Omics to Study Fungal Plant Pathogens

Subjects: Microbiology

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In plant pathology, multi-omics (genomics, transcriptomics, proteomics, and metabolomics) can help mainly in the prevention and management of diseases. The omics have been applied to elucidate the function of genes and the structure of the genome to provide insights into gene and protein expression and to understand the metabolic profiling of both the host and the pathogen during an infection process. The application of omics in the genus *Diaporthe* is still poorly explored, although metabolomics has been widely applied to explore endophytic *Diaporthe* natural products for their potential applications in pharmacology. Although the genus *Diaporthe* comprises important plant pathogens and endophytes, these species also have the ability to switch lifestyles.

Keywords: fungal-plant interactions ; genomics ; metabolomics ; pathogenicity ; proteomics

1. Genomics

Since the sequencing of the first fungal genome, the yeast *Saccharomyces cerevisiae* in 1996 [1], advances in next-generation sequencing (NGS) technology have led to an increase in genomes [2], specifically from fungal pathogens that affect agriculture and forestry [3]. NGS is a rapid and high-throughput approach, and it is represented by different sequencing platforms such as AB SOLiD, Illumina HiSeq System, PacBio RS, and Oxford Nanopore Technology PromethION [4][5]. In 2011, the 1000 Fungal Genomes Project started with plans to sequence at least two reference genomes from each fungal family (http://1000.fungalgenomes.org; acceded on 5 February 2023). A search at the NCBI database (https://www.ncbi.nlm.nih.gov/; accessed on 10 February 2023) and the Genome Portal (https://genome.jgi.doe.gov/portal/; accessed on 10 February 2023) retrieved more than 12,200 and 2600 fungal genomes, respectively. Among these, over 11,550 genomes belong to the phylum *Ascomycota* that comprises the highest number of sequenced fungal genomes [6]. Despite the increasing number of fungal genomes over the last years, there are only a few genomes available in the genus *Diaporthe*. **Table 1** sums up all species of *Diaporthe* with sequenced genomes deposited in NCBI and JGI databases.

Table 1. Synopsis of all *Diaporthe* strains with genomes sequenced. (Note: NA stands for 'not applicable' meaning that the genome is available at the JGI Portal but has no Project ID).

Species	Strain	Host	JGI Project	GenBank Accession Number	Sequencing Platform	References
Diaporthe ampelina	DA912	*Vitis vinifera*	NA	LCUC00000000	Illumina HiSeq	[7]
	S3MP	*Commiphora wightii*	-	LWAD00000000	Illumina HiSeq	[8]
Diaporthe amygdali	CAA958	*Vaccinium corymbosum*	-	JAJATV000000000	Illumina HiSeq	[9]
	DUCC20226	*Malus* sp.	-	JAJJOG000000000	PacBio Sequel and Illumina	-
Diaporthe aspalathi	MS-SSC91	*Glycine max*	-	LJJS00000000	Illumina HiSeq	[10]
Diaporthe batatas	CRI 302-4	*Ipomoea batatas*	-	JAHWGW000000000	Oxford Nanopore and PromethION	[5]
Diaporthe capsici	GY-Z16	*Juglans regia*	-	WNXA00000000	PacBio RSII	[11]
Diaporthe caulivora	D57	*G. max*	-	JAMPTR000000000	PacBio Sequel	[12]

Species	Strain	Host	JGI Project	GenBank Accession Number	Sequencing Platform	References
Diaporthe cf. *heveae*	LGMF1633	-	1251927	-	-	-
Diaporthe destruens	CRI305-2	*Ipomoea batatas*	-	JACAAM010000000	Oxford Nanopore and PromethION	[13]
Diaporthe citrisiana	ZJUD30	*Citrus unshiu*	-	JADAZS000000000	Illumina HiSeq	[14]
			-	JADWDH000000000		
Diaporthe citrichinensis	ZJUD34	*C. unshiu*	-	JADAZR000000000	Illumina HiSeq	[14]
Diaporthe citri	NFHF-8-4	*Citrus* sp.	-	JACTAD000000000	PacBio Sequel	[15]
	ZJUD2	*C. reticulata*	-	JADAZQ000000000	Illumina HiSeq	[14]
	ZJUD14	*C. reticulata*	-	JADAZP000000000	Illumina HiSeq	
	Q7	*C. reticulata*	-	JADAZO000000000	Illumina HiSeq	
Diaporthe eres (syn. *D. phragmitis*)	NJD1	*Actinidia deliciosa*	-	JACDXY000000000	PacBio RS	[16]
Diaporthe eres (syn. *D. vaccinii*)	CBS 160.32	*V. corymbosum*	-	JAJATR000000000	Illumina HiSeq	[9]
Diaporthe eres	Phoaprs 18-02	*Malus* sp.	-	JAKJXL000000000	Illumina NovaSeq	[17]
	Phoaprs 18-03	*Malus* sp.	-	JAKJXM000000000	PacBio Sequel	
Diaporthe helianthi	7/96	*Helianthus annuus*	NA	MAVT02000001	Illumina MiSeq	[18]
Diaporthe ilicicola	FPH2015-502	*Ilex verticillata*	-	JALPVH000000000	Illumina and Oxford Nanopore	[19]
Diaporthe inconspicua	LGMF1612	-	1251935	-	-	-
Diaporthe longicolla	MSPL 10–6	*G. max*	-	AYRD00000000	Illumina HiSeq	[20]
	TWH P74		-	JUJX00000000	Illumina HiSeq	[21]
Diaporthe vexans	PV 4	*Solanum melongena*		JAJLLZ000000000	Oxford Nanopore	[22]
Diaporthe vochysiae	LGMF1583	*Vochysia divergens*	1251933	-	Pacbio	-
Diaporthe sp.	DP-2020a	*Sequoia sempervirens*	-	JACVEP000000000	Illumina HiSeq	-
Diaporthe sp.	HANT25	*Hydnocarpus anthelminthicus*	-	JACBFG000000000	Illumina HiSeq	[23]

The low number of genomes and annotations available impairs researchers to unveil key genes involved in the infection process of *Diaporthe*, as well as mechanisms involved in the dual lifestyle (pathogen-endophyte). To bridge this, studies on the genome sequencing of *Diaporthe* species have focused on genomic signatures that allow them to successfully invade and colonize the host plant through the presence of:

(1)Hydrolytic enzymes to degrade plant cell wall polysaccharides (e.g., pectins, celluloses, and lignins) to ensure a successful entry into the host [9][12][14][20].

(2)Biosynthetic gene clusters encoding for toxic metabolites that injure plant cells and enhance disease progression. (e.g., fusicoccin A, fusarin, and ACT-toxin II) [9][20].

(3)

Cellular transporters of ions (e.g., zinc, sulfur, copper), molecules that enhance pathogenicity (e.g., peroxiredoxin, tetraspanin), and sugars from plant polysaccharides degradation (e.g., xylose, inositol, and glycerol) [9].

(4)Pathogenicity-related genes (e.g., acid aspartate and aminopeptidase) and candidate effectors (e.g., carboxylesterases, CFEM-domain, and laccases) that facilitate the host to be infected and manipulate the host immune defense [9][12].

Moreover, genome-wide association studies could be implemented to identify the genomic regions potentially associated with aggressiveness, through the analysis of single nucleotide polymorphism (SNP) data [24]. The analysis of SNPs between pathogenic and non-pathogenic fungi/endophytes is also a promising tool for the identification of candidate effectors underlying the pathogenicity of species of *Diaporthe*, as well as to understand the ecological and evolutionary dynamics of plant pathogens [25].

The integration of omics approaches can also speed up the identification of putative effectors in the genus *Diaporthe* and the characterization of their virulence functions in their host plants. Effectors are secreted proteins by fungal pathogens that modulate and interfere with plant defense responses [26]. Recently, Mena et al. [12] defined a set of proteins considered within the secretome of six *Diaporthe* species through a comparative analysis of available genomes. Moreover, Hilário et al. [9] have also identified candidate effectors from two *Diaporthe* species, through sequencing and analysis of their genomes (**Table 2**). This suggests that the genomes of species of *Diaporthe* have a large array of candidate effectors involved in pathogenicity, and some of them are common to other *Diaporthe* pathogens while others are *Diaporthe*-specific [12]. Nevertheless, future studies should be undertaken aiming to reveal effector functions during the infection process and to understand how effectors alter plant physiology, thus underpinning *Diaporthe* lifestyles [27]. Overall, genomic studies on *Diaporthe* intend to deepen the knowledge on:

(1)Ecological selection and adaptation of species of *Diaporthe* to degrade the available biomass as carbon source [9][12][20].

(2)Gene functions related to pathogenicity [9][12].

(3)Phylogenomic studies to offer insights into phylogenetic inference of *Diaporthe* [2][14].

(4)Genetic basis for multi-omics analyses to provide a thorough overview on plant-pathogen interactions [12][28][29][30][31].

Table 2. Overview of some effector proteins identified in the genomes of species of *Diaporthe*.

Species	Effector Candidate	Effector Location	References
D. amygdali	**glycosyl hydrolase family 61**	**Apoplastic**	**[9]**
	aldehyde reductase 1	**Apoplastic**	
	putative cfem domain-containing protein	**Cytoplasmic**	
	putative metalloprotease	**Apoplastic**	
	murein transglycosylase	**Apoplastic**	
	acetyl xylan esterase	**Apoplastic**	
	putative cerato-ulmin	**Apoplastic**	
	putative gas1-like protein	**Apoplastic**	
	putative secreted aspartic proteinase precursor	**Apoplastic**	
	Pectate lyase H	**Apoplastic**	
	glycosyl hydrolase family 61	**Apoplastic**	

Species	Effector Candidate	Effector Location	References
D. capsici	sterigmatocystin biosynthesis peroxidase stcC	Apoplastic	[12]
	pectate lyase F	Apoplastic	
	putative 1,4-beta-D-glucan cellobiohydrolase A	Apoplastic	
	putative proline-rich antigen	Apoplastic	
	chitin deacetylase	Apoplastic	
	xylanase G1	Apoplastic	
	putative chitin binding protein	Apoplastic	
	putative mannose binding	Apoplastic	
	putative gas1-like protein	Apoplastic	
	glycoside hydrolase family 11 protein	Apoplastic	
	Cell wall glyco protein	Cytoplasmic	
	Poly(rC)-binding protein 4	Cytoplasmic	
D. caulivora	putative sterigmatocystin biosynthesis peroxidase stcC	Apoplastic	[12]
	putative proline-rich antigen	Apoplastic	
	putative cytochrome p450	Apoplastic	
	xylanase G1	Apoplastic	
	glycoside hydrolase	Apoplastic	
	pectate lyase	Apoplastic	
	peptidase S41 family protein	Apoplastic	
	chitin deacetylase	Apoplastic	
	putative aldehyde dehydrogenase	Apoplastic	
	pectate lyase F	Apoplastic	
	putative 1,4-beta-D-glucan cellobiohydrolase A	Apoplastic	
	chitin deacetylase	Apoplastic	
	Glucan endo-1,3-beta-glucosidase	Apoplastic	
	putative 1,4-beta-D-glucan cellobiohydrolase A	Apoplastic	
	putative sterigmatocystin biosynthesis peroxidase stcC	Apoplastic	
D. citri	cholera enterotoxin subunit A2	Apoplastic	[12]
	pectate lyase	Apoplastic	
	polysaccharide lyase family 3 protein	Apoplastic	
	Chitin binding protein	Apoplastic	
	Acetylxylan esterase-like protein	Apoplastic	
	pectate lyase F	Apoplastic	
	xylanase G1	Apoplastic	
	putative riboflavin-aldehyde forming enzyme protein	Apoplastic	

Species	Effector Candidate	Effector Location	References
D. destruens	pectate lyase	Apoplastic	[12]
	NPP1 domain-containing protein	Apoplastic	
	xylanase G1	Apoplastic	
	cellulose binding CEL1	Apoplastic	
	putative pectate lyase F	Apoplastic	
	Poly(rC)-binding protein 4	Apoplastic	
	chitin deacetylase	Apoplastic	
	ribosomal protein s17	Cytoplasmic	
	Protein CAP22	Apoplastic	
	fungal cellulose binding domain-containing protein	Apoplastic	
D. eres (syn. *D. phragmitis*)	pectate lyase	Apoplastic	[12]
	Acetylxylan esterase 2	Apoplastic	
	putative glutamine-serine-proline rich	Apoplastic	
	putative rhamnogalacturonan acetylesterase	Apoplastic	
	xylanase G1	Apoplastic	
	Protein CAP22	Apoplastic	
	lytic polysaccharide monooxygenase	Apoplastic	
	pectate lyase F	Apoplastic	
	putative proline-rich antigen	Apoplastic	
	chitin deacetylase	Apoplastic	
	putative metalloprotease	Apoplastic	
	carbohydrate-binding module family 50 protein	Apoplastic	
	putative glycoside hydrolase family 61 protein	Apoplastic	
D. eres (syn. *D. vaccinii*)	acetylxylan esterase	Apoplastic	[9]
	putative ricin b lectin	Apoplastic	
	putative pectate lyase b	Apoplastic	
	aldehyde reductase 1	Apoplastic	
	putative npp1 domain	Cytoplasmic	
	putative pectinesterase	Cytoplasmic	
	putative pectate lyase	Apoplastic	
	disulfide-isomerase erp38	Cytoplasmic	

Species	Effector Candidate	Effector Location	References
D. longicolla	polysaccharide lyase family 3 protein	Apoplastic	[12]
	putative carbohydrate-binding module family 1 protein	Apoplastic	
	carbohydrate esterase family 5 protein	Apoplastic	
	starch binding domain-containing protein	Apoplastic	
	putative pectate lyase F	Apoplastic	
	Acetylxylan esterase 2	Apoplastic	
	pectate lyase	Apoplastic	
	cell wall protein PhiA	Apoplastic	
	xylanase G1	Apoplastic	
	cellulose binding CEL1	Apoplastic	
	fungal cellulose binding domain-containing protein	Apoplastic	
	Protein CAP22	Apoplastic	

2. Transcriptomics

The RNA-Seq technique has revolutionized the way in which transcriptomes are analyzed [32]. It promotes the understanding of gene expression under different conditions and allows for the discovery of new genes and transcription patterns, which helps to understand cell function and metabolic mechanisms [33]. As a result, it has been considered one of the most important applications of NGS technology, and one of the most important tools in plant pathology [32] since it allows to investigate the transcriptomic profiles of plant pathogens during infection [34][35]. As the interaction between plants and their pathogens is a dynamic process, these interactions should be analyzed as a dual process [34]. Hence, dual RNA sequencing allows to study host and pathogen transcriptomes simultaneously, detecting pathogen-specific transcripts as well as provides a more complete insight into the host defense mechanisms [36]. This approach has already been applied in studies of plant-pathogen interactions in crops such as grapevines [31][35]; peach [37] and potato [38]; medicinal plants [39]; and forest trees such as *Eucalyptus* sp. [40] and *Pinus* sp. [41].

However, the utilization of transcriptomics data is often hampered by the lack of annotations and genomes available, which is reflected in the scarce transcriptome studies, for example, in the genus *Diaporthe*. The few studies regarding the transcripts characterization in this genus are mainly based on quantitative PCR (qPCR). For example, Książkiewicz et al. [42] have used this technique to target genes on *Lupinus angustifolius* that confer resistance to *D. toxica*, the causal agent of lupinosis. Moreover, Elverson et al. [43] developed two qPCR assays to detect and quantify *D. helianthi* and *D. gulyae* on sunflower, the causing agents of *Phomopsis* stem canker. Hosseini et al. [44] have also established a multiplex qPCR to distinguish *D. longicolla*, *D. caulivora*, *D. eres*, and *D. novem* on soybean, which are responsible for seed decay, pod, and stem canker on this host. In another study, Fujiwara et al. [45] demonstrated that the qPCR assay they developed is useful to diagnose and quantify *D. batatas* and *D. destruens* in sweet potato, as they are the main causal agents of foot rot disease.

To the knowledge, Mena et al. [12] applied for the first time the dual RNA-Seq approach to the genus *Diaporthe* to evaluate how *D. caulivora* may affect soybean plants. The authors stated that the infected soybean with *D. caulivora* induces the reinforcement of cell walls, evidenced by the incorporation of phenolic compounds. Moreover, several defense genes were also upregulated, including those encoding a pathogenesis-related (PR) protein-1 (*PR-1*), a *PR-10*, a β-1,3-glucanase, two chitinases, two lipoxygenases, a phenylalanine-ammonia lyase, and a chalcone synthase [12][46]. Given the cosmopolitan behavior of species of *Diaporthe*, their ability to infect a wide range of hosts and their different lifestyles (e.g., endophytes and pathogens), transcriptome analyses of both the host and the pathogen, and the validation of the differentially expressed genes (DEGs) should be considered to understand the regulatory networks and mechanisms involved in infection processes. Such an approach would thus contribute to unravelling host-pathogen interactions to provide helpful information for the development of disease control strategies [39][40][41][47].

3. Proteomics

Profiling the protein expression can unravel the functions of different proteins by assessing the plant responses to environmental stresses, such as pathogen attack [48]. After the plant is stimulated by external stresses, their defensive response is rapidly generated, followed by changes in some physiological and biochemical characters (e.g., decrease in chlorophyl A and photosynthesis) [49]. For example, studies have demonstrated that *Arabidopsis* infected by *Fusarium* [50], rice infected by *Magnaporthe oryzae* [51], and strawberry leaves inoculated with *Colletotrichum* [52] showed an overexpression of peroxidase levels after pathogens infection, to scavenged reactive oxygen species (ROS). Additionally, PR proteins such as chitinases and plant β-1,3-glucanases are considered important components of plant defense mechanisms under a pathogen attack [53]. For instance, the above-mentioned proteins were upregulated in *Triticum aestivum* inoculated with *F. equiseti* [54] and in *Populus trichocarpa* after infection with *Botryosphaeria dothidea* [55].

When the fungus infects host plants, a series of effector proteins (e.g., cell wall degrading enzymes) are secreted into the host tissue to destroy intracellular components, interfering with their defense response [50][53]. The analysis of the proteome has been successfully made for some fungal plant pathogens. For instance, some studies have shown that cell wall degrading enzymes such as pectin, esterases, xylanases, pectate lyases, or galacturonases are upregulated in *L. theobromae* [52], *M. oryzae* [51], and *F. graminearum* [56], suggesting their pathogenicity on grapevines, rice, and barley, respectively. Moreover, the hydrolase glucan-β-glucosidase was found to be involved in the virulence of *C. higginsianum* [57] and *Alternaria alternata* [58]. Some effector proteins secreted by fungal pathogens, such as avirulence proteins (Avr), are delivered into the host plant, which have the potential to suppress pathogen-associated molecular patterns (PAMPs)-triggered immunity [59]. However, these pathogen-derived avirulence proteins are recognized by plant receptor proteins encoded by R genes, resulting in effector-triggered immunity that leads to fast responses [60].

As mentioned above, proteomics has been applied to unveil key proteins of several plant pathogens as well as those involved in plant defense under a pathogen attack. Nevertheless, no proteomic studies have been performed with members of the genus *Diaporthe* nor for their interaction with plants. As the proteome profiling during infection can identify specific proteins involved in plant disease resistance and pathogenicity processes [61], in-depth studies and comparative proteomics should be undertaken to reveal molecular mechanisms of *Diaporthe*—plant interactions as well as the susceptibility or resistance in plants. These studies will assist in the discovery of novel proteins that might be potential candidates for the enhancement of tolerance to fungal diseases.

4. Metabolomics

Currently, mass spectrometry (MS) has become a highly sensitive tool used for the identification and quantification of metabolites. Nuclear magnetic resonance (NMR) and types of mass analyzers are commonly used for metabolomic studies, such as capillary electrophoresis mass spectrometry (CE-MS), gas chromatography mass spectrometry (GC–MS), liquid chromatography mass spectrometry (LC–MS), and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [62].

The increasing number of sequenced genomes and the scalable metabolomics approaches have largely expanded the access to the metabolite repertoire of fungi [63]. The awareness that fungi are a source of beneficial compounds came up after Sir Alexander Fleming in 1928 discovered penicillin [64]. This first broad-spectrum antibiotic was produced by the fungus *Penicillium notatum* (syn. *P. rubens*) and was considered as the ‘wonder drug’ of World War II [65]. After this event, the study of microorganisms as sources for antibiotics gave rise to the golden era for the discovery of natural products from fungi [66]. Species of the genus *Diaporthe*, for instance, are well known as producers of several compounds (e.g., polyketides, indoles, and terpenes) with potential applications in pharmacology and biomedicine [67].

Besides that, metabolomics research of plant pathogenic fungi has gained attention, since it allows the identification of metabolites, their functions, and metabolic pathways involved in pathogenicity [68][69][70]. Moreover, metabolomics profiling of host plants has also been performed to elucidate plant responses to abiotic and biotic stresses and to evaluate plant adaptations to such conditions [71]. For instance, Dickinson et al. [72] applied the LC–MS method to investigate metabolite changes of *Medicago tranuclata* under drought stress and infection with *F. oxysporum*. The authors stated that under pathogen infection, an increase in flavonoids, sucrose relocation from leaves to roots, and a decrease in organic acids were observed. Also, Jones et al. [73] used a meta-analytical method based on GC–MS/MS, LC–MS/MS, and NMR to evaluate rice plants at different time points after infection by *M. grisea*. These authors proposed that the production of a large amount of alanine caused by fungi may lead to cell death and thereby promoting *M. grisea* infection, suggesting that metabolomics may help evaluating the overall effects of pathogen infection on plant hosts [74].

It has also been suggested that metabolomic profiling in fungal-plant interactions provide important information for the early diagnosis of several fungal plant pathogens [71][74][75]. Hu et al. [74] used the GC–MS method to analyze the metabolic profiling of strawberry infected with *Botrytis cinerea* and identified biomarkers in the early stage of disease development. Moreover, Zeiss et al. [75] analyzed the metabolic profiling of tomato plants infected with *Ralstonia solanacearum* using the LC–MS method and detected metabolites that may be used as biomarkers for an early infection diagnosis (e.g., phenylpropanoids, phenolic acids, and flavonoids). Additionally, plant metabolomics can also help identify and link genes associated with resistance to fungal pathogens. For instance, Kage et al. [76] have also reported an increase in the metabolite coumaroylagmatine in a tolerant wheat variety to *Fusarium* head blight. The analysis of these compounds and their metabolic pathways paved the way for the detection of a gene (agmatine coumaroyl transferase) that confers resistance against *F. graminearum* [76].

Several studies have been focused to identify a wide range of metabolites produced by species of *Diaporthe* with biotechnological applications [67]. Nevertheless, there is still a lack of metabolomic studies on the interaction between species of *Diaporthe* and their hosts. Therefore, metabolomic approaches should be performed in *Diaporthe*-infected plants to elucidate the metabolic pathways involved in pathogenicity, as well as secreted metabolites as potential biomarkers for early disease diagnosis [68]. Moreover, unveiling metabolic features responsible for plant survival under stress conditions (e.g., pathogen attack) could facilitate crop improvement for biotic-stress tolerance, through the application of unique metabolites in formulations [48][71].

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