was found between the virulence and CWDE activity of *C. lunata* along with the repeated generation of strains on the host plant.[38] The activity of cellulase varied more significantly along with the repeated inoculations on the host plant than the activities of other enzymes, suggesting that cellulase is an important virulence factor.[46,47] Genes related to the virulence of *C. lunata* are listed in Table 1.

Table 1. Summary of Identified Virulence Genes in *Curvularia lunata*

gene	GenBank accession number	biological function	reference
CLCUT7	[a]	Cutinase	Liu et al. 2016
CLT1	GQ292557	Toxin (M5HF2C)	Gao et al. 2014
BRN1	DQ358052		Liu et al. 2011
VELB	KY435512		Gao et al. 2015
CLPKS18	MF114294		Gao and Chen 2017
BRN1	DQ358052	Melanin	Liu et al. 2011
CLT1	GQ292557		Gao et al. 2014
CLPKS18	MF114294		Gao and Chen 2017
CLNPS6, *CLFTR1*	JQ698337, AHN52042.1	Iron ion channel	Lu et al. 2021
NOX1, *NOX2*	[a]	NADPH oxidases	Mao 2018 and Wang et al. 2020
CLK1	JN657517.1	MAPK Signaling pathways	Gao et al. 2013
CLM1	HQ851366		Wang and Chen 2011
CLH1	[a]		Ni et al. 2018
CLG2P, *CLF*	HQ655805, KT336108	RAS protein family	Liu et al. 2016

[a]Indicates no submission to the GenBank database.

Toxins and Melanin. Cell-free extract from *C. lunata* grown in Fries medium has been shown to be toxic to maize leaves. This extract damaged plant cell organelles, such as the chloroplast membrane, grana, matrix lamella, and mitochondria, which leads to the leakage of leaf cell electrolytes, thereby decreasing chlorophyll synthesis and causing leaf necrosis.[48,49] Notably, this extract from *C. lunata* also inhibited the germination of weed seeds, such as crabgrass (*Digitaria sanguinalis*), barnyard grass (*Echinochloa crusgali*), redroot amaranth (*Amaranthus retroflexus*), and green bristlegrass (*Setaria viridisas*) but was safe for seeds of rice, soybean, wheat, and cotton.[50] Two substances in the extracts from *C. lunata* grown in Fries modified medium were separated using chromatography.[51] They were found to be soluble in water and methanol and insoluble in chloroform and diethyl ether. One of these substances had an aromatic ring in its structure with a molecular weight of 350. This compound was determined to be a nonprotein-like substance containing soluble saccharide.[51] Another substance which was a thermal and light-stable substance was identified with a peak at 190 nm using the UV spectrum.[48,52] The virulence activity of this substance in maize is increased by dialysis under acidic conditions.[52] The chemical nature of toxic substances produced by *C. lunata* is unclear.

Liu et al.[53] purified and characterized the structure of one toxin from *C. lunata* using thin-layer chromatography (TLC) and high-performance liquid chromatography−mass spectrometry (HPLC-MS) techniques and identified the toxin as methyl 5-(hydroxymethyl) furan-2-carboxylate (M5HF2C) with the molecular formula $C_7O_4H_8$ and molecular weight of 156. This toxin induced leave spots not only on the host, maize (*Zea mays* L.), rice (*Oryza sativa* L.), sorghum (*Sorghum vulgare* L.),

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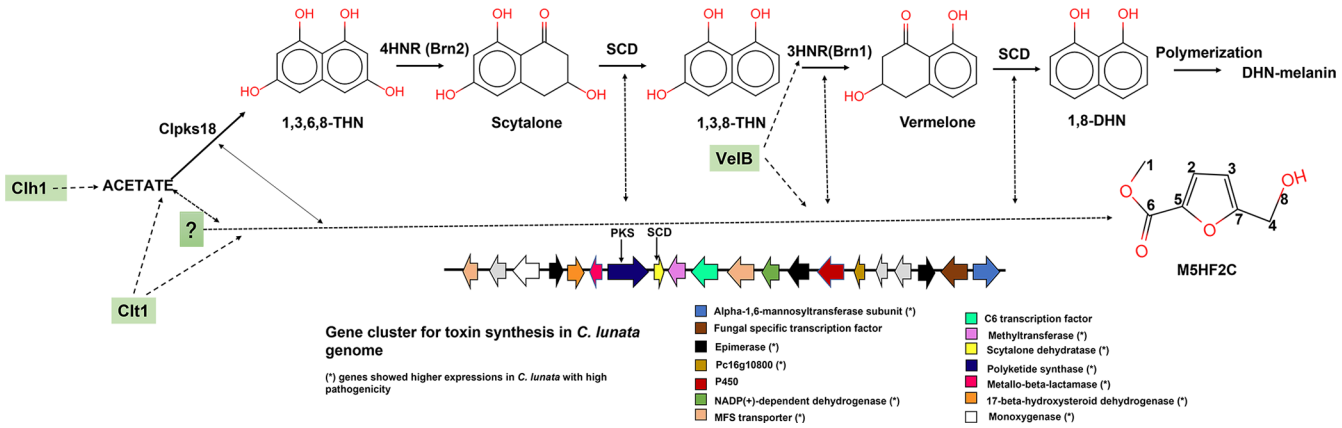

Figure 3. Genes shared between the biosynthesis of dihydroxynaphthalene (DHN) melanin and the production of the methyl 5-(hydroxymethyl) furan-2-carboxylate (M5HF2C) toxin in *Curvularia lunata*. Along the melanin biosynthesis pathway, positions indicated by arrows are those of related genes also involved in toxin production. The precursor of melanin is acetate, and *CLH1* is involved in the regulation of acetate production, influencing the synthesis of both toxins and melanin. Genes involved in the subsequent synthesis of melanin, such as *PKS*, *SCD*, and *BRN1*, also influence the production of the toxin M5HF2C. These genes are predicted in the gene cluster to be possibly related to toxin synthesis according to a *C. lunata* genome analysis. Reprinted in part with permission from reference Genome sequence and virulence variation-related transcriptome profiles of *Curvularia lunata*, an important maize pathogenic fungus. *BMC Genomics* **2014**, *15*, 627. Copyright [BioMed Central, 2014]. VelB can physically interact with BRN1, regulating melanin synthesis and influencing M5HF2C production. The regulator *CLT1* is involved in M5HF2C biosynthesis but also regulates the expression of genes related to melanin production.

tomato (*Lycopersicum esculentum* Mill.PE), and pepper (*Capsicum annuum* L.), but also on the nonhost plants, tobacco (*Nicotiana tabacum* L.), wheat (*Triticum aestivum* L. em, Thell.), spinach (*Spinacia oleracea* L.), and sunflower (*Helianthus annuus* L.), demonstrating that M5HF2C was a non-host-specific toxin. In a further attempt to explore the mechanism by which this toxin is synthesized or produced in *C. lunata*, Gao et al.[54] found gene *CLT1* (GenBank accession: GQ292557), which was predicted to encode a Brica-brac, Tramtrack, and Broad Complex (BTB) domain-containing protein comprised of 745 amino acids that occur as a single copy in the genome of *C. lunata*.[55] The BTB domain is an evolutionarily conserved protein−protein interaction motif. The BTB-containing proteins bind to other domains and can perform different functions, such as transcriptional regulation and protein degradation.[56] Recently, two CLT1-interacting proteins, which, respectively, correspond to xylanase (Clxyn24) and acetyl xylan esterase (Claxe43), were identified by a yeast two-hybrid system (Y2H). CLT1 physically interacted with Clxyn24 and Claxe43 through its BTB domain to degrade xylan which was used as a carbon source for *C. lunata* growth. The utilization of xylan provides acetyl-CoA for the synthesis of melanin and toxin as well as energy and other intermediate metabolites for conidiation.[57]

Melanin is a secondary metabolite that widely exists in animals, plants, and microorganisms.[58] Treatment of maize leaves with melanin extracted from *C. lunata* grown in potato dextrose medium induced leaf electrolyte leakage.[59] Melanin in fungi includes four types: polydihydroxynaphthalene (DHN), dopa (DOPA), catechol, and γ-glutamyl-3,4-hydroquinone (GBDH).[60,61] In *C. lunata*, Wang et al.[59] identified the intracellular melanin type as DHN using UV spectrum and infrared spectrum analysis. The biosynthesis of DHN melanin requires five key enzymes: PKS, BRN1, tetrahydroxy-naphthalene reductase (BRN2), SCD, and laccase.[62] Rižner and Wheeler[62] described the function of *BRN1* in the melanin biosynthesis of *C. lunata*. Gao et al.[38] detected three melanin synthesis genes, *BRN1*, *BRN2*, and *SCD*, that were significantly expressed in pathogenicity-enhanced strains. Liu et al.[63] cloned the full-length cDNA of *BRN1* (GenBank accession: DQ358052) of *C. lunata* and found that *BRN1* was involved in the production of M5HF2C in addition to melanin biosynthesis. Similarly, deletion of *CLT1* also inhibited melanin production, implying a gene network between the biosynthesis of *BRN1*-based DHN melanin and *CLT1*-related toxin[57] (Figure 3). However, the communication between the two biosynthesis pathways is unclear. In addition, Gao et al.[64] found that BRN1 physically interacts with VelB to regulate conidial formation, M5HF2C toxin biosynthesis, and the pathogenicity of *C. lunata*. VelB is a velvet protein that is involved in fungal secondary metabolism. However, VelB negatively regulates the melanization of mycelia in *C. lunata*.[65] Furthermore, a deletion mutant of *clpks18* (GenBank accession: MF114294) (a PKS gene from *C. lunata*) showed an albino phenotype and significantly reduced production of the M5HF2C toxin. This indicated that *CLPKS18* in *C. lunata* is not only involved in DHN melanin synthesis but also associated with M5HF2C toxin biosynthesis.[66,67]

Other Virulence Factors. Iron is an essential nutrient for maintaining the virulence of *C. lunata*. Lu et al.[68] demonstrated that nonribosomal peptide synthetase 6 (*ClNPS6*) (GenBank accession: JQ698337.1) and Fe transporter permease (*ClFTR1*) (GenBank accession: AHN52042.1) were involved in the iron acquisition and shown to be essential for virulence in infectious stages of *C. lunata*.

Wang et al.[69] clarified that a NADPH oxidase *NOX2* positively regulated sporulation, conidial germination, appressorium formation, melanin production, and reactive oxygen species generation and virulence in *C. lunata*. Additionally, small RNAs from *C. lunata* were identified by HiSeq deep sequencing and bioinformatics analysis.[70] Using the miRBase database, 1012 microRNA-like RNAs (milRNAs) were identified as similar to known microRNAs, whereas 48 potential novel miRNAs were identified as unique. Further studies on the expression pattern analysis and target prediction suggested that these milRNAs might be involved in the growth and virulence of *C. lunata*.[70]

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Signaling Pathways Involved in Pathogenicity. Three key genes of the mitogen-activated protein kinase (MAPK) pathway in *C. lunata*, *CLK1* (GenBank accession: JN657517), *CLM1* (GenBank accession: HQ851366), and *CLH1* genes (no accession number), were identified.[71−73] Functional analysis showed that *CLK1* regulates vegetative growth, sporulation, and virulence; *CLM1* regulates the production of conidia, cell-wall-degrading enzyme activity, and infectivity; and *CLH1* regulates antiosmotic capacity, the synthesis of toxins and melanin, and the activities of cellulase, pectinase, and cutinase.[71−73]

The RAS protein family is a family of important small GTPases that are involved in intracellular signal transduction pathways and function as organizers.[74] The key RAS protein Clg2P in *C. lunata* was identified and analyzed by Liu et al.[75] Clg2P physically interacts with CLF through Ras association (RA) to regulate appressorium formation and virulence in *C. lunata*. Additionally, the Fus3/Kss1-MAPK pathway plays an important regulatory role in conidial formation and virulence.[76] Three protein kinase genes, *CLF*, *Map2K*, and *CLK1*, and an anchor protein gene, *CLSTE50*, related to the Fus3/Kss1-MAPK pathway in *C. lunata* were identified by Zhao et al.[77]

DISEASE MANAGEMENT STRATEGIES

Chemical Control. Chemical fungicides can be used as a part of an integrated management strategy for CLS. A variety of chemical fungicides on the market, such as carbendazimzir-am-sulfur, thiophanate-methyl, iprodione, procymidone, polyoxins-carbendazim, chlorothalonil, difenoconazole propiconazole, and pyraclostrobin, have been reported to have good effective control against *C. lunata* in China.[78−82] Additionally, exogenous application of chemicals to maize can induce resistance against CLS. Nine exogenous chemical compounds that did not inhibit the growth of *C. lunata* were found to induce maize resistance against CLS: salicylic acid (SA) was the most effective compound, reducing disease severity by up to 49.6% ($p < 0.05$), followed by vitamin K_3 (41.5%, $p < 0.05$), riboflavin (38.8%, $p < 0.05$), K_2HP0_4 (37.8%, $p < 0.05$), and $H_2C_20_4$ (27.2%, $p < 0.05$) (Yi 2005). Although chemical fungicide control measures are effective against CLS, they must be considered environmental hazards.[83]

Biological Control. Biological control uses either microorganisms or their derivatives to control pathogens.[84] Either utilization of a biological control agent (BCA) alone or as part of an integrated management is a favorite strategy to control maize foliar diseases.[85−87] The application of *Trichoderma* strains (H6, D9, C40, and SH2303) increased expressions of defense-related enzymes POD and PAL in maize seedling leaves and decreased CLS disease severity by 50−70% in both pot and field experiments.[88] The molecular mechanism of induced systemic resistance (ISR) against CLS in maize by *Trichoderma* has been revealed. Two cell-wall-degrading related hydrolases (THPH1/THPH2) and one hydrophobin protein (HYD1) produced by *Trichoderma harzianum* T22 and T28, respectively, were found to be interacted with maize autophagocytosis-associated protein (ZmATG3)/germin-like protein (ZmGLP) and ubiquilin 1-like protein (UBL), to induce maize resistance to *C. lunata* via jasmonic-acid-dependent pathways.[89−92] Additionally, a platelet-activating factor acetylhydrolase-like (PAF-AH) of *T. harzianum* T28 was also found to induce maize resistance against *C. lunata*, but the mechanism is unclear.[93] In addition to fungi, bacterial BCA against CLS has been reported. *Pseudomonas putida* strain Sneb 2249 showed 55.0% control effect against CLS in pot experiments and 39.0% control effect in field trials, and Sneb 2249 significantly promoted plant height, dry weight of the overground part, and thousand kernel weight by 24.89%, 49.15%, and 7.97%, respectively.[94]

Nanomaterials (NMs) offer a promising alternative in plant disease management.[95] Choudhary et al.[96,97] found that biopolymer-derived Cu-chitosan and Zn-chitosan nanoparticle (NP) treated maize exhibited significant control of CLS. Cu-CNPs at 0.04−0.16% significantly decreased CLS disease severity by 43.8−48.4% ($p = 0.05$) in pot conditions, and a higher concentration of NPs (0.12 and 0.16%) showed significant disease control (30.4% and 33.8%) ($p = 0.05$) in field conditions. Similar results were shown with Zn-CNPs to control CLS disease: Zn-CNPs at (0.04−0.12%) suppressed CLS disease severity by 46.2−50.7% ($p = 0.05$) in pot conditions, and Zn-CNPs at (0.08−0.16%) suppressed CLS disease severity by 32.9−39.5% ($p = 0.05$) in field conditions.

In addition, studies have shown that plant-based antifungal formulations are used to control plant diseases.[98−100] The treatment of maize seeds with acetone extract (4 mL) of *Lawsonia inermis* combined with 4% clove bud oil cake and 100% cow dung (2 mL) was effective in controlling CLS (percent disease control: 89.47%, $P \leq 0.05$).[101]

Host Resistance. Deployment of resistance variety is expected to be the most effective way to control CLS. Precise knowledge of the genetic factors governing CLS resistance may increase the speed of CLS resistance breeding efforts. Numerous QTL mapping investigations of CLS resistance have been performed in different cultivars of maize. Five to six QTLs for CLS resistance with small to moderate effects have been repeatedly detected on chromosomes 6, 7, 8, and 10.[102−106] However, the underlying mechanisms of resistance to CLS in different maize cultivars are not well characterized.

microRNAs are involved in maize resistance to CLS. Liu et al.[107] screened and identified microRNAs in maize during infection by *C. lunata*. They found a much higher diversity of microRNAs in the resistant variety (Luyuan) than in the susceptible variety (Huangzao 4); the targets of the differentially expressed miRNAs included genes involved in oxidoreductase activity, signal transduction, substance transport, hydrolase, amino acid metabolism, photosynthesis, protein translation processing and degradation, hormone regulation, and synthesis and metabolism of disease-resistant substances. Among the differentially expressed miRNAs, one called PC732 was significantly downregulated in the resistant variety and upregulated in the susceptible variety. Maize with overexpression of PC732 (PC732-OX) showed higher susceptibility to *C. lunata*, while PC732-underexpressed (PC732-STTM) transgenic maize plants had increased resistance. The results of bioinformatics and degradome sequencing analysis suggested that PC732 might affect the protein translation of metacaspase1 (ClCMA1), thereby affecting maize resistance to CLS. Exploration of the role of ClCMA1 in the resistance to CLS is underway. These results provide a new perspective for increasing maize resistance to CLS.

CONCLUSIONS

The CLS surveys in different maize fields over the years showed that CLS is still an important maize disease in China. Therefore, it is imperative that preventive measures should be

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undertaken to minimize pathogen dispersion and economic losses. To allow early detection of CLS in the absence of visual symptoms, a detection method based on hyperspectral imaging technology was explored. Hyperspectral image data of inoculated leaves and noninoculated leaves were collected over a range of 400−1000 nm with a hyperspectral imaging system. The accuracy of the linear kernel function for the test set 3 days after inoculation reached a high score (88.8%).[108] This early monitoring technology needs to be further vigorously developed to be more stable, precise, and easier to operate for application on the market.

The level of disease incidence depends first on the maize cultivar's resistance. However, until now, the maize resistance mechanism to CLS is still not entirely understood, even though some cultivars have been evaluated as CLS-resistant cultivars,[14,109] which significantly hindered the application and extension of CLS-resistant cultivars for commercial production.[110] Second, CLS is a typical airborne disease, and secondary infections of CLS occur frequently.[9,22] The level of disease incidence depends on many factors of the epidemiology cycle, such as the accumulation of this pathogen from debris of the previous crop, planting density, temperature, and humidity, etc. Thus, comprehensive healthy cultivation measures need to be applied, in which rational deployment of disease-resistant varieties is one of the leading measures.[22−24]

The CLS causative agent, *C. lunata*, showed virulence differentiation among different strains. Moreover, comparative transcriptome analysis of low-virulence and high-virulence *C. lunata* strains revealed that host pressure increased pathogen virulence by increasing the pathogen's biosynthesis of toxins and melanin.[37,38] To prevent disease re-epidemics in maize-growing areas, more attention should be given to the dynamic changes of strains with varied virulence. The combination of different molecular approaches and biochemical methods could be applied to monitor pathogenic variation.[15,16] Additionally, *C. lunata* can produce many virulence factors, but the synthesis mechanisms of these factors, such as toxin and melanin, are still not clear. Therefore, further exploration of the precursors, intermediates, and node genes involved in virulence factor synthesis is required to propose corresponding effective methods to control *C. lunata* infection.

Biological control is receiving increasing attention as an alternative means of disease control; however, it is generally still in the experimental stage, and few BCAs are currently available for the maize production system.[111] Seed coating agents containing biocontrol microbes have shown ISR effects, reducing foliar disease infection, but their control efficiency is usually not as high as that of chemical fungicides. In addition, common environmental stress factors, such as high temperature and drought, often impact the BCA effect in fields.[112] It seems that biological control can play important roles in integrated disease management strategies to reduce the input of chemical pesticides and in organic plant production.

In conclusion, a comprehensive integrated management strategy that combines the use of early detection of disease occurrence, rational deployment of disease-resistant varieties, biobased seed-coating agents, and novel chemical or biological fungicides, is an effective approach for the prevention and control of CLS.

AUTHOR INFORMATION

Corresponding Author

Jie Chen − *School of Agriculture and Biology, Key Laboratory of Microbial Metabolism, and Ministry of Agriculture Key Laboratory of Urban Agriculture (South), Shanghai Jiao Tong University, Shanghai 200240, P. R. China;* orcid.org/0000-0002-7773-3160; Email: jiechen59@sjtu.edu.cn

Authors

Shaoqing Wang − *School of Agriculture and Biology, Key Laboratory of Microbial Metabolism, and Ministry of Agriculture Key Laboratory of Urban Agriculture (South), Shanghai Jiao Tong University, Shanghai 200240, P. R. China;* orcid.org/0000-0001-8792-3649

Zhixiang Lu − *School of Agriculture and Biology, Key Laboratory of Microbial Metabolism, and Ministry of Agriculture Key Laboratory of Urban Agriculture (South), Shanghai Jiao Tong University, Shanghai 200240, P. R. China*

Bo Lang − *School of Agriculture and Biology, Key Laboratory of Microbial Metabolism, and Ministry of Agriculture Key Laboratory of Urban Agriculture (South), Shanghai Jiao Tong University, Shanghai 200240, P. R. China*

Xinhua Wang − *School of Agriculture and Biology, Key Laboratory of Microbial Metabolism, and Ministry of Agriculture Key Laboratory of Urban Agriculture (South), Shanghai Jiao Tong University, Shanghai 200240, P. R. China*

Yaqian Li − *School of Agriculture and Biology, Key Laboratory of Microbial Metabolism, and Ministry of Agriculture Key Laboratory of Urban Agriculture (South), Shanghai Jiao Tong University, Shanghai 200240, P. R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c03013

Author Contributions

Shaoqing Wang, Zhixiang Lu, and Bo Lang wrote the original draft preparation. Shaoqing Wang wrote and edited the review. Xinhua Wang, Yaqian Li, and Jie Chen performed project administration and funding acquisition. All authors approved the article for publication.

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Notes

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