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Variability in *Sclerospora graminicola*, the Pearl Millet Downy Mildew Pathogen

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Abstract

Sclersopora graminicola, the causal agent of pearl millet downy mildew, is heterothallic and reproduces both sexually and asexually. Pathogenic variability in *S. graminicola* is known in both Africa and India. Host-specific evolution of virulence occurs in the pathogen, especially to genetically homogeneous single-cross F_1 hybrids in India. Virulence monitoring through a collaborative international virulence nursery and field surveys in India have provided useful information on virulence diversity. Genotypic differences in host-specific virulent isolates have been identified by using DNA fingerprinting markers. Quantitative trait locus (QTL) mapping for downy mildew resistance against different pathotypes from Africa and India has confirmed the presence of variable pathotypes. Developing cultivars with resistance to specific pathotypes and resistance gene pyramiding against multiple pathotypes through marker-assisted backcrossing could lead to cultivars with durable resistance.

Introduction

Genetic variability in plant pathogens is important for survival of their species and biotypes. The primary objective of many studies of variation in pathogenicity or virulence is to enable the exploitation of host plant genetic resistance for disease management (3). When selecting breeding material for disease resistance, it is important to know which pathotype to use in the screening process, how the resistance is expressed and inherited, and whether the resistance is likely to be adequate and durable. It also is important to determine whether the pathogen can change in response to the selection pressure resulting from host plant resistance and how rapidly such changes can occur.

The pearl millet downy mildew pathogen, *S. graminicola* (Sacc.) Shröt, is highly variable and reproduces both sexually and asexually (16). Physiological specialization in the pathogen was first reported in 1981 (19). Isolates from Africa and India are known to vary in pathogenicity and to be sexually compatible (1, 2, 10, 27, 29, 34). In India, increased virulence in *S. graminicola* has been attributed to large-scale cultivation of a number of genetically homogeneous single-cross F_1 hybrids (25, 29). Increased virulence diversity in the pathogen population has shortened the useful life of several commercial hybrids, and farmers have suffered losses due to reduced grain and fodder yields. Monitoring virulence changes in the pathogen population, identifying resistance to specific and multiple pathotypes, and directing breeding programs towards strategic utilization and deployment of resistance genes form the basis of long-term downy mildew disease management in pearl millet. In this chapter, we review the results of virulence monitoring, the status of virulence patterns in the pathogen, possible mechanisms of virulence evolution, and strategies for containing the disease through genetic resistance.

Monitoring Virulence Diversity

Pathogenicity is the genetic marker of greatest interest and has been studied intensively since 1981. Pathogenic and genetic variability in *S. graminicola* have been studied through a collaborative International Pearl Millet Downy Mildew Virulence Nursery (IPMDMVN), disease surveys in farmers' fields, inoculations of host differential lines in the greenhouse, and DNA fingerprinting markers.

The IPMDMVN. This collaborative virulence nursery was established in 1992 and contains 12 known resistant and susceptible pearl millet lines (inbreds and hybrids) of diverse geographic origin (24). Locations for the nursery were selected in the major pearl millet growing parts of the world where downy mildew is an endemic problem and active pathology research is in progress. These locations cover pearl millet areas from latitudes of 7.6° (Samaru, Nigeria) to 14.8° (Bambey, Senegal) in western and central Africa, and from 11.3° (Coimbatore) to 30.9° (Ludhiana) in India.

The IPMDMVN was planted in a replicated field experiment at four locations in 1992 and at 21 locations in 1998, in both downy mildew sick plots and/or by using an infector-row inoculation system (36). Downy mildew data on disease incidence were recorded at 30 days (preboot stage) and 60 days (soft-dough stage) after seedling emergence. Some pearl millet lines have higher disease incidence at 60 days than at 30 days. Daily maximums and minimums for temperature and relative humidity were recorded for this 60-day period. Collaborators sent infected leaf tissue containing oospores from the heavily infected lines from each location to the coordinating centers in India (ICRISAT, Patancheru, India) and west central Africa (ICRISAT, Bamako, Mali) for studies of variability under controlled environmental conditions.

The results of disease incidence data across 23 locations (3-8 years of data from each location) documents virulence diversity among *S. graminicola* populations (Table 8-1). Seven pearl millet lines (IP 18292, IP 18293, 700651, P310-17, P7-4, MBH 110, and 852B) had differential reactions that could be used to classify 23 populations into one of 15 putative pathotypes. Pathogen populations at several locations in India and Africa behaved similarly and were classified as the same pathotype. For example, the pathogen populations at Bambey (Senegal), and at Jamnagar and Gwalior (India) were in the least virulent category, whereas those at Bagauda (Nigeria) and Cinzana (Mali) were in the most virulent category. These results provide preliminary information on pathogenicity as it relates to spatial and genetic diversity in the population. However, further testing under controlled conditions is needed to understand and appreciate the spectrum of diversity. The field data also are limited by the number of locations used in the various countries, except for India where 14 locations were used. Within India, the 14 populations were classified into nine pathotypes. In Mali, Samanko and Cinzana have different populations, as do Bengou and Sadoré in Niger, and Bambey and Nioro-du-Rip in Senegal. A Western African Downy Mildew Variability Nursery also identified virulence diversity among *S. graminicola* populations at Bambey, Bengou, Kamboinsé, and Samaru (35). This nursery led in 1991 to the West African Downy Mildew and Smut Observation Nursery, in which promising lines from regional breeding programs are evaluated for stability of resistance to downy mildew. Screening locations for this nursery include Bambey, Bengou/Sadoré, Cinzana, Kam-

TABLE 8-1. *Sclerospora graminicola* populations from 23 locations in seven countries divided into 15 putative pathotypes based on seven host differential lines and 3-8 years of field testing from 1992-1999.

Location[a]	Pathogen Group	Host Differential Line						
		IP 18292	IP 18293	700651	P310-17	P7-4	MBH 110	852B
Bambey, Jamnagar, and Gwalior	I	R[b]	R	R	R	R	R	R
Bengou and Ludhiana	II	R	R	R	R	S[c]	S	S
Kamboinse, Durgapura, and Jodhpur	III	S	S	R	S	S	S	S
Mysore, Pune, and Dhule	IV	R	R	S	S	S	R	S
Bagauda and Cinzana	V	S	S	S	S	S	S	S
Patancheru	VI	R	R	S	R	S	R	R
Hisar	VII	R	R	R	S	R	R	S
Jalna	VIII	R	R	S	R	R	S	R
Coimbatore	IX	R	R	R	R	S	S	R
Aurangabad	X	R	R	S	R	R	R	S
Nioro-du-Rip	XI	S	R	R	R	R	R	S
Samanko	XII	R	S	R	S	R	S	S
Sadore	XIII	S	R	R	S	S	S	S
Mandor	XIV	S	R	S	S	S	S	S
El-Obeid	XV	R	R	R	R	R	S	S

[a]Locations: Bambay and Nioro, Senegal; Bengou and Sadore, Niger; Samanko and Cinzana, Mali; Bagauda, Nigeria; Kamboinse, Burkina Faso; El-Obeid, Sudan; and Jamnagar, Gwalior, Patancheru, Hisar, Jalna, Coimbatore, Aurangabad, Mysore, Ludhiana, Durgapura, Jodhpur, and Mandor, India.

[b]R, < 10% mean disease incidence.

[c]S, > 10% mean disease incidence.

boinsé, Tamale/Bawku, and Samaru/Zaria.

Virulence survey. Farmers' fields in India have been systematically surveyed for downy mildew incidence in pearl millet for the past seven years. Surveys in Maharashtra state documented both the diversity of hybrid cultivars grown by the farmers and the extent of downy mildew incidence (Table 8-2). In 1993 there were three popular commercial hybrid cultivars in Maharashtra. This number increased to 24 in 1999. If a new hybrid is grown on the same piece of land for three consecutive years, then the hybrid generally becomes susceptible if the weather has been favorable for disease development. Hybrids for which very high disease incidence has been reported (up to 80%) in farmers' fields (29), *e.g.*, MLBH 104, MBLBH 267, GK 1004, Proagro 7701, Eknath 201, BK 560, are now slowly being replaced by new resistant hybrids. Similar results have been obtained from limited surveys in the states of Rajasthan and Gujarat (Thakur *et al.*, *unpublished*). In contrast to genetically uniform single-cross hybrids, heterogeneous open-pollinated varieties, e.g., ICTP 8203 and Mallikarjuna, have had durable resistance to downy mildew (29; Table 8-2).

TABLE 8-2. Field survey of pearl millet cultivar diversity and downy mildew incidence (percentage)[a] on selected major hybrids and varieties in Maharashtra from 1993-1999.

Hybrid / Variety	93	94	95	96	97	98	99
BK 560[b]	90	70	-	-	50	-	80
Eknath 201[b]	-[c]	-	15	90	50	-	70
GK 1004[b]	-	-	80	20	75	95	60
MLBH 104[b]	53	20	90	80	70	75	75
MLBH 267[b]	0	40	20	90	60	75	52
Proagro 7701[b]	-	-	40	-	75	55	80
ICTP 8203[d]	0	0	2	0	1	2	0
Mallikarjuna[d]	0	-	0	0	0	-	-
Total entries planted	3	3	6	14	16	21	24

[a]Maximum incidence recorded in any of the observed fields.
[b]Hybrid.
[c]- Entry not planted.
[d]Open-pollinated variety.

Mechanisms of Virulence Evolution

Evolution of virulence in plant pathogens occurs by mutation, genetic drift, recombination, gene flow, and selection. In *S. graminicola*, recombination is important in creating and maintaining haplotype diversity, and considerable pathogenic variation has been reported among single-oospore and single-zoospore isolates from several populations (26, 28, 31). Molecular genetic variation in oospore- and zoospore-derived progeny has been demonstrated using DNA fingerprinting (18, 33) and Random Amplified Polymorphic DNA (RAPD; 15, 22). Host availability serves as a powerful force on pathogen populations and can result in the evolution of new virulence pathotypes (29, 32). For example, a highly virulent pathotype of *S. graminicola* from Jodhpur, Rajasthan appears to have evolved from a *S. graminicola* strain that can attack a local land race, *Nokha* (28). This pathotype caused a downy mildew epidemic during the 1997 rainy season crop at the farm of Central Arid Zone Research Institute, Jodhpur. Another virulent pathotype, specific to commercial hybrid MLBH 104, was identified in the state of Maharshtra (29) and resulted in the withdrawal of this hybrid.

As hypothesized by Leonard (14), selection intensities under greenhouse and field conditions for the same pathogen are not the same. In *S. graminicola*, the differences may reflect more intense selection pressure in the greenhouse due to higher pathogen densities, or that the field estimates include selection during the interval between seasons. The genetic composition of the host genotype has considerable impact on virulence selection, and this process is much faster with inbred lines than with hybrids or composite populations (32). Also, in greenhouse populations of *S. graminicola*, the fungus reproduces only asexually and only on seedlings. This process stabilizes allele frequencies specific to the particular host genotype(s) on which the pathogen is maintained and multiplied. In contrast to the greenhouse situation, sexual recombination can occur in the field, and new virulence gene combinations can result. In a host-parasite system, the amount of genetic variation for resistance and virulence can alter the population dynamics and equilibrium of the interacting species (20).

Use of Host Differential Lines. In host-pathogen systems where near-isogenic lines with known resistance genes are available, the use of host differentials has been very successful in monitoring and identifying new pathotypes or races of the pathogen. There are no such well-defined differential lines available in pearl millet, but we have identified pearl millet lines, *e.g.*, the IPMDMVN, that can discriminate between pathogen populations based on disease incidence. A number of downy mildew resistance QTLs specific to particular pathogen populations have been mapped (7). We are developing near-isogenic lines for several of these QTLs by using molecular marker-assisted backcrossing into a common, agronomically elite background. These inbred lines will be used as differentials for monitoring pathogen virulence.

There has been limited characterization of the pathogen variability present at many locations, and we currently have no good data sets from a number of representative locations in west and central Africa. Temporal

and spatial variation in data from field nurseries is commonly reported (24), but these observations need to be confirmed in greenhouse tests.

Use of Molecular Markers. DNA markers are being used to characterize pathogen isolates and to determine the genetic structure of populations and its relationship to virulence diversity. The molecular methods have the advantages of precise scoring, economy when conducted on a large scale, and the simultaneous measurement of variation at several loci including those with co-dominant alleles. At ICRISAT, DNA fingerprinting with Simple Sequence Repeats (SSRs) has been used to detect genetic diversity among pathotypes of *S. graminicola* (18). Of the SSRs tested, $(GATA)_4$ identified the most polymorphism amongst the host genotype-specific pathogen isolates of *S. graminicola*. The same SSRs also were used to confirm the emergence of a new virulent pathotype specific to a recently introduced commercial hybrid cultivar MLBH 104 (29). More recently, Amplified Fragment Length Polymorphism (AFLP) markers also have been used to detect genetic variation among *S. graminicola* isolates from India. We often can score 50-70 bands with each primer-pair combination, and the proportion of polymorphic bands per primer pair ranges from 35-60%.

Studies of *S. graminicola* populations from Africa and India are in progress, but there have been problems due to the need to work with small samples collected from natural lesions in Africa. Oospore samples must be air-dried before being sent to the United States in order to satisfy import regulations. These samples often yield no visible precipitated DNA following standard extraction protocols, which leaves only PCR-based amplification methods for use in detecting variation at the DNA level. Even AFLP analysis requires at least 50 ng of starting material, considerably more than is often available. Consequently, protocols that permit "whole genome amplification," originally developed for use in human diagnostics and forensics, are being tested for use with *S. graminicola*. Recently, our attention has focused on Degenerate Oligonucleotide Primer (DOP)-PCR (23), primarily because this method yields a fairly uniform amplification of sequences throughout the genome. Visible quantities of DNA (> 30 ng/ml) can be amplified from as little as 10 pg to 1 ng of purified *S. graminicola* target DNA. Subsequent amplifications of the DOP-PCR products with RAPD or microsatellite primers yield a high level of polymorphism between the control samples.

Since whole genome amplification primers are not genome specific and field samples are unlikely to contain only fungal material, special precautions must be taken to ensure that DNA polymorphisms that distinguish isolates are not the result of contaminating DNA from other plants or microbes. At present, we hybridize polymorphic bands to *S. graminicola* genomic DNA to confirm the origin of the amplified sequences.

Management of Virulence

Pathogen variation is a major cause of failure for control programs that rely on fungicides (4, 5, 12, 25). For example, studies of late blight of potato, caused by *Phytophthora infestans*, over the last 20 years have shown that resistance can be readily developed to fungicides that target a single active site (6, 12). This resistance can result in decreased or lost activity by such products under field conditions, *e.g.*, the failure of metalaxyl to control late blight of potato (5, 6, 12). Metalaxyl is widely used for seed treatment to control downy mildew in pearl millet, but effective control is obtained for only 30-40 days. Depending on the weather conditions and the susceptibility of the cultivar(s), downy mildew symptoms may appear on late tillers and the ear heads may be malformed. However, neither the failure of metalaxyl nor the emergence of metalaxyl-resistant strains of *S. graminicola* has yet been reported.

Effective disease management can be obtained with cultivars that have durable resistance. The most effective utilization of host plant resistance requires knowledge of the genetics of resistance, the population genetics and evolutionary biology of the pathogen, and the interaction of crop management practices with host plant resistance. The use of multiple resistance genes to produce durable resistance often is effective because the probability that a pathogen will simultaneously mutate to virulence at multiple corresponding loci usually is small (17). Major gene resistance is most efficiently exploited when it is developed either to prevent the pathogen population from easily adapting or to minimize the selection pressure exerted on the pathogen population.

Molecular approaches can be employed either in (i) the pyramiding of two, or more, resistance genes in a host line through marker-assisted selection or (ii) through genetic modification of existing resistance genes. Good sources of resistance to different and/or multiple pathotypes are available (30), and the genetics and inheritance of resistance from some sources are known (21). Genes from several of these sources have been incorporated into elite breeding lines and hybrid parental lines through conventional breeding methods (9). Some of these resistance sources also have been used to develop populations, and the first DNA markers for QTLs against different pathotypes of the pathogen were identified during 1993-1994 (11). At least 25 QTLs have been mapped on seven pearl millet linkage groups. Some of these QTLs

account for a significant proportion (20-80%) of the observed genetic variation within a given mapping population and are being transferred into elite hybrid parental lines by using marker-assisted backcrossing. A strategy for gene pyramiding and deployment also has been developed to reduce the damage caused by downy mildew (8, 37). This strategy combines marker-assisted selection, the multiline approach, and hybrid seed production procedures to generate hybrid cultivars that are agronomically uniform but variable in their resistance gene complements (37).

Conclusions

Significant advances have been made in understanding virulence diversity in the pearl millet downy mildew pathogen through field surveys and the IPMDMVN since the last global conference (13). However, the highly heterogeneous nature of the pathogen populations, and their ability to rapidly adapt to new host cultivars, means that new virulence pathotypes can quickly emerge whenever a new host plant resistance gene is deployed uniformly within a popular single-cross hybrid. This diversity in virulence limits the effective life span of high-yielding single-cross F_1 hybrid cultivars in India. Host-directed selection appears to be the key mechanism of evolution of new pathotypes in the pathogen population. Further studies of pathogenic and genetic variation in pathogen populations from diverse pearl millet growing areas will provide better knowledge of virulence diversity. The incorporation of downy-mildew-resistant QTLs specific to pathotypes through marker-assisted backcrossing into elite parental lines of hybrid cultivars should reduce the damage caused by the disease. Virulence monitoring through the IPMDMVN, field surveys, and the characterization of pathogen isolates for their virulence spectrum should continue to ensure optimal utilization and deployment of downy mildew resistance genes in pearl millet.

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