Disease Management in the Genomics Era—Summaries of Focus Issue Papers

S. J. Klosterman, J. R. Rollins, M. R. Sudarshana, and B. A. Vinatzer

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First author: U.S. Department of Agriculture-Agriculture Research Service (USDA-ARS), 1636 E. Alisal Street, Salinas, CA 93905; second author: Department of Plant Pathology, University of Florida, Gainesville 32611; third author: USDA-ARS, One Shields Avenue, Davis, CA 95616; and fourth author: Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg 24061.

The genomics revolution has contributed enormously to research and disease management applications in plant pathology. This development has rapidly increased our understanding of the molecular mechanisms underpinning pathogenesis and resistance, contributed novel markers for rapid pathogen detection and diagnosis, and offered further insights into the genetics of pathogen populations on a larger scale. The availability of whole genome resources coupled with next-generation sequencing (NGS) technologies has helped fuel genomics-based approaches to improve disease resistance in crops. NGS technologies have accelerated the pace at which whole plant and pathogen genomes have become available, and made possible the metagenomic analysis of plant-associated microbial communities. Furthermore, NGS technologies can now be applied routinely and cost effectively to rapidly generate plant and/or pathogen genome or transcriptome marker sequences associated with virulence phenotypes in the pathogen or resistance phenotypes in the plant, potentially leading to improvements in plant disease management. In some systems, investments in plant and pathogen genomics have led to immediate, tangible benefits. This focus issue covers some of the systems. The articles in this focus issue range from overall perspective articles to research articles describing specific genomics applications for detection and control of diseases caused by nematode, viral, bacterial, fungal, and oomycete pathogens. The following are representative short summaries of the articles that appear in this Focus Issue.

PERSPECTIVES ON ADVANCES IN THE STUDY OF PLANT PATHOGENS IN THE GENOMICS ERA

With a wealth of new crop plant genome data, the discovery of new resistance and defense-related genes and the characterization of variation in resistance loci has become a data-rich reality. How do we best take advantage of this knowledge? The perspective piece from Burdon et al. (2016) provides a critical view of the past, present, and developing genomic era strategies for rationale improvement and management of disease resistance. The authors outline evolutionary and epidemiological principles underlying the identification and deployment of resistance genes that should serve well in guiding the discovery of new resistance genes, the development of resistance loci markers, and the deployment of gene cassette and other genome-empowered strategies for durable disease management.

Bacteria present their own peculiar challenges for disease management. Since the publication of the first genome sequence of a plant pathogenic bacterium in the year 2000, breath-taking progress in understanding bacteria–plant interactions has been achieved in a short time. Sundin et al. (2016) document the progress made in their

concise review, and lay out how this progress is being translated into real advances in disease management. Among others, applications include more sensitive and precise diagnostics, genome-edited disease resistance, and the development of small molecules that target virulence genes in the pathogens themselves.

NGS has not only allowed us to unravel the diversity of bacterial plant pathogens but has also opened new vistas into the analysis of microbial diversity associated with plants directly or within the environment in which plants live. The emerging field of plant microbiome—or phytobiome—research shows much promise in elucidating the intricate interactions between plants and their phytobiomes. This accumulating information could even be employed to engineer phytobiomes to reduce disease incidence and severity or by using phytobiome members as biopesticides to control specific pathogens. Poudel et al. (2016) propose in their article a systems framework to elucidate microbiome networks, and more importantly, identify candidate microbes to manage plant diseases.

The genomics revolution and associated tools have also advanced strategies for control of plant viruses. The last three decades saw enormous success in engineered resistance to plant viruses using transgenes derived from viral and nonviral sequences. In recent years, gene editing and functional genomics are being used to develop novel virus resistant plants. Fondong et al. (2016) reviewed these technologies, viz. artificial miRNA, transacting small interfering RNA, and some which are more popular by their acronyms, viz. Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated protein 9 (Cas9) technology, and Targeting Induced Local Lesions IN Genomes (TILLING). Some of these technologies do not need a selectable marker for the selection of transgenic plants and agroinfection.

With the increasing genome resources tools at our disposal, it has also become possible to tease apart complex interactions between pathogens and insect vectors. Plant viruses vectored by insects are often controlled by insecticides, which have come under increasing scrutiny in recent years. Kaur et al. (2016) review how NGS can be used to unravel insect genes that are up-regulated during insect–virus interactions following the acquisition of the virus. Their review specifically focuses on the interaction of a begomovirus, *Tomato yellow leaf curl virus*, and a crinivirus, *Tomato chlorosis virus*, with the whitefly vector *Bemisia tabaci*; and of a tospovirus, *Tomato spotted wilt virus*, with thrips *Frankliniella occidentalis*. In addition, they discuss how this new information may be exploited for the insect vector control by RNA interference.

IDENTIFICATION OF PATHOGEN EFFECTOR SEQUENCES

Examination of the complete effector repertoire in plant pathogens has become possible with the increasing availability of whole pathogen genome sequences, and represents an invaluable resource for development of durable resistance. Gibriel et al. (2016) provide a fascinating review of some pathogen effectors identified in recent years through genomic approaches. Third generation sequencing tools can overcome short read and error prone limitations of earlier

Corresponding author: S. J. Klosterman;
E-mail address: steve.klosterman@ars.usda.gov

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sequencing technologies, especially in repeat-rich regions of genomes, aiding in the correct identification of effector sequences and annotation. The authors also discuss the use of effectors as tools in screening for disease resistance and also the monitoring of effector diversity in pathogen populations. Knowledge of this diversity can lead to more focused and cost-effective development and deployment of host resistance.

Bacterial comparative genomics has been greatly facilitated by the decreasing cost of genome sequencing. This now allows sequencing of not just one representative strain of a pathogen but dozens or even hundreds of strains to identify the core repertoire of essential virulence genes of a pathogen or a group of related pathogens. Crop breeding can then focus on developing cultivars with resistance genes against these core pathogen genes that the pathogen is unlikely to modify or lose. Using such an approach in their article, Timilsina et al. (2016) find that the genes *xopJ4* and *avrBsT* are currently the best targets for breeding tomato to obtain durable disease resistance against bacterial spot.

The quantitative nature of virulence and the recalcitrant nature of manipulating fungal sexual cycles have placed limitations on the discovery and understanding of host–pathogen compatibility. Gao et al. (2016) take advantage of dominant host sensitivity to identify necrotrophic effectors using a genome-wide association study (GWAS) approach. Analysis of a global collection of 191 *Parastagonospora nodorum* isolates with 2,983 newly developed SNP and gene-specific markers not only revealed loci for previously characterized necrotrophic effectors as a proof of concept but also identified a new locus associated with virulence. Further characterizations of marker saturation and linkage disequilibrium decay serve as helpful guides to parameters influencing the application of GWAS for discovery of loci influencing quantitative traits in fungi.

GENOMICS APPLICATIONS FOR PATHOGEN POPULATION ANALYSES AND DIAGNOSTICS

Draft sequencing of pathogen genomes can provide a rapid and cost effective means to understanding life and disease cycle attributes and provide resources for tool development required for more detailed analyses. This strategy was used to characterize the genetic diversity and population structure of the gray leaf spot pathogen of maize in South Africa (Muller et al. 2016). Comparing draft genomes from South Africa and the United States provided a streamlined process for identifying polymorphic small sequence repeat (SSR) markers. In conjunction with species-specific mating type locus sequences, these markers were applied to population genetic analyses. The results indicate that sexual recombination is common and population differentiation among the sampled geographical areas is lacking. This kind of analyses powered by the establishment of draft genomes allow for knowledge-based management decisions concerning the deployment of resistant varieties and judicious application of fungicides.

We have begun to see how increased availability of genomic sequences has accelerated the development of diagnostic tools for individual plant pathogens. For those plant pathogens for which whole genome resources may not be available, application of inexpensive NGS can be used to generate necessary sequences quickly and cost effectively for development of diagnostic markers. Withers et al. (2016) describe the use of NGS to develop species-specific molecular diagnostics for the oomycete pathogen, *Pseudoperonospora cubensis*. The study first entailed generation of NGS data for two very closely related species, *Pseudoperonospora cubensis* and *Peronospora humuli*. Sequence comparisons through bioinformatics approaches led to the finding of seven sets of markers conserved in *Pseudoperonospora cubensis* but absent from the genomic sequence of *Peronospora humuli*. The markers were also tested against isolates of the same species and other oomycetes to provide further confirmation of specificity. Withers et al. (2016) envision the immediate usage of the markers in studies addressing epidemiological questions of *Pseudoperonospora cubensis* by species-specific detection in plant material or from spore traps.

Grapevine red blotch-associated virus (GRBaV) was recently identified as an emerging pathogen, which has impacted both the nurseries and premium wine grape production in the United States. In particular, the virus has impacted the wine industry in the state of California, where wine shipments totaled >\$30 billion in 2015 (www.wineinstitute.org). Since its discovery, the researchers are on the pursuit to identify a vector(s) capable of GRBaV transmission under vineyard conditions. Bahder et al. (2016) conducted a phylogenetic study on the amino acid sequence of the coat protein sequences of geminiviruses and found that the coat protein of GRBaV was closely related to that of *Tomato pseudo-curly top virus*, a geminivirus transmitted by treehoppers of the family Membracidae. Following this, they found that the three-cornered alfalfa hopper, *Spissistilus festinus* (Say), considered a minor pest of grapevines, was able to transmit the virus between grapevines. A quick diagnosis of the virus in the recipient plants was achieved using digital PCR technology.

GENOMICS ACCELERATES DEVELOPMENT OF PLANT DISEASE RESISTANCE

The broadening availability of high quality crop plant genomes is opening doors to an ever-increasing set of tools for plant breeders. This is particularly welcoming to breeders of crops with large and complex genomes such as wheat. With the wheat genome and advanced breeding genotype resources as a backdrop, Mahoney et al. (2016) describe taking advantage of next generation sequencing technologies to establish a genotyping-by-sequencing (GBS) strategy to map quantitative traits loci for tolerance to Rhizoctonia root rot. This soilborne disease is of economic concern in spring-planted wheat in the Pacific Northwest. Identification of markers for loci conferring increased levels of tolerance provides a foundation for cultivar improvement, marker development, and a new genetic resource for the management of root rot.

Also taking advantage of wheat genome resources, Pariyar et al. (2016) describe the use of association mapping to identify associations between phenotypic variations in susceptibility to the cyst nematode *Heterodera filipjevi* and single nucleotide polymorphisms (SNPs). Of seven quantitative trait loci (QTLs) identified by the authors, those on chromosomes 1AL, 2AS, 2BL, 3AL, and 4AL of wheat were linked to genes with predicted roles in biotic stress and plant pathogen interactions. The genome-wide association study included 161 modern winter wheat accessions, and the study of Pariyar et al. (2016) represents the first report of QTLs conferring resistance to *H. filipjevi* in wheat.

The most cost-effective way to control plant diseases is genetic resistance. However, breakdown of host resistance by new pathogen races is a perpetual threat. In a third article focused on wheat disease resistance, Xiang et al. (2016) show how genomics can greatly accelerate and facilitate breeding of stripe rust-resistant wheat cultivars. Using the excellent collection of molecular markers available in wheat, the authors were able to precisely map a stripe rust resistance locus in the wheat cultivar Tyee. This result will greatly facilitate breeding of new cultivars in which the mapped resistance locus Yr76 can be combined with other resistance loci for broad resistance to the majority of pathogen races. Such cultivars can be expected to significantly reduce yield losses from wheat stripe rust.

Chang et al. (2016) carried out genome-wide association mapping studies on the USDA-Agricultural Research Service Soybean Germplasm Collection using phenotypic data in the public Germplasm Resources Information Network (GRIN) and public SNP data. This approach identified SNPs associated with disease ratings from 11 different diseases, including those caused by a bacterium, multiple fungi, a nematode, and several viruses. Linkage disequilibrium analyses of SNPs revealed that some of the resistance phenotypes previously characterized were significantly associated with candidate leucine-rich repeat LRR-receptor-like kinase and NBS-LRR genes that may play a role in resistance to one or more of these pathogens. Importantly, Chang et al. (2016) carried out resistance

mapping for the first time using the approach for *Diaporthe* stem canker, caused by *Diaporthe phaseolorum* var. *caulivora* and *D. phaseolorum* var. *meridionalis* as well as for *Bean pod mottle virus*. The study illustrates the power of genome-wide association studies to discover disease resistance in soybean.

GBS was successfully deployed by Mhora et al. (2016) to predict resistance to lima bean downy mildew. In this study, the authors were able to map the race F resistance locus, associated with the downy mildew resistance phenotype. More specifically, the authors deployed Kompetitive allele specific PCR (KASP) assays which utilize allele-specific oligo extension and fluorescence resonance energy transfer for detection, to develop 12 GBS markers. Two of the markers were used to accurately predict downy mildew race F resistance in a diverse panel of 256 lima bean varieties, cultivars, and wild germplasm. The study of Mhora et al. (2016) demonstrates the power of such genomics-based approaches in accurately predicting disease resistance.

The correlation of genomic results with reproducible phenotypes can be challenging. Cadle-Davidson et al. (2016) undertook phenotyping studies of grape powdery mildew influenced by variables such as environment, inoculum dose, and pathogen genotype, which can impact phenotype observations. The phenotyping was conducted in relation to GBS data and previously identified *REN1* or *REN2* loci that confer powdery mildew resistance. The authors validated some of the bioassays in identifying *REN1* or *REN2* loci, demonstrating that the phenotype of pathogen colony density at 8 or 9 days postinoculation was most reproducibly associated with *REN1* or *REN2*. QTL mapping with GBS maps defined the *REN1* and *REN2* loci at high resolution. Because some phenotyping approaches did not correlate with the resistant genotype, this work indicates that results from studies focusing on the genotyping of plant resistance must still be grounded by expertise in phenotyping for field validation (Cadle-Davidson et al. 2016).

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