

Introduction

Plant Disease Resistance Research at the Dawn of the New Era

Awais Khan,^{1,†} Roi Ben-David,^{2,†} Jonathan Richards,^{3,†} Urmil Bansal,^{4,†} Congli Wang,^{5,†} Curt McCartney,^{6,†} Remco Stam,^{7,†} and Nian Wang^{8,†}

¹ Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, NY 14456, U.S.A.

² Department of Vegetable and Field Crops, Institute of Plant Sciences, Agricultural Research Organization–Volcani Institute, Rishon LeZion 7528809, Israel

³ Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, U.S.A.

⁴ The University of Sydney Plant Breeding Institute, Faculty of Science, School of Life and Environmental Sciences, 107 Cobbitty Road, Cobbitty, NSW 2570, Australia

⁵ State Key Laboratory of Black Soils Conservation and Utilization, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, Heilongjiang, P.R. China

⁶ Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada

⁷ Department of Phytopathology and Crop Protection, Institute of Phytopathology, Faculty of Agricultural and Nutritional Science, Christian Albrechts University, Kiel, Germany

⁸ Citrus Research and Education Center, Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Lake Alfred, FL 33850, U.S.A.

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Plant diseases caused by fungal, bacterial, viral, and nematode pathogens incur serious economic losses to crops every year, with yield losses estimated to be approximately 16% worldwide, and pose a major threat to global food and nutritional security (Oerke et al. 2006). Importantly, monocultures of susceptible varieties enhance agroecosystem vulnerability and would require intense and costly disease management strategies in the event of severe epidemics. Farmers' reliance on repeated chemical sprays raises environmental and food safety concerns and can potentially lead to emergence of new chemical resistant pathogen strains. Utilization of host disease resistance is considered the most sustainable approach to protect plants against various pathogens (Khan and Korban 2022).

Identification and utilization of resistance mechanisms that can be used in plant breeding programs remains one of the most thriving research areas of the plant pathology field. This has been supported by recent developments of novel technologies and their application in plant pathology including lost-cost and high throughput genome sequencing (Hamim et al. 2022), artificial intelligence (Sperschneider 2020), and CRISPR genome editing (Wheatley and Yang 2021), which expedite the laborious and time-consuming process of identification of novel genes underlying disease resistance and development of disease resistant cultivars.

There is a growing interest within the scientific community in developing durable and sustainable disease management strategies for current and future climate change, as well as cultural practice scenarios centered on resistant cultivars. Understanding plant disease resistance mechanisms is critical for such a task. Significant progress has been made in understanding the gene-for-gene model, effector-triggered immunity, and pathogen-associated molecular pattern triggered immunity. At the same time, advances have been made in understanding cell death, production of reactive

oxygen species (ROS) and secondary metabolites, pathogenesis-related genes, systemic defense responses, and callose deposition in disease resistance. Furthermore, links have been established between plant–pathogen interactions and different modes of resistance and susceptibility. These breakthroughs continuously result in novel approaches to reshaping and engineering plant resistance.

Plant disease resistance research is entering a new era, facilitated by new technologies and multidisciplinary interactions that are transforming our understanding of host resistance for long-term sustainable disease management. Plant disease resistance is the topic of the May issue of *Phytopathology*. This Focus Issue solicited and encouraged submission of research and review articles on topics related to plant disease resistance described above.

A review article by Amas et al. (2023) summarizes key advances in the era of genomics-assisted disease resistance improvement of *Brassica* species. This review discusses how genomics has paved the way for understanding the complex genomes of *Brassica* spp., which has been critical in dissecting the genetic underpinnings of traits and for the development of superior cultivars. This review also discusses advances in the development of genomic resources for *Brassica* spp., such as long-read sequencing and pangenome assemblies, as well as developments in pathogen genomics, pathogenicity factor discovery, and disease resistance gene identification in the host. Finally, this review considers how combining these key advances with new breeding techniques and improved phenotyping through the use of advanced data analysis platforms will make disease resistance improvement in *Brassica* spp. more efficient and responsive to current and future demands.

The field of metabolomics has also seen much technical improvement. The review by Muñoz-Hoyos and Stam (2023) summarizes the progress on secondary metabolite research in plant defense against pathogens. Firstly, the authors described major historically described secondary metabolites and their role in immune responses during plant–pathogen interactions. The authors classified them into constitutive (phytoanticipins) and inducible (phytoalexins) secondary metabolites and presented detailed introduction on saponins, glucosinolates, cyanogenic glycosides, benzoxazinone glycosides, benzoxazolinones, phenylalanine-derived phytoalexins, terpenes, terpenoids, indole phytoalexins, and oxylipins. Furthermore, the authors reviewed how targeted and untargeted metabolomics have been used in investigating secondary metabolites in plant–microbe interactions and how advances in these technologies can speed up

† Corresponding authors: A. Khan; awais.khan@cornell.edu, R. Ben-David; roib@volcani.agri.gov.il, J. Richards; JRichards@agcenter.lsu.edu, U. Bansal; urmil.bansal@sydney.edu.au, C. Wang; wangcongli@iga.ac.cn, C. McCartney; Curt.McCartney@umanitoba.ca, R. Stam; remco.stam@phytomed.uni-kiel.de, and N. Wang; nianwang@ufl.edu

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the discovery of new compounds. They also pointed out the promise of integrating multi-omics approaches with new advances in omics technologies to help identify functional compounds and the associated genes, to allow using these findings in future resistance breeding efforts.

This special issue includes four papers on identification of resistance genes or loci. Verma et al. (2023) reported the discovery of a major-effect quantitative trait loci (QTL) linked to begomovirus resistance in pumpkin (*Cucurbita moschata* Duchesne), which is a major cause of quantitative and qualitative losses. To identify the genomic region linked to begomovirus resistance, they phenotyped 229 F_{2:3} progeny plants derived from a resistant source and performed whole-genome resequencing of resistant and susceptible bulks together with their parents. Two genomic regions with significant associations to begomovirus resistance were identified: one was a 1.52 Mb region on chromosome 7 and the other a 0.87 Mb region on chromosome 17. The highly significant QTL on chromosome 7 region had nine polymorphic single nucleotide polymorphisms (SNP) that were converted into KASP markers. The QTL interval on chromosome 7 was reduced by KASP genotyping of F₂ individuals. One of the KASP markers can accurately predict disease reaction in 91% of diverse *Cucurbita* genotypes. These results will contribute to the understanding of genetic mechanism of begomovirus resistance and marker-assisted breeding in *C. moschata*.

The research article by Essenberg et al. (2023) demonstrated that the bacterial blight resistance gene *B₅* confers strong and broad-spectrum resistance to bacterial blight and increases the production of sesquiterpenoid phytoalexins in cotton (*Gossypium hirsutum*). The authors generated a near-isogenic line (NIL), AcB₅, with *B₅* in a bacterial blight-susceptible genetic background. Under field conditions, test cross segregation patterns revealed that AcB₅ is likely homozygous for resistance at two loci with partial dominance gene action. Two of the four copies of *B₅* were required for effective resistance in a controlled environment. AcB₅ conferred high resistance to isogenic strains of *Xanthomonas citri* subsp. *malvacearum* carrying cloned five avirulence genes and weaker resistance to one strain carrying a different avirulence gene, contrary to gene-for-gene theory predictions. The authors also investigated the production of sesquiterpenoid phytoalexins in five NILs carrying different B genes by measuring cadalene and lacinilene phytoalexins during resistant responses.

Rasool et al. (2023) used a 50K Axiom SNP Array to dissect the genetic basis and identify marker-trait associations (MTAs) for Fusarium wilt, a devastating disease of chickpea (*Cicer arietinum* L.) caused by *Fusarium oxysporum* f. sp. *ciceri*. In this study, 179 chickpea genotypes were tested for Fusarium wilt response under field and controlled conditions at seedling and reproductive stages. Genotyping with the Axiom® *Cicer*SNP Array and trait data identified 26 significant MTAs for Fusarium wilt resistance, with these MTAs explaining 11.75 to 15.86% of phenotypic variation. Five MTAs were designated as major, explaining more than 15% of the phenotypic variation in Fusarium wilt, and two MTAs were designated as stable. The study suggests that these stable and major MTAs can be used in chickpea breeding programs.

Newman et al. (2023) utilized association mapping and genome sequencing data to characterize the genetic basis of Sclerotinia stem rot, caused by the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*, which can significantly reduce the yield of canola (*Brassica napus*). They identified regions in the *B. napus* genome significantly associated with resistance to Sclerotinia stem rot and validated the contribution of these genomic regions to resistance through additional disease screening experiments. These screening experiments also confirmed the high levels of Sclerotinia stem rot resistance in previously studied genotypes. Subsequently, they used publicly available whole-genome sequencing data from a panel of 83 *B. napus* genotypes and identified non-synonymous polymorphisms linked to the Sclerotinia stem rot resistance loci. Additionally, they utilized qPCR to demonstrate that two of the genes con-

taining these polymorphisms were transcriptionally responsive to *S. sclerotiorum* infection. These candidate genomic loci associated with resistance will be useful to breed canola varieties with improved genetic resistance to Sclerotinia stem rot.

Screening of disease resistant resources is critical for resistance breeding and identification of resistance genes. Hamershlak et al. (2023) evaluated the resistance of 97 carrot accessions to the phloem-colonizing bacterial pathogen '*Candidatus Liberibacter solanacearum*' in both field trials and controlled conditions. '*Ca. L. solanacearum*', like many other '*Ca. Liberibacter*' species, such as '*Ca. L. asiaticus*' has been expanding to new locations worldwide (Wang et al. 2017). The authors identified five accessions showing significantly lower disease incidence compared with the reference cultivar, which provide a useful resource for resistance breeding programs and for better understanding of the resistance mechanisms to '*Ca. L. solanacearum*'.

Ma et al. (2023) made use of a broad set of available genetic resources to screen for new resistances. They screened 260 melon accessions for their resistance against Gummy stem blight (GSB) caused by *Didymella bryoniae*, a devastating ascomycete fungus disease. In their study, they identified a tentative major resistance gene that they dubbed *Gsb-7(t)*. Using an F₂ mapping population of resistance and susceptible bulk, they narrowed down the resistance locus to a 140-kb region that contains 10 candidate genes. Using transcription analyses, they show that the T2 splice variant of one of the genes, a putative wall-associated receptor kinase (WAK) gene *MELO3C010403*, is highly upregulated after infection in resistant, but not in susceptible accessions, making it the most likely R-gene candidate.

Lathyrus sativus (grass pea) is an important legume crop as food, fodder, and forage. Martins and colleagues investigated the resistance of 189 grass pea accessions against powdery mildew pathogens *Erysiphe pisi* and *E. trifolii* (Martins et al. 2023). The authors combined grass pea disease severity with SNP identified previously via the genotype-by-sequencing approach. This study revealed putative common and unique genetic components responsible for the partial resistance against *E. pisi* and *E. trifolii* and provided a useful resource for breeding disease resistant grass pea.

In addition to resistance genes, susceptibility genes also play critical roles in determining the outcome of plant–pathogen interactions and knocking out of susceptibility genes has shown promises in generating disease resistant plants. By using several molecular biology techniques, R. Li et al. (2023) characterized the function of a newly identified susceptibility factor of wheat, which makes it susceptible against the mildew fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*). They cloned the so-called *TaPOD70* gene, which codes for a class III peroxidase. Using transient assays in *Nicotiana benthamiana*, they show that the protein is translocated to the cell membrane and able to inhibit BAX-induced cell death. *TaPOD70* is strongly transcriptionally upregulated in a susceptible wheat cultivar, whereas virus induced gene silencing increased the resistance against *Bgt* and was coupled to increased H₂O₂ levels in the cell. Combined, this shows that *TaPOD70* is a genuine susceptibility factor and thus important to account for in *Bgt* resistance breeding programs.

How advances in molecular biology can help improve understanding for resistance breeding is further illustrated by Y. Li et al. (2023). They conducted a quantitative analysis of ROS accumulation in the apoplast (apoROS) and in the cytosol (intraROS) during the *Bgt* life cycles across a panel of wheat accessions that carry different powdery mildew (*Pm*) resistance genes. The authors studied ROS accumulation and cell death processes in response to infection with virulent and avirulent *Bgt* isolates on wheat carrying major R gene compared with their susceptible counterparts. This research is basically derived from microscopic observations of *Bgt* infections in wheat leaf tissues and stained for ROS accumulation and localized cell death. Authors found that compared with apoROS, intraROS activation significantly triggers hypersensitive response (HR)-mediated cell death of the infected cells and upregulated

pathogenesis-related (PR) gene expression in resistant wheat lines. Y. Li et al. (2023) concluded that HR response followed by intraROS accumulation is part of the resistance mechanism activated by NLR genes with exception of some unconventional *Pm* genes generating different immune responses. This paper sheds new light on the contribution of cytosolic intraROS-mediated localized cell death due to the activation of *R* gene(s) and the resultant wheat local resistance immune signal responses against *Bgt*.

Figueiredo et al. (2023) used an innovative approach to characterize the proteomes of grapevine and *Plasmopara viticola* in the apoplast during early infection. A downy mildew tolerant grapevine cultivar was inoculated with *P. viticola* and apoplastic fluid was extracted at 6 h postinoculation. Comparison of the apoplastic proteomes of inoculated and mock-inoculated grapevine revealed a total of 264 differentially accumulated proteins. A suite of host proteins involved in signaling, oomycete recognition, stress responses, transport, and ROS production were found. Additionally, 60 proteins belonging to *P. viticola* were identified, including 32 predicted effector proteins. These results reveal early events occurring during grapevine–*P. viticola* interactions in the extracellular space and may provide novel targets for breeding programs or disease management tactics.

CRISPR/Cas9-mediated gene editing has the potential to transform crop productivity and resistance. Shnaider et al. (2023) used CRISPR/Cas9 to disrupt the *CsaMLO8* powdery mildew susceptibility gene in cucumber. The authors designed three sgRNA sequences to target different *CsaMLO8* exons and incorporated them into a single construct. A susceptible cucumber cultivar was transformed and one transgenic T0 line was identified and backcrossed to the susceptible cultivar. Two mutants were selected, one harboring a 5-bp deletion in exon 1 which results in a premature stop codon and another mutant that had a 10 bp insertion and a 1,280 bp between exons 1 and 5. Following removal of the transgene via backcrossing, the mutant plants were phenotyped and found to be resistant to powdery mildew. This research illustrates the utility of CRISPR/Cas9 for susceptibility gene disruption and provides a promising approach for resistant cultivar development.

Pyramiding host resistance genes has been deemed as a durable and effective strategy to manage crop diseases. Zhang et al. (2023) examined the CIMMYT wheat line Parula, which is resistant to multiple rust diseases, using genetic approaches to characterize the deployed resistance gene pyramid. The authors developed a double haploid population derived from a cross of Parula × Thatcher and genotyped the population with the Infinium iSelect 90K wheat chip. Construction of a high-density genetic map and subsequent QTL mapping identified rust resistance gene loci *Lr34*, *Lr46*, *Sr2*, and *Lr68* derived from Parula. Additionally, two novel rust resistance QTL were identified from Parula. Grouping individuals based on the presence or absence of specific QTL identified specific combinations of resistance loci that provided stable resistance against all three rust diseases.

Zhu et al. (2023) developed an efficient method that will allow cotton breeders to assess resistance to *F. oxysporum* f. sp. *vasinfectum* race 4 at the seed germination stage. Fusarium wilt is an important vascular disease that limits cotton production around the world. Four inoculation methods were compared based on a disease severity rating of the taproot. Taproot dipping after seed germination accurately differentiated resistant and susceptible cotton genotypes. The assay takes 2 weeks to complete and is scalable for testing large numbers of lines in a breeding program.

Overall, this Focus Issue includes 13 research and 2 review articles covering various aspects of 15 diseases that cause significant economic losses to crops. These articles will be of great interest to the plant pathology, genetics, and breeding community in the development of sustainable disease management strategies based on resistant cultivars. The issue features articles ranging from the characterization of resistance mechanisms, identification of the resistance gene loci, to the deployment of host disease resistance

in plant breeding programs. To identify and utilize disease resistance mechanisms and develop resistant cultivars, these articles describe cutting-edge technologies such as genome sequencing, high-throughput genotyping platforms, comparative genomics, transcriptomics, marker assisted selection, and CRISPR/Cas9-based genome editing. This Focus Issue also highlights significant progress in understanding plant disease resistance mechanisms, including the gene-for-gene model, quantitative resistance, and susceptibility genes, and encourages multidisciplinary research to characterize and utilize host genetics for developing disease-resistant cultivars.

Literature Cited

- Amas, J. C., Thomas, W. J. W., Zhang, Y., Edwards, D., and Batley, J. 2023. Key advances in the new era of genomics-assisted disease resistance improvement of *Brassica* species. *Phytopathology* 113:771-785.
- Essenberg, M., McNally, K. L., Bayles, M. B., Pierce, M. L., Hall, J. A., Kuss, C. R., Shevell, J. L., and Verhalen, L. M. 2023. Gene *B5* in cotton confers high and broad resistance to bacterial blight and conditions high amounts of sesquiterpenoid phytoalexins. *Phytopathology* 113:812-823.
- Figueiredo, J., Santos, R. B., Guerra-Guimarães, L., Leclercq, C. C., Renaut, J., Sousa, L., and Figueiredo, A. 2023. Deep into the apoplast: Grapevine and *Plasmopara viticola* proteomes reveal the secret beneath host and pathogen communication at 6 h after contact. *Phytopathology* 113:893-903.
- Hamershlak, D., Assoline, N., Dror, O., and Bahar, O. 2023. Assessing carrot accessions susceptibility to the bacterial pathogen '*Candidatus Liberibacter solanacearum*' and its associated symptoms. *Phytopathology* 113:791-799.
- Hamim, I., Sekine, K. T., and Komatsu, K. 2022. How do emerging long-read sequencing technologies function in transforming the plant pathology research landscape? *Plant Mol. Biol.* 110:469-484.
- Khan, A., and Korban, S. S. 2022. Breeding and genetics of disease resistance in temperate fruit trees: Challenges and new opportunities. *Theor. Appl. Genet.* 135:3961-3985.
- Li, R., Zhang, X., Zhao, B., Song, P., Zhang, X., Wang, B., and Li, Q. 2023. Wheat class III peroxidase TaPOD70 is a potential susceptibility factor negatively regulating wheat resistance to *Blumeria graminis* f. sp. *tritici*. *Phytopathology* 113:873-883.
- Li, Y., Roychowdhury, R., Govta, L., Jaiwar, S., Wei, Z.-Z., Shams, I., and Fahima, T. 2023. Intracellular reactive oxygen species-aided localized cell death contributing to immune responses against wheat powdery mildew pathogen. *Phytopathology* 113:884-892.
- Ma, J., Li, C., Tian, J., Qiu, Y., Geng, L., and Wang, J. 2023. Identification and fine mapping of gummy stem blight resistance gene *Gsb-7(t)* in melon. *Phytopathology* 113:858-865.
- Martins, D., Santos, C., Sampaio, A. M., Rubiales, D., and Vaz Pato, M. C. 2023. *Lathyrus sativus* resistance against the existing and emerging pathogens *Erysiphe pisi* and *E. trifolii*: A case of commonalities or total discrepancy? *Phytopathology* 113:866-872.
- Muñoz-Hoyos, L., and Stam, R. 2023. Metabolomics in plant pathogen defense: From single molecules to large-scale analysis. *Phytopathology* 113:760-770.
- Newman, T. E., Khentry, Y., Leo, A., Lindbeck, K., Kamphuis, L. G., and Derbyshire, M. C. 2023. Association mapping combined with whole genome sequencing data reveals candidate causal variants for Sclerotinia stem rot resistance in *Brassica napus*. *Phytopathology* 113:800-811.
- Oerke, E. C., Steiner, U., Dehne, H. W., and Lindenthal, M. 2006. Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions. *J. Exp. Bot.* 57:2121-2132.
- Rasool, B., Summuna, B., Djalovic, I., Shah, T. A., Sheikh, P. A., Gupta, S., Tyagi, S., Bilal, S., Varshney, R. K., Abidi, I., Kumar, J., Penmetsa, R. V., Khandey, I., Kumar, U., Sofi, P. A., Khan, M. A., Bhat, M. A., Wani, F. J., Thudi, M., and Mir, R. R. 2023. Delineating marker-trait associations for Fusarium wilt in chickpea using Axiom® *Cicer*SNP Array. *Phytopathology* 113:836-846.
- Shnaider, Y., Elad, Y., Rav-David, D., Pashkovsky, E., Leibman, D., Kravchik, M., Shtarkman-Cohen, M., Gal-On, A., and Spiegelman, Z. 2023. Development of powdery mildew resistance in cucumber using CRISPR/Cas9-mediated mutagenesis of *CsaMLO8*. *Phytopathology* 113:786-790.
- Sperschneider, J. 2020. Machine learning in plant–pathogen interactions: Empowering biological predictions from field scale to genome scale. *New Phytol.* 228:35-41.
- Verma, N., Garcha, K. S., Sharma, A., Sharma, M., Bhatia, D., Khosa, J. S., Kaur, B., Chuuneja, P., and Dhatt, A. S. 2023. Identification of a major-effect quantitative trait loci associated with begomoviruses resistance in *Cucurbita moschata*. *Phytopathology* 113:824-835.
- Wang, N., Pierson, E. A., Setubal, J. C., Xu, J., Levy, J. G., Zhang, Y., Li, J., Rangel, L. T., and Martins, J., Jr. 2017. The *Candidatus Liberibacter*-host

- interface: Insights into pathogenesis mechanisms and disease control. *Annu. Rev. Phytopathol.* 55:451-482.
- Wheatley, M. S., and Yang, Y. 2021. Versatile applications of the CRISPR/Cas toolkit in plant pathology and disease management. *Phytopathology* 111:1080-1090.
- Zhang, W., Boyle, K., Gao, P., Polley, B., Brost, J. M., Francis, T., Sidebottom, C., McCallum, B. D., Kutcher, H. R., Randhawa, H., Fetch, T. G., Ferrie, A. M. R., and Fobert, P. R. 2023. Systematic characterization of multi-rust resistance genes from a 'Parula × Thatcher' population with high-density genetic map. *Phytopathology* 113:847-857.
- Zhu, Y., Willey, K., Wheeler, T., Dever, J. K., Whitelock, D., Wedegaertner, T., Hake, K., Bissonnette, K., and Zhang, J. 2023. A rapid and reliable method for evaluating cotton resistance to Fusarium wilt race 4 based on taproot rot at the seed germination stage. *Phytopathology* 113:904-916.