Tar Spot of Maize: Current knowledge of genetic interactions and future research prospects to improve disease resistance

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Abstract

The emergence and spread of crop plant pathogens pose a significant risk to global food and nutritional security. Of particular concern are those fungal pathogens that can significantly reduce yields of staple crop plants such as maize (*Zea mays* subsp. *mays*). One such plant pathogen is *Phyllachora maydis* Maubl (*P. maydis*), which causes tar spot disease on maize. Though it was only recently identified within the United States, *P. maydis* is now considered one of the most economically important foliar diseases of maize and has since spread to most maize production areas within the United States and Canada, resulting in substantial yield losses. In this mini-review, we aim to summarize recent advancements in our understanding of the genetic interactions between maize and *P. maydis* whilst also highlighting future research prospects for developing genetic-based resistances.

Keywords: Maize, *Phyllachora maydis* , tar spot, disease resistance

Introduction

Maize (*Zea mays* subsp. *mays*) is one of the most widely cultivated cereal grains and serves as a significant source of calories for human and animal consumption. Though there are a multitude of threats to global maize production, substantial yield losses caused by diseases significantly challenge our ability to achieve global food and nutritional security sustainably and impose significant financial constraints to growers (Savary et al., 2019). Of particular concern are fungal pathogens capable of causing global pandemics and significantly reducing maize yields (Savary et al., 2019). One such fungal pathogen is the ascomycete *Phyllachora maydis* Maubl (*P. maydis*), a foliar pathogen of maize that causes tar spot disease (Ruhl et al., 2016; Figure 1A-B). First documented in Mexico in 1904, *P. maydis*is endemic to Central and South America where it has the potential to significantly reduce grain yields, especially under favorable environmental conditions (Maublanc 1904; Valle-Torres et al., 2020). Though *P. maydis* is often associated with tropical maize production areas, it emerged in Illinois and Indiana during 2015 and has since spread to several other maize-growing areas

within the United States and Canada (Rocco da Silva et al., 2021; Ruhl et al., 2016; Valle-Torres et al., 2020). Such rapid emergence and spread of *P. maydis* across varying geographical climates strongly suggests commercial maize hybrids in the United States are susceptible to this fungal pathogen. To this end, Telenko et al. (2019) evaluated a subset of commercially grown maize hybrids for their responses to *P. maydis* and showed that all hybrids tested were susceptible. Notably, a severe tar spot epidemic within the United States in 2018 resulted in an estimated ˜$840 million USD economic loss, demonstrating *P. maydis* is capable of imposing severe financial constraints to growers (Crop Protection Network, 2021). The current lack of durable, genetic-based resistance coupled with its potential to significantly reduce maize yields makes *P. maydis* one of the most economically important foliar diseases of maize (Mueller et al., 2020). Hence, future research should, in part, investigate the genetic mechanisms underlying maize–*P. maydis* interactions to improve tar spot resistance.

Population screens and linkage mapping have contributed to our understanding of the maize–*P. maydis* pathosystem

Our general knowledge regarding *P. maydis* resistance in temperate-derived maize remains limited given its recent emergence within the continental United States and Canada. However, sources of resistance to *P. maydis* have been identified in diverse maize germplasm collections. Mahuku et al. (2016) and Cao et al. (2017) performed Genome-Wide Association Studies (GWAS) on diverse mapping populations and identified a large-effect Quantitative Trait Locus (QTL) on chromosome 8 (bin 8.03), referred to as *qRtsc8-1* . Fine-mapping of this locus revealed two candidate genes GRMZM2G063511 and GRMZM2G073884, encoding an integral membrane protein and a putative leucine-rich repeat receptor-like protein, respectively, as likely having a functional role in *P. maydis* recognition (Ren et al., 2022). It is worth noting that integral membrane proteins and leucine-rich repeat receptor-like proteins are known to have a functional role in Surface Receptor-Mediated Immunity (SRMI) against several plant pathogens (Ding et al., 2021; Yadeta et al., 2013). It remains unclear, however, what pathogen-derived proteins are recognized by these host proteins and how their activation induces resistance to*P. maydis* .

To identify new allelic diversity against *P. maydis* , Lipps et al. (2022) screened several maize accessions developed by the Germplasm Enhancement of Maize (GEM) project. Though no line showed complete resistance, two accessions, GEMS-0066 and GEMS-0226, consistently displayed partial resistance to *P. maydis* across multiple geographical locations. These lines may thus serve as important sources of new resistance specificities (Lipps et al., 2022). Furthermore, 200 GEM accessions and a subset of 600 inbred lines from the Wisconsin Diversity Panel-942 (WiDiv-942) were evaluated from their responses to*P. maydis* (A. Thompson, *personal communication*). GWAS analysis revealed more than 100 Single Nucleotide Polymorphisms (SNPs) were associated with *P. maydis* tolerance (A. Thompson,*personal communication*). Intriguingly, several of the identified SNPs were linked to defense-related genes and may thus be important for*P. maydis* resistance (A. Thompson, *personal communication*).

The maize Nested Association Mapping (NAM) recombinant inbred lines (RILs) representing global maize diversity were derived from 26 inbred founder lines and are a valuable genetic resource that may contain additional allelic diversity against *P. maydis* (Gage et al., 2020). Indeed, the 26 inbred NAM founders were recently evaluated for their responses to *P. maydis* with the goal of identifying inbreds that are either resistant or susceptible to this fungal pathogen (R. Singh, *personal communication*). Though all inbred lines evaluated were susceptible, there were significant variations in their responses (Figure 1C). Nine inbred lines were scored as tolerant without complete resistance, six as moderately tolerant, two moderately susceptible, and the remaining eight were susceptible (R. Singh, *personal communication*). Intriguingly, most of the CIMMYT-derived maize lines (CMLs) were evaluated as tolerant or moderately tolerant. In contrast, the North American-derived lines, including B73, displayed varying levels of susceptibility but most inbreds were susceptible (R. Singh,*personal communication*). Variations in susceptibility of the 26 founder lines to *P. maydis* could help guide the selection of appropriate NAM mapping populations for future genetic studies. Future research efforts should thus focus on fine mapping the genetic loci responsible for *P. maydis* resistance via GWAS and/or SNP linkage map analysis. Collectively, these studies demonstrate that diverse maize

germplasm collections harbor genetic-based disease resistances to*P. maydis* and have been instrumental in our initial understanding of the genetic architecture underlying resistance to *P. maydis* .

Research prospects to improve disease resistance to P. maydis

The data thus far suggest *P. maydis* can cause significant yield losses in maize and should thus be considered a serious concern for maize growers. We, therefore, would like to propose high-priority research avenues likely to advance our fundamental understanding of the maize–*P. maydis* pathosystem.

Screen additional maize germplasm to identify new sources of resistance to *P. maydis*

Immediate efforts should focus, in part, on evaluating additional maize germplasm collections for resistance to *P. maydis* . In addition to screening the aforementioned natural germplasm collections, artificially generated germplasms also may serve as a useful resource for identifying new sources of resistance to *P. maydis* (Figure 2A). Ethyl methanesulfonate (EMS)-mediated mutagenesis of pollen grains is often used to generate mutagenic populations of maize that can then be subsequently used to screen for specific phenotypes (Candela and Hake, 2008). For example, Marla et al. (2018) used an EMS-mutagenized population of maize to generate and functionally characterize novel mutant alleles of the *Hm1* resistance gene. It is thus likely a similar chemical mutagenesis-based strategy could be deployed to identify new sources of genetic resistance against multiple *P. maydis* isolates. One advantage of screening an EMS-mutagenized population is the likelihood of identifying susceptibility genes, which are host proteins required by the pathogen to facilitate infection (Garcia-Ruiz et al., 2021; Langner et al., 2018). For this reason, inactivation of host susceptibility genes often compromises pathogen infection, thereby conferring resistance (Garcia-Ruiz et al., 2021; Langner et al., 2018). Therefore, screening an EMS-mutagenized population of maize for loss of susceptibility to *P. maydis* may be an attractive approach for identifying new resistance loci.

Investigate the infection strategy of *P. maydis* using high-resolution microscopy

In parallel to screening natural and artificial maize populations for new resistance alleles, additional studies should also aim to investigate the infection strategy used by *P. maydis* at the individual plant scale (Figure 2B). Currently, *P. maydis* is presumed an obligate biotrophic fungal pathogen as it likely requires photosynthetically active tissue for survival and is unable to be cultured in the laboratory (Rocco da Silva et al., 2021; Telenko et al., 2020). However, *P. maydis* has also been shown to overwinter on maize debris in the United States (Kleczewski et al., 2019b; Groves et al., 2020), suggesting it can survive as a saprophyte and may exhibit a bi-phasic lifestyle. Hence, *P. maydis* may be considered a hemibiotroph. Furthermore, our current understanding regarding the mechanisms underlying initial host infection and systemic spread is limited but is urgently needed to understand host immune mechanisms. High-resolution microscopy, in which the host and associated microbe are clearly observed at cell-type resolution (Caldwell et al., 2019), offers the potential to help elucidate the pathogen lifestyle and infection strategy of *P. maydis* within the host plant. For example, confocal microscopy revealed that *Fusarium oxysporum* f. sp.*lycopersici* first infects tomato root hairs and colonizes junctions between root epidermal cells (Lagopodi et al. 2002). Confocal microscopy also revealed differences in the root infection strategy between highly and weakly virulent strains of *Fusarium oxysporum*f. sp. *phaseoli* in susceptible common bean (Nino-Sanchez et al. 2015). The highly virulent strain reached the root xylem faster than the weakly virulent strain, which predominantly colonized the root cortex (Nino-Sanchez et al. 2015). Microscopy has also revealed differences in*Fusarium* colonization between resistant and susceptible cultivars in several species, including banana (Chen et al. 2019) and chickpea (Jimenez-Fernandez et al. 2013). Using such microscopy methods in plants with differing responses to *P. maydis* will help us elucidate whether this fungal pathogen is biotrophic or hemibiotrophic as well as shed light on the underlying resistance mechanisms in maize.

P. maydis **genomic surveillance and isolate profiling using a field pathogenomics strategy**

A detailed understanding of the current genetic diversity and population structure of *P. maydis* populations is limited. Such information is critical for monitoring *P. maydis* outbreaks and for the emergence of new *P. maydis* populations with potential resistance to fungicides. To this end, Broders et al. (2021) assessed

the sequence diversity of *P. maydis* collected from 186 single stroma derived from 16 hosts in an effort to initially understand the genetic diversity within *P. maydis* populations. Intriguingly, sequencing of the internal transcribed spacer (ITS) region from these samples revealed that there are likely three genetically distinct groups of *P. maydis* in the United States capable of infecting maize (Broders et al., 2021). However, the phylogenetic analysis performed was based only on sequencing of the ITS region and historical representatives from herbaria and may not be representative of current populations. Alternatively, whole transcriptome sequencing of *P. maydis* -infected tissue collected directly from the field offers a more thorough and detailed understanding of host-pathogen interactions (Figure 2C). This approach, termed field pathogenomics, presents a detailed analysis of the population structure and genetic diversity of*P. maydis* as well as informative gene expression data for both the host and pathogen. Field pathogenomics has been used to identify new lineages of wheat yellow rust populations in the United Kingdom (Hubbard et al., 2015) and the origins of wheat blast in Bangladesh (Islam et al., 2016; Kamoun et al., 2019). For these reasons, the field pathogenomics strategy may be an attractive approach to actively monitor*P. maydis* populations for the emergence of novel isolates.

Characterize *P. maydis* virulence (effector) proteins and their host targets in maize

Virulence proteins translocated by fungal pathogens often have a functional role in promoting plant disease either by suppressing or activating host immune responses and, therefore, can serve as important resources for introducing novel host resistance specificities (Figueroa et al., 2021). Though *P. maydis* is predicted to secrete effector proteins (Telenko et al., 2020), our general understanding of how*P. maydis* utilizes its effector repertoire for virulence as well as their host targets in maize remain unknown. Such studies are complicated by the observation that *P. maydis* is genetically intractable and culturing of this pathogen has not been successful (Rocco da Silva et al., 2021; Telenko et al., 2020). Hence, genetic manipulation of the fungal pathogen is not yet possible. Furthermore, controlled inoculation protocols using *P. maydis* have yet to be developed, thus greenhouse-based disease assays aimed at evaluating host responses to *P. maydis* cannot be performed. Despite these limitations, screening the effector repertoire of *P. maydis* can be performed through the use of heterologous expression systems in non-host plants such as *Arabidopsis thaliana* and *Nicotiana benthamiana* (Figueroa et al., 2021; Lorrain et al., 2018). This strategy has been effective at revealing the specific plant cell compartments targeted by effectors from other obligate biotrophic fungi as well as their host protein interactors (Figueroa et al., 2021; Lorrain et al., 2018). Future work should thus aim to exploit the use of heterologous plant systems to investigate the subcellular compartments targeted by *P. maydis* effector proteins as well as identify their host targets in maize (Figure 2D). Such information is likely to advance our understanding of the molecular interactions between *P. maydis*and host as well as provide initial insight into the pathogenicity mechanisms used by *P. maydis* to promote disease.

Concluding remarks

The rapid emergence and spread of *P. maydis* within the United States poses a significant threat to maize production. Most commercial U.S. maize hybrids are susceptible to *P. maydis* , and naturally occurring resistance genes have yet to be identified and functionally characterized. For these reasons, we aspire to synergize collaborations among multidisciplinary research experts in an attempt to: 1) evaluate additional germplasm collections for *P. maydis* resistance; 2) determine the lifestyle and host infection strategy of this fungal pathogen; 3) assess its population diversity; and 4) characterize the*P. maydis* effector repertoire and identify their host targets in maize (Figure 2). Such multidisciplinary research collaborations will likely accelerate the development and deployment of maize hybrids with genetic-based disease resistance to *P. maydis* .

Author Contributions

We sincerely apologize to colleagues whose works we were not able to cite due to space limitations. MH conceived this minireview. MH produced the first draft, and all authors critically revised the manuscript. All authors approved the final version of the manuscript.

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Figure Legends

Figure 1. *Phyllachora maydis* disease symptoms on maize.A, Tar spot symptoms on foliage and husk leaves. Severe*P. maydis* infection often leads to premature senescence and blighting. **B,** Cross section of *P. maydis* -infected leaf tissue depicting a single perithecium with ascospores. **C,**Representative leaves from four maize accessions phenotyped as tolerant and susceptible to *P. maydis* .

Figure 2. Proposed research strategies to improve disease resistance to *P. maydis*. A, Screening of additional maize germplasms may lead to the identification of new sources of resistance. **B,** High-resolution microscopy will help shed light on the pathogen lifestyle and infection strategy. **C,**Whole-transcriptome sequencing of *P. maydis* -infected tissue may be an effective approach to monitor pathogen populations for the emergence of novel isolates. **D,** Functionally characterizing the*P. maydis* effector repertoire and identifying their host targets in maize will help elucidate the virulence mechanisms that promote disease. Figure created with*www.BioRender.com*.

Figure 1

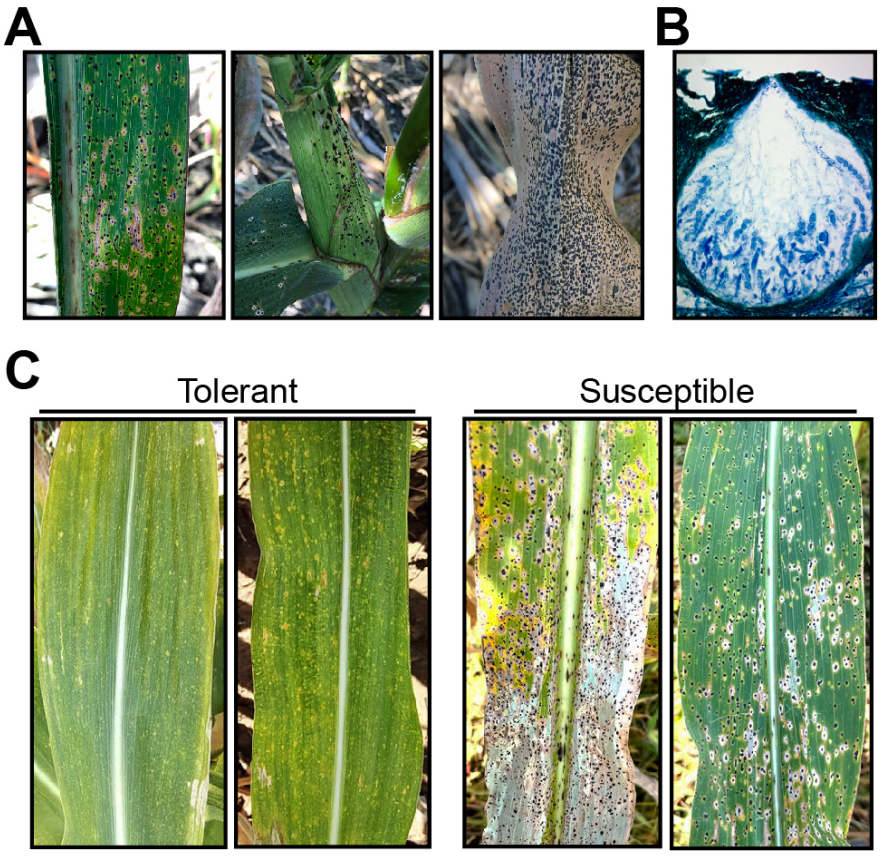

Figure 2