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De la caractérisation fine du dialogue moléculaire entre le blé et *Fusarium graminearum* à l'identification des facteurs de sensibilité à la fusariose de l'épi

Florian Rocher

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Florian Rocher. De la caractérisation fine du dialogue moléculaire entre le blé et *Fusarium graminearum* à l'identification des facteurs de sensibilité à la fusariose de l'épi. *Biologie végétale*. Université Clermont Auvergne, 2022. Français. NNT : 2022UCFAC091 . tel-04215583

HAL Id: tel-04215583

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ECOLE DOCTORALE DES SCIENCES DE LA VIE, SANTE, AGRONOMIE, ENVIRONNEMENT

Thèse présentée à l'Université Clermont-Auvergne pour
l'obtention du grade de Docteur d'Université

Spécialité : *Biologie végétale*

Soutenue le 28 novembre 2022

Par **Florian ROCHER**

De la caractérisation fine du dialogue moléculaire entre le blé et *Fusarium graminearum* à l'identification des facteurs de sensibilité à la fusariose de l'épi

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UMR 1095 INRAE – UCA « Génétique, Diversité et Écophysiologie des Céréales »

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Résumé

Fusarium graminearum, l'agent principal de la fusariose de l'épi (FHB), est l'un des agents pathogènes les plus nuisibles des céréales, en particulier du blé. Avec des impacts directs sur le rendement, la qualité du grain et par l'accumulation de mycotoxines cancérigènes, la fusariose est considérée comme un facteur limitant majeur pour la production de blé en Europe, en Amérique du Nord et en Asie, entraînant d'importantes pertes économiques. Avec la hausse des températures associée à des augmentations occasionnelles de l'humidité de l'air dues au changement climatique, les épidémies de FHB devraient être plus fréquentes et intenses. Actuellement, les méthodes de lutte, basées sur l'utilisation combinée de cultivars résistants, de pratiques agronomiques adaptées et de traitements fongicides, sont inefficaces pour contrôler totalement les épidémies de FHB. Il est ainsi nécessaire de poursuivre les travaux de recherche pour développer de meilleures stratégies de gestion. La résistance du blé au FHB est un caractère strictement quantitatif qui implique de nombreux « Quantitative trait loci » (QTL) présentant des effets relativement faibles, en particulier lorsque les conditions environnementales sont favorables à l'agent pathogène, rendant nécessaire la recherche de sources alternatives de résistance. Au cours des vingt dernières années, les multiples preuves de l'existence des gènes des plantes requis pour l'infection par des agents pathogènes ont ouvert de nouvelles opportunités pour le contrôle de l'immunité des plantes. Ces gènes, les gènes de sensibilité, sont impliqués dans une large gamme de processus fondamentaux de la plante qui sont manipulés par les effecteurs sécrétés par l'agent pathogène. Au sein du pathosystème blé - *F. graminearum*, le rôle des gènes de sensibilité dans l'issue de l'interaction a déjà été démontré dans plusieurs études. Par conséquent, élucider les mécanismes de sensibilité du blé à *F. graminearum* représente une approche prometteuse pour améliorer la résistance au FHB. Dans ce but, un profilage, par RNA-seq, des gènes de *F. graminearum* codant les effecteurs putatifs et des gènes de réponse du blé au FHB a été réalisé au cours d'une cinétique d'infection et en tirant avantage de la variabilité génétique des deux espèces. Cette approche a mis en évidence un programme d'infection complexe et très conservé, basé sur un core-effectome de 357 gènes exprimés de manière séquentielle au cours de l'infection. La caractérisation du réseau de régulation contrôlant les réponses du blé à la fusariose a mis en évidence un ensemble de régulateurs principaux. Ensuite, l'intégration des réponses du blé avec les données d'expression des gènes codant les effecteurs de *F. graminearum* a permis l'identification de facteurs de sensibilité putatifs impliquant principalement l'inhibition des mécanismes de défense des plantes et incluant plusieurs gènes correspondant à des régulateurs très connectés précédemment identifiés. Enfin, une analyse approfondie de l'état physiologique des épis de blé lors de l'infection par le FHB par une double approche, la mesure des échanges gazeux et les réponses transcriptomiques du blé au FHB, a démontré une forte augmentation de la respiration parallèlement à une altération des processus photochimiques et biochimiques de la photosynthèse. L'ensemble de nos résultats ont fourni un corpus robuste et conservé d'effecteurs et de facteurs de susceptibilité proposés comme étant nécessaires au succès de l'infection du blé par *F. graminearum* et constituant une ressource d'intérêt pour la sélection du blé.

Mots clefs : *Fusarium graminearum*; Blé; Dialogue moléculaire; Effecteurs; Facteurs de sensibilité; Transcriptomique à l'échelle du système; Ecophysiologie

Abstract

Fusarium graminearum, the main causal agent of Fusarium Head Blight (FHB), is one of the most damaging pathogens in small grain cereals, especially wheat. With direct impacts on yield, grain quality and through the accumulation of carcinogenic mycotoxins, FHB is considered as a major limiting factor for wheat production in Europe, North America and Asia resulting in substantial economic losses. With rising temperatures and occasional increases in air humidity due to climate change, FHB outbreaks are expected to be more frequent and intense. Currently, control methods, based on the combined use of resistant cultivars, adapted agronomical practices and fungicide treatments, are ineffective to fully control FHB outbreaks. Thus, further research is needed to develop better management strategies. Wheat resistance to FHB is a strictly quantitative trait which involves multiple Quantitative Trait Loci (QTLs) displaying relatively minor effects especially when environmental conditions are conducive to the pathogen, making it necessary to search alternative sources of resistance. For the last twenty years, the multiple evidences of plant genes required for pathogen infection have opened new opportunities to control immunity in plants. These genes, the so-called susceptibility genes, are involved in a wide range of plant fundamental processes that are hijacked by the pathogen through the delivery of effectors. In the wheat - *F. graminearum* pathosystem, the role of susceptibility genes in the interaction outcome has already been demonstrated in several studies. Hence, elucidate the mechanisms of wheat susceptibility to *F. graminearum* represents a promising approach to improve FHB resistance. For this purpose, a RNA-seq based profiling of *F. graminearum* genes encoding putative effectors and wheat's FHB responsive genes was performed during a time course infection and by taking advantage of the genetic variability from both species. This approach shed light on a highly conserved and complex infection program based on a core-effectome of 357 genes sequentially expressed along infection progress. Through the characterization of the regulation network orchestrating wheat responses to FHB, a set of master regulators was evidenced. Then, the dual-integration of wheat's responses with the expression data of *F. graminearum* effector genes led to the identification of putative susceptibility factors mostly involved the inhibition of plant defense mechanisms, of which several genes corresponded to highly connected regulators afore-described. Finally, an in-depth analysis of the physiological status of wheat's spikes along FHB infection in a two-pronged approach, gas exchange measurements and wheat's transcriptomic responses to FHB, demonstrated a strong increase of the respiration rate parallel to an alteration of the photochemical and biochemical processes involved in the photosynthesis. Taken together, our findings provided a robust corpus of the conserved effectors and susceptibility factors proposed to be necessary for the success of *F. graminearum* infection in wheat and will be a valuable resource for wheat breeding.

Keyword: *Fusarium graminearum*; Wheat; Molecular crosstalk; Effectors; Susceptibility factors; System-level transcriptomics; Ecophysiology

Remerciements

Je voudrais tout d'abord remercier l'ensemble des membres de mon jury de thèse qui ont accepté d'évaluer ce travail : **Claire Veneault-Fourey, Sylvain Raffaele, Laurence Lejay et Marie-Laure Martin.**

Je souhaiterais ensuite remercier mes encadrants de Thèse. Un grand merci à **Thierry Langin** et **Philippe Label** pour tous leurs conseils et les discussions intéressantes. Je tiens tout particulièrement à remercier mon encadrant principal, **Ludovic Bonhomme**, qui m'a donné ma chance sur ce projet passionnant et exigeant, a su me guider dans les méandres de la data et m'a poussé à me dépasser. Petite pensée particulière pour ce voyage/congrès à San Francisco, pour les kms parcourus à pied et pour la découverte des fameux crabes de la baie.

Un grand merci à **Pierre Bancal, Marie-Odile Bancal, Alain Fortineau et Jérôme Ngao** pour m'avoir aiguillé et apporté leur expertise sur l'ensemble de la partie écophysiologie. Je remercie également **Hélène Rimbert** et **Andréa Garavito-Guyot** pour tous leurs conseils en bio-informatique. Merci à **Richard Blanc, Stéphane Bernard** et **Marc Deffradas** pour leur aide en chambre de culture lors des expérimentations. J'espère que les sessions de techno ne vous auront pas trop traumatisés. Merci **Tarek Alouane** pour ta bonne humeur au bureau et ton aide plus que précieuse pour l'analyse des données de Fusa. Ce fut un réel plaisir de travailler avec toi et vive le SuperMégaPangenomeDeLaMuerta !

Je tiens à remercier le trio infernal des techniciens de l'équipe MDC, **Olivier**, l'expert sporulateur au petit accent du sud et toujours prêt à boire une bière, Maître **Cambon** qui, sur son falcon perchée, m'a appris à tenir une pipette et à ne pas colorier le scotch d'autoclave et enfin **Géraldine** (pain au Gérald) petit esprit facétieux au rire démoniaque (mes tympans s'en souviendront) qui m'aura suivi dans de nombreux délires costumés. Merci aussi aux autres membres de l'équipe que j'ai eu la chance de côtoyer, ceux partis vers d'autres horizons et ceux qui tiennent encore la baraque, en particulier **Cyrille, Samir** (je m'excuse pour tous les DOUX jeux de mots), **Larissa, Jean-Noël, Andréa, Mélanie, Juliette, Francis** et **Shimlal.**

Je tiens également à remercier plusieurs personnes de l'unité qui ont contribué à créer cet environnement convivial dans lequel j'ai eu la chance d'évoluer au cours de ces 4 années. Merci tout d'abord à la team *In Bascula Veritas* pour tous les moments de jovialité partagés pendant la crise. Merci **Mélissa**, lady stomata la bresom du triangle des bermudes, pour ta bonne humeur le matin aux pauses cafés, les batailles de nerfs, les coups de balai et tant d'autres délires que je ne peux pas citer ici mais qui auront révolutionné la science des membranes. On a débuté cette aventure ensemble et on la termine à une semaine d'écart, c'est beau. Piou piou piou **Loriane** pour ta folie, ta bonne humeur et tes cris d'oiseau, c'était vraiment CHOUQUETTE d'avoir partagé ce bureau avec toi. Merci **Maxime**, artisan mêmeur et régulateur des pauses déjeuners, on aura beaucoup ri à défaut de produire de la valeur. TchuTchu **Lena** pour ton implication au sein du triangle des bermudes, tes jeux de mots aiguisés, tes compositions musicales de génie et les pauses prune (et si tu crois que c'est fini... Jamais, Dans le lin, 2022). L'album du duo Albedo/La Calotte est bientôt disponible dans les bacs. Merci **Aline**, l'esprit libre aux chaussettes dépareillées, je te souhaite pleins de bons 11h52. Merci **Jean** et **Emile** pour tous les bons moments passés en votre compagnie. Et enfin peut-être la plus importante du groupe, merci à toi **Jeanne**, tant pour ton rôle de masseuse que d'happiness manager. Sans toi on ne se serait jamais autant amusé et je serais probablement courbé à angle droit aujourd'hui.

Merci à tous les copains de l'ENSAT pour toutes ces années passées à vos côtés. Merci pour tous ces week-end mémorables sous le signe de la folie et de la bienveillance. Longue vie à l'amicale du rôti ! Je n'ai malheureusement pas la place de tous vous citer, mais je tiens en particulier à remercier **Alex, Vincent, Tang, Marion, Pauline, Vicky, Marie, Victor, Anne, Eloi** et **Gabrielle**.

Petite pensée particulière à **Pierre** qui me supporte depuis maintenant plus de 10 ans et sans qui la vie n'aurait pas été la même.

Merci **Sandra** pour toutes les séances ciné, certaines plus surprenantes et perchées que d'autres (j'oublierais jamais mon premier contact avec le cinéma roumain). Merci **Laura** pour tous les bons moments avec une pensée particulière pour toutes nos parties de Perpebebe game.

Pour terminer, je tiens à remercier du fond du cœur mes parents qui ont toujours été là pour me soutenir et sans qui je ne serais jamais arrivé jusque-là. Merci à mes deux petites sœurs qui supportent ma personne depuis si longtemps. Enfin, merci à ma mamie qui m'a toujours encouragé.

Maintenant passons aux choses sérieuses, voici le plan du manuscrit.

Table des abbreviations

9-LOX	9-lipoxygenase
ABA	Acide abscissique
AP2/ERF	Apetala2/ethylene responsive factor
ARN	Acides ribonucléique
BAK1	Brassinosteroid insensitive 1-associated receptor kinase 1
Bax	Protéine Bcl-2-associated X
BiFC	Biomolecular fluorescence complementation
BIK1	Botrytis-insensitive kinase 1
BR	Brassinostéroïdes
CAMTA	Calmodulin-binding transcription activator
CBP60g	Cam-binding protein 60-like g
CK	Cytokinine
CRISPR	Clustered regulatory interspaced short palindromic repeats
CWDE	Cell wall degrading enzyme
DEG	Gène différentiellement exprimé
DMR6	Downy mildew resistance 6
DON	Deoxynivalenol
ERC	Early root compatibility
ET	Ethylène
ETI	Effector-triggered immunity
ETS	Effector-triggered susceptibility
FHB	Fusarium head blight
FS	Facteur de Sensibilité
GA	Gibbéreline
GFP	Green fluorescent protein
GH12	Glycoside hydrolases 12
HIGS	Host-induced gene silencing
hpi	Heures post infection
IAA	Auxine
IC	Infection cushions
ICS1	Isochorismate synthase 1
IP	Invasion pattern
IPR	Invasion pattern receptors
JA	Jasmonate
MAMP	Microbe-associated Molecular Pattern
MAPK	Mitogen-activated protein kinase
MLO	Mildew locus o
MTI	Mamp-triggered immunity
NIS1	Necrosis-inducing secreted protein 1
NLR	Nucleotide-binding site–Leucine-rich repeat Receptor
NPR1	Nonexpressor of pathogenesis-related genes 1
PRR	Patterns recognition receptor

QTL	Quantitative trait locus
RACB	Rho-related protein racb
RIPb	ROP INTERACTIVE PARTNER b
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
S genes	Susceptibility genes
SA	Acide salycilique
SARD1	Sar deficient 1
STP	Sugar transporter protein
SUT	Sucrose transporter
SWEET	Sugars Will Eventually be Exported Transporter
TAL	Transcription activator-like effector
TALEN	Transcription activator-like effector nuclease
TF	Facteur de Transcription
TRI	Trichothecene biosynthetic gene

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Avant-propos : Les champignons phytopathogènes, une menace pour la sécurité alimentaire

Dans leur environnement et au sein des systèmes agricoles, les plantes sont soumises à de nombreuses pressions biologiques, générées par une grande diversité de parasites : bactéries, virus, nématodes, oomycètes et champignons, qui compromettent leur développement optimal. Avec plus de 8,000 espèces phytopathogènes affectant la quasi-totalité des espèces cultivées, les champignons parasites sont responsables d'environ 30% de pertes agricoles, représentant ainsi la menace biotique la plus importante pour la sécurité alimentaire mondiale (Gladieux et al., 2017; Steinberg and Gurr, 2020).

Fusarium graminearum, l'agent principal responsable de la fusariose de l'épi (FHB) chez les céréales et plus particulièrement le blé tendre en est un exemple frappant (Parry et al., 1995; Goswami and Kistler, 2004). Avec des impacts directs sur le rendement, la qualité technologique du grain, mais principalement par l'accumulation de mycotoxines carcinogènes (i.e., deoxynivalenol, DON) (Boyacıoğlu and Hettiarachchy, 1995; Argyris et al., 2003; Chen et al., 2019), la FHB est considérée comme un facteur limitant majeur de la culture du blé en Europe, Amérique du Nord et Asie (McMullen et al., 2012; Dahl and Wilson, 2018) qui a engendré 1.176 milliard de dollars de pertes économiques aux Etats-Unis sur les années 2015 et 2016 (Wilson et al., 2018). Les hausses de température associées aux pics ponctuels d'humidité relative, conséquences du changement climatique, représentent des facteurs de risque d'épidémies intenses et sévères de FHB (Vaughan et al., 2016; Mylonas et al., 2020). L'utilisation combinée de génotypes de blé partiellement résistants, de fongicides, de pratiques culturales adaptées et de technologies de surveillance de la pression parasitaire permet de limiter cette dernière (Wegulo et al., 2015) sans toutefois contrôler totalement les épidémies, rendant ainsi nécessaire la recherche de stratégies de contrôles plus performantes et durables (Xia et al., 2020). Dans un contexte de réduction des produits phytosanitaires en France, matérialisé par les plans Ecophyto, l'identification de sources de résistances plus durables et efficaces représente un levier important dans la lutte contre la FHB (Lamichhane et al., 2019).

La sensibilité d'une plante à un agent pathogène résulte de la capacité de ce dernier à s'établir et compléter son cycle biologique au détriment de son hôte tandis que la résistance d'une plante résulte de sa capacité à empêcher l'infection ou à limiter le développement de l'agent pathogène. Ainsi, le devenir de l'interaction entre un agent pathogène et sa plante hôte repose sur un dialogue moléculaire complexe entre les deux protagonistes et est la conséquence de la combinaison de leurs caractéristiques génétiques, biochimiques et physiologiques. De par la complexité et l'aspect multidimensionnel du dialogue moléculaire en œuvre au sein des pathosystèmes, il est nécessaire de décrypter et de comprendre les déterminants moléculaires sous-jacents pour identifier de nouveaux leviers pour l'amélioration de la réponse des plantes aux stress biotiques.

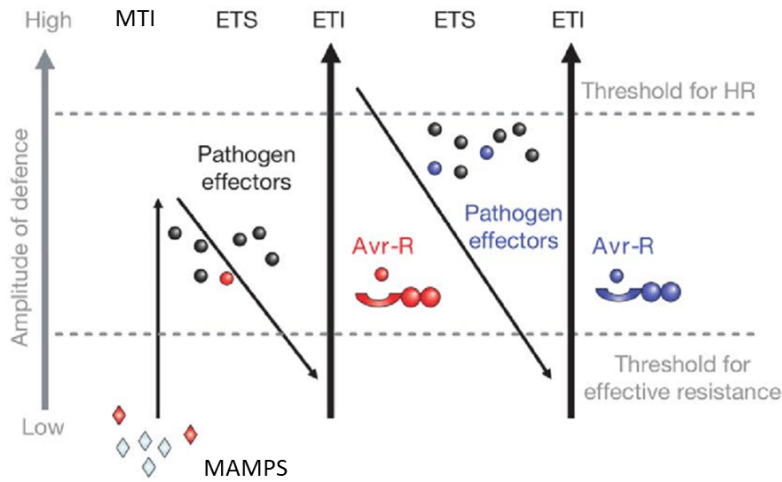
Introduction

I. Le pathosystème blé-*Fusarium graminearum* est un modèle de la limite de l'étude des mécanismes de défense

1. Les interactions plantes-pathogènes, des systèmes biologiques complexes principalement étudiés par le prisme de l'immunité

Outre les mécanismes de défenses préformés, i.e. les barrières physiques et chimiques, les interactions antagonistes établies entre les plantes et les agents pathogènes engendrent des processus complexes de coévolution, qui se traduisent par la mise en place de mécanismes de reconnaissance sophistiqués chez les plantes et de stratégies de contournement chez l'agent pathogène (Flor, 1971; Woolhouse et al., 2002; Han, 2019). Cette vision des interactions plantes-pathogènes est notamment matérialisée par le modèle « zig-zag » (Jones and Dangl, 2006). Cette description du système immunitaire repose sur quatre phases successives : (1) la reconnaissance de motifs conservés, les Microbe-associated Molecular Patterns (MAMPs) par les Patterns Recognition Receptors (PRR) membranaires de la plante qui aboutit à la MAMP-Triggered Immunity (MTI), (2) la désactivation de la MTI par la sécrétion de molécules par l'agent pathogène, les effecteurs, aboutissant à l'Effector-Triggered Susceptibility (ETS), (3) la reconnaissance des effecteurs par des récepteurs intracellulaires de l'hôte, les protéines nucleotide-binding site-leucine-rich repeat receptor (NLR), déclenchant l'Effector-Triggered Immunity (ETI) et enfin (4) le rétablissement de l'ETS par le contournement de l'ETI suite à la mutation ou la perte de l'effecteur reconnu ou sa suppression par l'utilisation d'un nouvel effecteur.

(A)



(B)

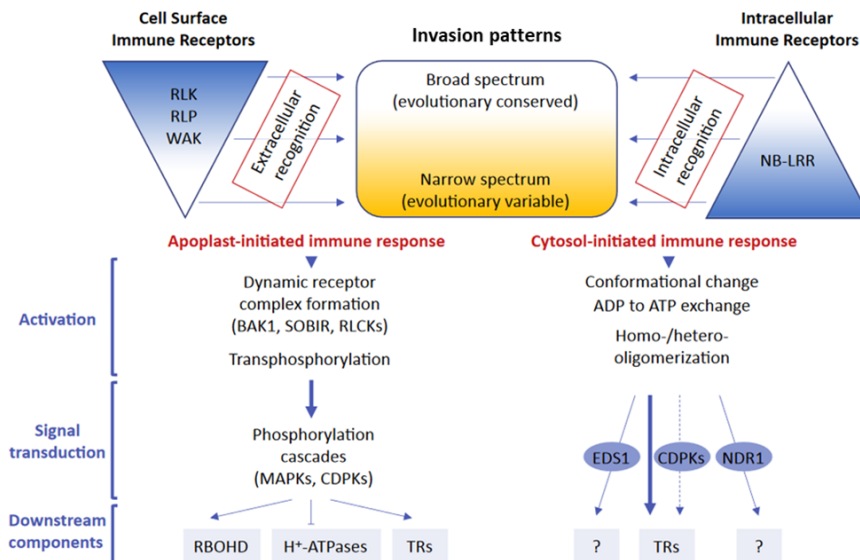


Figure 1. Evolution des modèles de l'immunité des plantes. Le modèle zigzag proposé en 2006 par Jones et Dangl (A) repose sur quatre étapes (1) la reconnaissance des Microbe-associated Molecular Patterns (MAMPS) déclenchant la MAMP-Triggered Immunity (MTI), (2) la désactivation de la MTI par la sécrétion de molécules par l'agent pathogène, les effecteurs, aboutissant à l'Effector-Triggered Susceptibility (ETS), (3) la reconnaissance des effecteurs par des récepteurs intracellulaires de l'hôte, les protéines nucleotide-binding site-leucine-rich repeat (NLR), déclenchant l'Effector-Triggered Immunity (ETI) et (4) le rétablissement de l'ETS par le contournement de l'ETI. Le modèle actuel de l'immunité est le modèle spatial d'invasion (B) proposé pour la première fois par Cook et collaborateurs et décrivant l'immunité comme un système de surveillance basé sur la reconnaissance d'Invasion Patterns (IPs) qui peuvent être conservés (reconnaissance à large spectre) ou spécifiques (reconnaissance spectre réduit) par des Invasion Pattern Receptors (IPRs) localisés au niveau de la membrane plasmique ou dans le cytoplasme des cellules. La reconnaissance de ces IPs active des voies de signalisation très complexes aboutissant aux réponses de défense. (Adapté de Jones and Dangl, Nature, 2006 ; Kanyuka and Rudd, Curr. Opin. Plant Biol., 2019)

Le cadre spatial et temporel réducteur qui conduit à une dichotomie des processus de MTI et d'ETI, l'aspect pathogène voire souche spécifique de l'ETI et la non prise en compte de la complexité et de la diversité des interactions plantes-pathogènes, ont remis en cause le « zig-zag » model, remplacé par le modèle spatial d'invasion basé sur la localisation du site de reconnaissance de l'agent pathogène (*Figure 1*) (Cook et al., 2015; Kanyuka and Rudd, 2019; van der Burgh and Joosten, 2019). Ce modèle décrit l'immunité comme un système de surveillance basé sur la reconnaissance d'Invasion Patterns (IPs) issus de l'agent pathogène ou de l'hôte et qui peuvent être conservés (reconnaissance à large spectre) ou spécifiques (reconnaissance spectre réduit) par des Invasion Pattern Receptors (IPRs) localisés au niveau de la membrane plasmique ou dans le cytoplasme des cellules. De plus, ce modèle propose une vision quantitative de l'immunité avec la mise en place de réponses d'intensité variable et permet de prendre en compte l'ensemble des interactions trophiques des plantes avec les micro-organismes, i.e. biotrophes, nécrotrophes, hémibiotrophes et symbiontes.

De plus, quatre études de 2021 ont mis en évidence des interconnexions fortes entre la MTI et l'ETI qui partagent des processus et agissent de manière synergique, complexifiant ainsi notre vision du système immunitaire des plantes (Ngou et al., 2021; Pruitt et al., 2021; Tian et al., 2021; Yuan et al., 2021). La reconnaissance de l'agent pathogène par la plante hôte s'intègre dans un réseau de régulation complexe avec la transduction du signal d'infection via les cascades mitogen-activated protein kinase (MAPK), les voies hormonales, la signalisation calcique, les facteurs de transcription et les processus de régulation épigénétique qui permettent d'initier une vaste gamme de réponses de défense allant de la synthèse de composés de défenses de nature extrêmement variée, aux mécanismes de détoxification et au renforcement des barrières physiques (Andersen et al., 2018; Kanyuka and Rudd, 2019).

Bien que plus complet, le modèle spatial d'invasion ne permet d'appréhender les interactions plantes-pathogènes que par le prisme de l'application en sélection variétale et donc par le prisme de la reconnaissance de l'agent pathogène par la plante. Ainsi, bien que particulièrement adaptés pour décrire le système immunitaire de la plante, ces modèles n'offrent qu'une vision partielle du dialogue moléculaire établi lors de l'interaction et excluent son instigateur, à savoir l'agent pathogène et ses besoins.

2. Le devenir d'une interaction entre une plante et un agent pathogène ne se limite pas au système immunitaire de l'hôte

a. La sensibilité, un levier pour l'identification de résistances alternatives plus durables

Bien que le contournement ou la suppression des différentes couches du système immunitaire de l'hôte soit essentiel pour la compatibilité de l'interaction, le succès de cette dernière repose également sur la capacité de l'agent pathogène à exploiter ou manipuler les processus essentiels de l'hôte, i.e. des fonctions biologiques nécessaires au développement de la plante en l'absence de stress, aussi appelés facteurs de compatibilité ou facteurs de sensibilité. Ainsi, la plante hôte, via la présence de gènes de sensibilité (Susceptibility genes, S genes) dans son génome, participe de manière active au succès de l'interaction (Vogel et al., 2002; Eckardt, 2002; Lapin and Van den Ackerveken, 2013; van Schie and Takken, 2014). Par conséquent, la perte de fonction ou la mutation de ces gènes de sensibilité réduit la compatibilité de l'interaction et contribue à augmenter la résistance de la plante. La résistance conférée par la reconnaissance de l'agent pathogène est codée par des gènes de résistance qui sont généralement hérités de manière dominante. Cependant, l'existence de résistances héritées de manière récessive indique la perte de facteurs de sensibilité clef manipulés par l'agent pathogène. Ainsi ces gènes de résistance correspondent donc à des versions mutées et récessives de gènes de sensibilité (Pavan et al., 2010; Kourelis and van der Hoorn, 2018).

Ces sources de résistance issues de la perte de facteurs de sensibilité sont exploitées depuis des décennies dans les processus de sélection variétale. C'est notamment le cas de la résistance récessive et quasi-complète de l'orge à *Blumeria graminis*, agent causal de l'oïdium, conférée par la mutation naturelle du gène MLO dont la version dominante est nécessaire pour le développement du champignon (Jørgensen, 1992). Identifiée en 1942 et introduite dans les variétés élités dès 1979, la résistance mlo confère une résistance pré-invasion à large spectre à la quasi-totalité des souches de *B. graminis* qui malgré une utilisation intensive est restée durablement efficace au champs (Lyngkjær et al., 2000). De plus, le gène MLO est conservé chez l'ensemble des plantes mais aussi chez des algues vertes et la mousse (Devoto et al., 2003; Kusch et al., 2016) et la version dominante du gène est nécessaire à l'établissement de *B. graminis* chez toutes ses espèces hôtes.

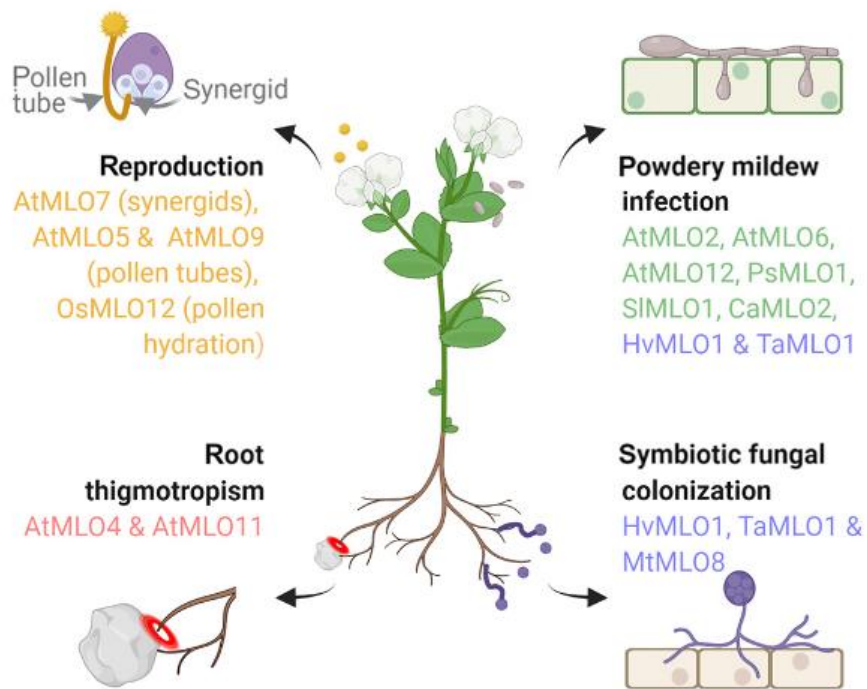


Figure 2. Fonctions exercées par le gène Mildew Resistance Locus (MLO). Outre son rôle clef dans la sensibilité à l'oïdium, le gène MLO joue un rôle de mécano-perception et intervient dans de nombreux processus fondamentaux des plantes tels que la reproduction, le développement racinaire et la mise en place de symbioses. (Adapté de Jacott et al., Trends Plant Sci., 2021)

Ainsi, la résistance mlo représente une ressource génétique universelle importante, et est notamment largement utilisée chez le pois et la tomate (Kusch and Panstruga, 2017). L'émergence du gène MLO très tôt chez les plantes, son haut degré d'homologie de séquence (Kusch et al., 2016) ainsi que les effets pléiotropiques associés à sa mutation (Kusch and Panstruga, 2017) suggère qu'il y joue un rôle fondamental exploité ensuite par *B. graminis*. Cloné en 1997, le gène MLO code une protéine transmembranaire (Büschges et al., 1997) dont la fonction moléculaire précise reste encore aujourd'hui non connue (Jacott et al., 2021). Cette dernière est cependant associée à la signalisation par les ions calcium (Ca^{2+}) et les Reactive Oxygen Species (ROS) (Hückelhoven et al., 2001; Kim et al., 2002; Cui et al., 2018). Longtemps principalement perçu comme un régulateur négatif des mécanismes de défense (Engelhardt et al., 2018), le gène MLO intervient dans de nombreux tissus et dans de nombreux processus de développement impliquant la mécano-perception dont la formation du tube pollinique, la croissance et la forme du système racinaire, les réponses à la fertilisation mais aussi la mise en place de symbioses (Figure 2) (Jacott et al., 2021).

Enfin, les nouvelles technologies d'édition des génomes, telles que les systèmes « Transcription Activator-Like Effector Nuclease » (TALEN) ou « Clustered Regulatory Interspaced Short Palindromic Repeats » (CRISPR)/Cas9, représentent des outils puissants pour modifier les gènes de sensibilité et développer rapidement de nouveaux cultivars résistants et ce sans introduction de transgènes (Nejat et al., 2017; Zaidi et al., 2018). Ces deux technologies ont notamment été appliquées avec succès chez le blé pour éditer le gène MLO et ont abouti au développement de mutants résistants à *B. graminis*, un caractère non présent naturellement chez le blé (Wang et al., 2014; Li et al., 2022). Le gène MLO constitue un exemple frappant de l'intérêt d'identifier les facteurs de sensibilité et du succès lié à leur utilisation comme source alternative pour développer des résistances efficaces, à large spectre et durables.

Ainsi, caractériser un pathosystème sous l'angle de la sensibilité pour identifier le catalogue de facteurs de sensibilité gouvernant le succès de l'interaction prend tout son sens. La multiplicité des rôles des gènes de sensibilité dans les processus fondamentaux de la plante rend nécessaire d'acquérir des connaissances sur leur place dans le dialogue moléculaire établi au cours de l'interaction, mais nécessite également de comprendre leurs effets sur les caractéristiques intrinsèques de la plante.

b. FHB chez le blé, un modèle pour l'étude de la sensibilité

Le pathosystème blé-*F. graminearum* atypique présentent de nombreuses particularités rendant difficile la recherche de sources de résistance.

- Spécificités de l'interaction entre le blé tendre et *F. graminearum*

F. graminearum est un pathogène hémibiotrophe généraliste capable d'infecter de nombreuses espèces de graminées dont le blé, l'orge, l'avoine, le maïs et le riz, de se développer de manière saprophyte sur les résidus de culture mais aussi de s'installer sans causer de maladies dans des hôtes alternatifs appartenant à de nombreuses familles de plantes dont entre autres certaines graminées, des genres *Agrostis* et *Bromus* par exemple, des cucurbitacées, des légumineuses, des astéracées et des chénopodiacées (Goswami and Kistler, 2004; Mourellos et al., 2014; Suproniene et al., 2019). De plus, chez le blé, *F. graminearum* est capable de s'installer dans de nombreux organes, présentant des caractéristiques physiologiques très différentes, tels que l'inflorescence, le collet, les racines et les graines causant ainsi des maladies distinctes : la fusariose de l'épi, la fusariose du collet, la fusariose des racines et la fonte des semis (Kazan et al., 2012). Cette capacité à interagir avec de nombreuses espèces de plantes et à se propager dans différents tissus suggère une plasticité génomique importante permettant l'ajustement de sa stratégie d'infection et de son mode de vie en fonction du contexte moléculaire auquel il est confronté. L'analyse du transcriptome de *F. graminearum* lors de l'infection de trois graminées différentes, le blé, l'orge et le maïs, a notamment mis en évidence d'importantes spécificités hôtes caractérisées par l'expression de gènes hôte-spécifiques et des patterns d'expression spécifiques pour les gènes exprimés chez plusieurs hôtes (Harris et al., 2016). De plus, chez une même espèce hôte, ici le blé, des spécificité associées aux organes infectés sont observées. Au cours de la fusariose des racines, *F. graminearum* met en place une stratégie d'infection en deux temps, une première phase caractéristique des pathogènes racinaires suivie d'une deuxième phase similaire au FHB en terme de structure d'infection et de chronologie (Q. Wang et al., 2015).

Enfin, dans le cas de la fusariose de l'épi, *F. graminearum* infecte un organe reproducteur complexe composé de six tissus différents (glume, paléa, lemma, ovaire, anthère et rachis). *F. graminearum* infecte les épillets au cours de l'anthèse et se développe en parallèle de la formation et du développement du grain (Pugh et al., 1933; Guenther and Trail, 2005). Le champignon fait ainsi face à un environnement moléculaire en évolution rapide et profonde, induite par l'ontogénie du grain. Cela se manifeste en outre par l'implication d'un grand nombre de voies de signalisation hormonales qui jouent un rôle à un moment précis du développement, avec les cytokinines et l'auxine qui régulent la phase de cellularisation tandis que la gibbérelline et l'acide abscissique sont impliqués dans les étapes de remplissage et de maturation (Nadaud et al., 2010; Kong et al., 2015). L'analyse du développement de *F. graminearum* dans l'épi a mis en évidence l'existence de deux phases d'infection avec une première phase asymptomatique caractérisée par la progression intercellulaire des hyphes suivie d'une seconde phase symptomatique caractérisée par l'apparition de nécroses et une colonisation intracellulaire des tissus (Brown et al., 2011, 2010; Chetouhi et al., 2016). Les résultats obtenus dans le cadre des travaux de C. Chetouhi et collaborateurs semblent indiquer que les phases d'infection sont intimement liées à l'ontogénie du grain, la phase asymptomatique se déroulant lors de la phase de cellularisation donc de formation du grain et le début de la phase symptomatique lors du remplissage (Chetouhi et al., 2015, 2016). De nombreux événements moléculaires renforcent l'hypothèse du maintien de l'intégrité de la formation du grain. L'expression des gènes TRI impliqués dans la synthèse de la DON est fortement induite lors de la phase asymptomatique, leur expression étant maximale au niveau du front de colonisation, et est fortement réduite lors de la phase symptomatique (Brown et al., 2011, 2017). Or en deçà d'un certain seuil, cette mycotoxine est associée à l'inhibition de la mort cellulaire (Diamond et al., 2013). Ainsi, la DON joue un rôle ambivalent et concentration-dépendant au cours de l'interaction en favorisant dans un premier temps la colonisation des tissus par *F. graminearum* puis en déclenchant dans un second temps la mort cellulaire (Brown et al., 2011). Du côté du blé, l'inhibition de la mort cellulaire a également été mise en évidence chez le génotype sensible 'Récital' et se manifeste par la surexpression de gènes impliqués dans l'inhibition de la mort cellulaire et la sous-expression de gènes impliqués dans son induction (Chetouhi et al., 2016).

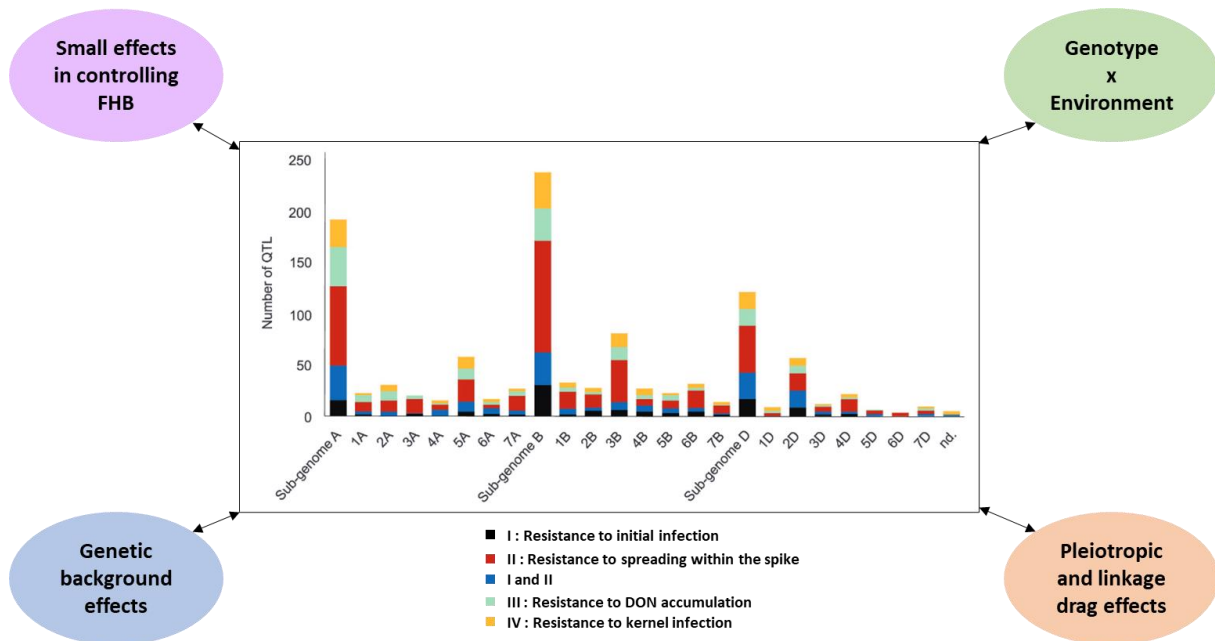


Figure 3. Structure de la résistance du blé tendre au FHB et limites à son utilisation dans les programmes de sélection. La résistance au FHB est un caractère strictement quantitatif impliquant plus de 625 QTLs répartis sur l'ensemble du génome et appartenant à 5 grands types de résistance: (Type I) résistance à la pénétration, (Type II) résistance à la propagation, (Type III) la résistance à l'accumulation de mycotoxines, (Type IV) la résistance du grain à l'infection, Type (V) la tolérance à la perte de rendement (aucun QTL de ce type n'a été détecté à ce jour). Ces QTLs présentent des effets relativement faibles qui sont en partie dépendant du contexte génétique et environnemental. (Adapté de Venske et al., *Front. Plant Sci.*, 2019)

De plus, la complexité de l'interaction dans l'épi est associée à une spatialisation des déterminants moléculaires de *F. graminearum* et des réponses du blé. En effet, l'induction de l'expression du gène TRI5, indicateur de la synthèse de DON, est dépendante de l'organe se manifestant par une expression forte au niveau du grain en développement et des nœuds du rachis et l'absence d'expression dans les anthères (Ilgen et al., 2009). Il en va de même du côté du blé avec des réponses fortes et similaires pour les organes végétatifs tandis que des réponses très spécifiques sont observées pour les organes reproducteurs, en particulier dans le cas de l'ovaire correspondant à l'organe le moins impacté (Golkari et al., 2007). La phase biotrophe de *F. graminearum* associée au maintien de la phase de cellularisation apparaît ainsi comme une étape clef de la stratégie d'infection permettant l'installation durable du champignon dans l'épi et le détournement de la phase de remplissage qui suit. Cependant, au-delà d'une stratégie unilatérale de la part de *F. graminearum*, la complexité de l'interaction pourrait en partie résulter d'une stratégie de tolérance de la part de la plante hôte pour préserver sa capacité reproductive plutôt que d'une stratégie de résistance, c'est-à-dire réduire l'effet de l'infection sur la fitness plutôt que d'inhiber l'infection (Best et al., 2010; Pagán and García-Arenal, 2018). L'ensemble de ces spécificités font de la fusariose de l'épi une maladie complexe et pourrait en partie expliquer l'absence de mécanismes de défense efficaces du blé contre *F. graminearum*.

- La résistance du blé tendre au FHB, un caractère complexe

A ce jour, aucune source de résistance totale au FHB n'a été identifiée. La résistance du blé au FHB est un caractère strictement quantitatif possédant une structure polygénique extrêmement complexe et ne présentant aucune spécificité liée à la souche de *F. graminearum* (Figure 3) (Bai and Shaner, 2004; Ren et al., 2019; Ghimire et al., 2020; Ma et al., 2020). A la complexité de la structure génétique de la résistance, vient s'ajouter la complexité des mécanismes de résistance répartis en 5 types de résistance (Buerstmayr et al., 2020). Les cinq types de résistance sont les suivants : (Type I) résistance à la pénétration de l'agent pathogène qui inclue également des mécanismes dits passifs qui sont associés à des caractéristiques morphologiques et phénologiques (taille de la plante, extrusion des anthères et période de floraison) (Schroeder and Christensen, 1963), (Type II) résistance à la propagation du champignon dans l'épi (Miller et al., 1985),

(Type III) la résistance à l'accumulation de mycotoxines par inhibition de leur synthèse ou par leur dégradation (Miller et al., 1985), (Type IV) la résistance du grain à l'infection associée au paramètre du taux de grains infectés (Mesterhazy, 1995), Type (V) la tolérance à la perte de rendement (Mesterhazy, 1995). En 2021, après deux décennies consacrées à la détection de Quantitative Trait Loci (QTLs) de résistance, un total de 625 QTLs associés aux résistances de type II et III ont été répertoriés et sont répartis sur l'ensemble du génome du blé (T. Zheng et al., 2021), illustrant ainsi la complexité de la résistance au FHB chez le blé. Malgré ce vaste catalogue de sources de résistance, la grande majorité des QTLs, en particulier ceux identifiés à partir de matériel biologique localement adaptés, présentent des effets faibles et ne sont pas utilisés dans les programmes de sélection (Steiner et al., 2017; Venske et al., 2019) et les cultivars commercialisés ne présentent qu'une résistance modérée au FHB provenant en général de matériel exotique (Ma et al., 2020). En effet le QTL Fhb1, premier QTL de résistance au FHB identifié (chez les cultivars 'Sumai 3' et 'Ning7840' en 1999) (Bai et al., 1999; Waldron et al., 1999), reste encore aujourd'hui, de par son efficacité (réduction de la sévérité de l'infection de l'ordre de 20 à 50%) et sa stabilité dans différents fonds génétiques (G. Bai et al., 2018; T. Li et al., 2019), la source de résistance la plus utilisée dans les programmes de sélection (Buerstmayr et al., 2020; Ma et al., 2020).

Au-delà des effets relativement faibles des QTLs, de nombreux autres freins au développement de cultivars résistants viennent s'ajouter (*Figure 3*). Le fond génétique des cultivars utilisés dans les croisements, que ce soit le matériel élite receveur ou le matériel résistant donneur, joue notamment un rôle non négligeable sur l'efficacité de la résistance (Bai and Shaner, 2004; Handa et al., 2008; Brar et al., 2019a; T. Li et al., 2019). L'utilisation de 'Sumai 3', principale source de résistance au FHB, dans les croisements nécessite une attention particulière. En effet, en 1997, des lignées de 'Chinese Spring' dont le chromosome 2D a été substitué par le chromosome 2D de 'Sumai 3' présentent un niveau de sensibilité supérieur à 'Chinese Spring', suggérant l'existence d'un allèle de sensibilité chez 'Sumai 3' (Yao et al., 1997). Une étude similaire réalisée en 2002 avec les deux mêmes cultivars a montré une augmentation de la concentration de DON dans les lignées 'Chinese Spring' dont le chromosome 2D a été substitué par celui de 'Sumai 3' (Zhou et al., 2002). En 2008, l'utilisation de lignées haploïdes doublés issues du croisement entre 'Gamenya' (génotype sensible) et 'Sumai 3' (génotype résistant) a permis de positionner le QTL sur le

bras court du chromosome 2D et de montrer que la combinaison de l'allèle de 'Sumai3' pour le QTL Fhb 1 avec l'allèle du parent sensible 'Gamenya' pour le QTL 2DS présente un niveau de résistance plus élevé que le cultivar 'Sumai 3'. Un gène codant une protéine impliquée dans la résistance aux toxines (Multidrug Resistance-associated Protein, MRP), dont l'expression dans le génotype sensible est significativement plus élevée que chez 'Sumai 3', a été proposé comme candidat (Handa et al., 2008). Ainsi malgré son haut niveau de résistance, 'Sumai 3' porte des allèles de sensibilité pour certains QTLs de résistance, ce qui complexifie grandement son utilisation dans les schémas de sélection.

La transmission de caractères délétères associés à des effets pléiotropiques des gènes sous-jacents des QTLs ou à du déséquilibre de liaison fort avec des gènes impliqués dans d'autres processus biologiques (Bai and Shaner, 2004; Suzuki et al., 2012; Brar et al., 2019b) constitue un autre frein important à l'introgession des QTLs de résistance. Dans le cas de l'introgession du QTL Fhb5 dans des lignées élites canadiennes, l'augmentation du niveau de résistance a été accompagnée d'une augmentation de la taille des plantes, d'une réduction du rendement mais aussi de l'altération du contenu en protéines (Brar et al., 2019b).

Enfin, l'efficacité des QTLs est soumise à des effets importants de l'environnement et leurs effets sont fortement diminués en cas de fortes pressions parasitaires (Bai and Shaner, 2004; Verges et al., 2006; Osman et al., 2015; Vaughan et al., 2016; Steiner et al., 2017; Ghimire et al., 2020; Goddard et al., 2021). A titre d'exemple, sur 14 QTLs de résistance au FHB identifiés dans du matériel brésilien, seuls 2 QTLs présentaient des effets stables dans plusieurs environnements (Goddard et al., 2021). L'ensemble de ces verrous rend difficile le développement de cultivars élites résistant au FHB et nécessaires la recherche de sources de résistance alternatives et complémentaires aux QTLs, telles que les gènes de sensibilité.

- Place de la sensibilité dans le pathosystème blé-*F. graminearum*

Le fort impact du fond génétique sur l'efficacité des QTLs de résistance au FHB pose également la question de la présence de facteurs de sensibilité dans les génomes et de leurs potentiels impacts antagonistes sur le niveau de résistance conféré par les QTLs. Depuis deux décennies, de nombreuses études ont mis en évidence la présence de facteurs de sensibilité au FHB dans le génome du blé. Leur implication dans le devenir de l'interaction a été montrée pour la première fois en 2006 par l'analyse de 30 lignées ditélosomiques du cultivar 'Chinese Spring' par Ma et collaborateurs (Ma et al., 2006). En effet, six lignées ditélosomiques, arborant des délétions des chromosomes 7AS, 3BL, 6BL, 7BS, 4DS et 6DL, présentaient une forte réduction (jusqu'à 29%) de la proportion d'épilletés infectés par rapport à la lignée mère, suggérant ainsi la présence de facteurs de sensibilité sur certaines portions du génome dont l'élimination permet d'augmenter significativement le niveau de résistance au FHB (Ma et al., 2006). De la même manière, la délétion d'une portion du bras long du chromosome 3D du cultivar sensible 'Apogée' a permis de fortement réduire sa sensibilité au FHB, jusqu'à 59% par rapport à la lignée mère (Garvin et al., 2015). L'analyse de lignées ditélosomiques représente ainsi une méthode robuste pour identifier les portions du génome associées à la sensibilité du blé au FHB et a permis de mettre en avant l'importance des facteurs de sensibilité. A ce jour deux facteurs de sensibilité proposés par Ma et collaborateurs ont été validés (Ma et al., 2006). En effet, les facteurs de sensibilité identifiés sur les chromosomes 4DS et 7AS (Sf-Fhb-7AS) ont été réduits à des intervalles de 31.7Mb (Hales et al., 2020) et de 50 Mb (Chhabra et al., 2021) respectivement. Ces deux délétions permettent de réduire la propagation de *F. graminearum* dans l'épi (résistance de Type II) et ce de l'ordre de 50 à 60% dans le cas du facteur de sensibilité Sf-Fhb-7AS (Chhabra et al., 2021). Dans le cas de Sf-Fhb-7AS, l'évaluation des réponses au FHB de lignées de 'Chinese Spring', dont le chromosome 7A a été substitué par celui de cinq lignées de blé et d'une lignée de *Triticum dicoccoides*, a également montré un niveau de sensibilité similaire ou supérieur à 'Chinese Spring', indiquant ainsi que ce FS est conservé chez le blé mais aussi chez d'autres espèces. De plus, la délétion de ce FS n'entraîne pas d'effets morphologiques et phénologiques délétères. Pour toutes ces raisons, la délétion du facteur de sensibilité Sf-Fhb-7AS représente une alternative intéressante et efficace pour augmenter la résistance au FHB (Chhabra et al., 2021).

Depuis 2015, des gènes de sensibilité au FHB ont été clairement identifiés et validés (Nalam et al., 2015; Gordon et al., 2016; Hu et al., 2018; G. Li et al., 2019; Su et al., 2019; Brauer et al., 2020; Su et al., 2021). C'est par exemple le cas du gène TaLpx-1 codant pour une 9-lipoxygénase (9-LOX) dont l'inactivation s'accompagne d'une augmentation du niveau de résistance. L'inactivation chez *Arabidopsis thaliana* des gènes LOX1 et LOX5, homologues de TaLpx-1, engendre également une réduction de la sévérité de la maladie, indiquant ainsi la conservation des gènes LOXs en tant que gènes de sensibilité à *F. graminearum* (Nalam et al., 2015). Bien qu'encore discutée, la recherche des gènes sous-jacents au QTL Fhb1 illustre parfaitement l'intérêt de l'utilisation des gènes de sensibilité comme source de résistance alternative. Deux études publiées de manière indépendante en 2019 ont démontré que la mutation du gène TaHRC, codant pour une protéine nucléaire de liaison au calcium riche en histidine, est une des composantes de la résistance conférée par Fhb1 (G. Li et al., 2019; Su et al., 2019). En effet, l'allèle résistant du gène TaHRC présente une délétion naturelle au niveau du codon start par rapport à l'allèle sensible qui engendrerait une perte (Su et al., 2019) ou un gain (G. Li et al., 2019) de fonction responsable de la résistance au FHB. De plus, TaHRC est un gène conservé chez les plantes, ce qui suggère que ce gène est impliqué dans des processus biologiques fondamentaux. Bien que la fonction précise du gène TaHRC reste à être élucidée, son rôle dans l'interaction blé-*F. graminearum* est celui d'un FS (G. Li et al., 2019; Su et al., 2019) dont la mutation représente à ce jour le premier exemple d'application directe de ce type de gène en sélection variétale (T. Li et al., 2019).

Par conséquent, l'identification suivi de l'édition génétique des gènes de sensibilité représentent une alternative pertinente à l'introgession des QTLs dans le cas du pathosystème blé-*F. graminearum*.

II. La sensibilité, la prise de contrôle de l'hôte par l'agent pathogène

1. Effecteurs et facteurs de sensibilité, deux faces indissociables de la sensibilité

La capacité de l'agent pathogène à exploiter les ressources de son hôte nécessite de pirater ses processus biologiques tels que les mécanismes de défense, sa physiologie et ses processus métaboliques. La manipulation parasitaire est un des moteurs clefs de la sensibilité et implique la sécrétion d'effecteurs, principalement de nature protéique, dans les tissus de l'hôte. Ces effecteurs altèrent notamment la structure des cellules et ciblent des fonctions précises de l'hôte, les FS, et ce à un moment précis de l'infection. (O'Connell and Panstruga, 2006; Selin et al., 2016; Zeilinger et al., 2016; M. Khan et al., 2018; He et al., 2020; Jaswal et al., 2020). Ainsi leur rôle dans le processus infectieux est déterminé par le timing de leur sécrétion, leur localisation sous-cellulaire chez l'hôte, i.e. l'apoplasme ou les compartiments intracellulaires, mais surtout par les fonctions exercées par les FS ciblés (Brown and Hammond-Kosack, 2015; Lo Presti et al., 2015; Sperschneider et al., 2015; Arroyo-Velez et al., 2020; Jaswal et al., 2020). Ainsi, la compréhension précise de la fonction d'un effecteur nécessite l'identification de sa ou ses cibles chez l'hôte.

La sensibilité d'une plante est contrôlée par une large gamme de processus biologiques décrits pour la première fois de manière extensive par Van Schie et collaborateurs en 2014 (van Schie and Takken, 2014). Ces FS ont également fait l'objet de deux nouvelles revues en 2018 (Engelhardt et al., 2018) et 2021 (Garcia-Ruiz et al., 2021) qui viennent apporter de nouveaux éléments sur leur nature et leur fonction au sein des interactions plantes-pathogènes. Les FS peuvent être regroupés en trois grandes catégories : (1) les processus permettant la reconnaissance et la pénétration par l'agent pathogène, (2) la régulation des mécanismes de défense et (3) les processus permettant de subvenir aux besoins métaboliques de l'agent pathogène (van Schie and Takken, 2014; Engelhardt et al., 2018; Garcia-Ruiz et al., 2021).

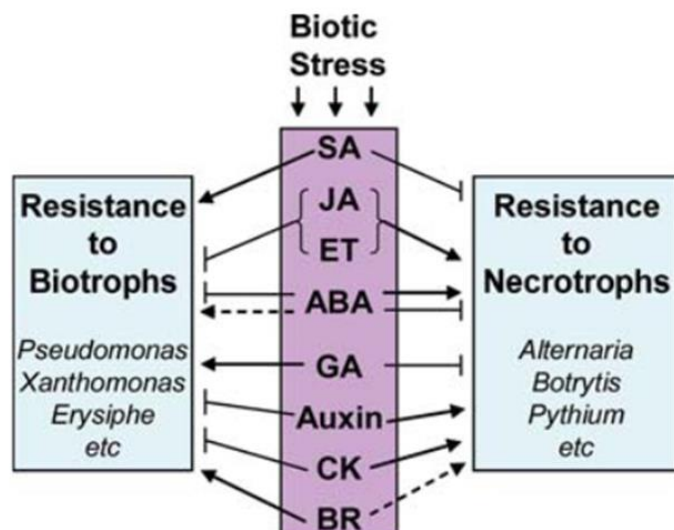
a. Reconnaissance et pénétration de l'hôte par l'agent pathogène

Lors de la rencontre entre les deux protagonistes, la capacité de l'agent pathogène à reconnaître un hôte permet la germination de ses spores et le développement des hyphes. Il s'agit ainsi de la première étape essentielle et déterminante pour le succès de l'infection. Ces signaux de l'hôte peuvent être de différentes natures. Ainsi la structure de la surface de contact entre les deux organismes, i.e. la topologie des stomates, la dureté et l'hydrophobicité de la surface, la composition de la cuticule, i.e. la cire et la cutine ainsi que la présence de certains métabolites secondaires de surface, i.e. composés volatiles et certains flavonoïdes, représentent des signaux de reconnaissance de l'hôte (Kou and Naqvi, 2016; Engelhardt et al., 2018). Par exemple, en réponse aux signaux d'hydrophobicité et de monomères de cutine, *Ustilago maidis* active un vaste programme génétique impliquant la synthèse d'enzymes de dégradation des parois (Cell Wall Degrading Enzymes, CWDEs) et d'effecteurs et ce même avant l'étape de pénétration (Lanver et al., 2014). De plus, l'absence ou la modification de ces signaux empêchent la mise en place d'une interaction entre la plante et l'agent pathogène et sont responsables de la résistance de type non hôte. Dans le cas du pathosystème orge-*B. graminis*, l'altération de la structure de la cire cuticulaire de l'orge résultant de la mutation des gènes HvKCS1 et HvKCS6 empêche la germination des spores de *B. graminis* et bloque ainsi la mise en place de l'infection (Li et al., 2018).

b. Régulation des mécanismes de défenses

Pour se défendre contre les agents pathogènes, les plantes disposent d'un système immunitaire complexe reposant sur la reconnaissance des signaux spécifiques de l'infection et l'activation de cascades de signalisation permettant la mise en place efficace des réponses de défenses. Leur mise en place détourne l'énergie et les nutriments alloués en condition optimale aux processus de développement des plantes. Les ressources étant limitées, ces deux processus ont des impacts, généralement antagonistes, directs ou indirects l'un sur l'autre conduisant à des compromis entre croissance et immunité. Ainsi, les plantes nécessitent une régulation fine et concertée de ces processus pour s'adapter à un environnement versatile.

(A)



(B)

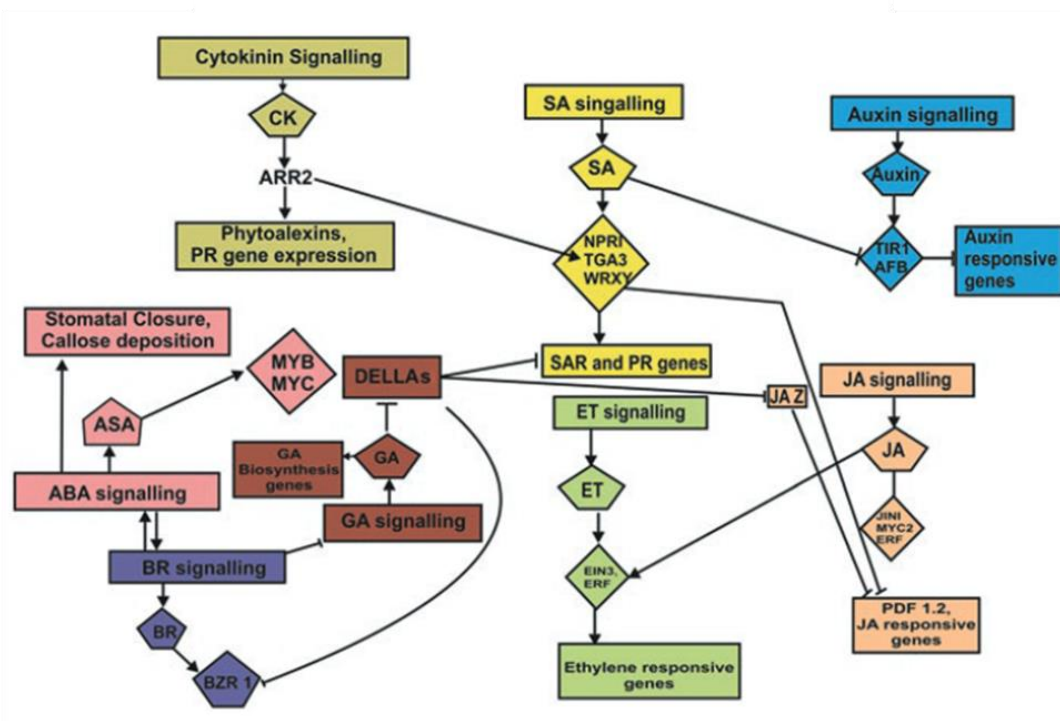


Figure 4. Modèle simplifié (A) du rôle des voies hormonales dans la tolérance des plantes aux stress biotiques selon la nature de l'interaction et (B) des interactions entre les différentes voies. Les flèches indiquent une régulation positive et les lignes bloquées une régulation négative. (Bari et al. Plant Mol. Biol., 2009 ; Checker et al., Molecular Aspects of Plant-Pathogen Interaction, 2018)

Cette régulation est notamment permise par une interconnexion étroite des voies de signalisation du système immunitaire et du développement des plantes qui partagent de nombreux déterminants moléculaires, tels que les protéines kinases, les voies hormonales et les facteurs de transcription (Tsuda and Somssich, 2015; Couto and Zipfel, 2016; Ning et al., 2017). Les processus de régulation des plantes s'organisent en de vastes réseaux dont la complexité peut être illustrée par le rôle des voies hormonales dans les processus immunitaires. Les voies hormonales jouent un rôle pivot dans la régulation des différents processus de la plante dont l'immunité et interagissent de manière synergique ou antagoniste entre elles (*Figure 4*). Classiquement, les voies hormonales associées à la régulation des processus immunitaires sont les voies de l'acide salicylique (SA), du jasmonate (JA) et de l'éthylène (ET) dont les rôles dépendent du mode trophique de l'agent pathogène. De manière générale, la voie du SA est impliquée dans la mise en place des défenses contre les agents pathogènes biotrophes et hémibiotrophes tandis que les voies du JA et de l'ET sont impliquées dans la résistance contre les agents pathogènes nécrotrophes. Les voies du JA et de l'ET étant antagonistes de celle du SA et vice versa, l'équilibre du dialogue hormonal impacte fortement le devenir des interactions avec les agents pathogènes (Bari and Jones, 2009; Glazebrook, 2005; De Vleeschauwer et al., 2014). Cependant, les autres phytohormones, l'auxine (IAA), les cytokinines (CKs), les brassinostéroïdes (BRs), l'acide abscissique (ABA) et les gibbérelines (GAs), jouent également un rôle dans la régulation des processus immunitaires en agissant principalement comme des régulateurs des voies du SA, JA et ET mais aussi en agissant sur certains processus de défenses des plantes (Robert-Seilaniantz et al., 2011; De Vleeschauwer et al., 2014).

L'ensemble des mécanismes de régulation des processus de l'immunité, qu'ils soient négatifs ou positifs, constituent ainsi des cibles importantes des champignons pathogènes pour inhiber la mise en place de la mort cellulaire dans le cas des agents biotrophes ou la déclencher dans le cas des agents nécrotrophes (Engelhardt et al., 2018; M. Khan et al., 2018; He et al., 2020). En effet, la compilation des informations concernant les cibles des effecteurs fongiques dans la littérature a mis en évidence que les facteurs de sensibilité associés aux processus de l'immunité représentent 81% de leurs cibles (M. Khan et al., 2018). C'est par exemple le cas des kinases BAK1 et BIK1, associées aux récepteurs PRRs et impliquées dans la

transduction du signal après reconnaissance des IPs, ciblées par NIS1, un effecteur conservé au sein des champignons pathogènes ascomycètes et basidiomycètes, pour supprimer la mort cellulaire (Irieda et al., 2019). *Verticillium dahliae*, quant à lui sécrète l'effecteur VdSCP412 qui cible deux facteurs de transcription d'*A. thaliana*, CBP60g and SARD1, pour inhiber leurs fonctions d'activateurs du gène ICS1 impliqué dans la synthèse du SA et ainsi supprimer la mise en place des mécanismes de défense (Sun et al., 2015; Qin et al., 2018). Dans le cas des organismes hémibiotrophes et nécrotrophes, les CWDEs jouent, en plus de leur activité enzymatique, un rôle dans la manipulation de l'immunité. C'est le cas des glycoside hydrolases 12 (GH12), VdEG1 et VdEG3 sécrétées par *V. dahliae* et de la GH12 FoEG1 sécrétée par *Fusarium oxysporum* qui, de manière indépendante à leur activité enzymatique, induisent la mort cellulaire et participent au succès de l'infection (Zhang et al., 2021; Gui et al., 2017).

Enfin, certains effecteurs ne jouent pas de rôle dans la régulation des mécanismes de défense mais permettent de masquer la présence de l'agent pathogène ou de le protéger contre les réponses de défense de l'hôte, dont ses enzymes et métabolites de défense (Toruño et al., 2016).

c. Assurer les besoins métaboliques de l'agent pathogène

Plus particulièrement dans le cas des agents pathogènes biotrophes, la structure des cellules et des tissus de l'hôte ainsi que ses voies métaboliques sont manipulées pour permettre la mise en place des structures d'absorption et le détournement des ressources de la plante (Lapin and Van den Ackerveken, 2013; van Schie and Takken, 2014; Engelhardt et al., 2018). Lors de l'infection de l'orge par *B. graminis*, l'interaction entre les protéines codées par les gènes RACB et RIPb, impliquée dans les processus de polarisation cellulaire, est favorisée au niveau du site d'infection et participe activement à la mise en place de l'haustorium du champignon (Scheler et al., 2016; McCollum et al., 2020). Dans le cas du pathosystème maïs-*Ustilago maidis*, les tumeurs formées au cours de l'infection représentent d'importants puits de carbone qui détournent les sucres destinés au remplissage du grain. Pour accéder aux sucres c'est-à-dire les rendre disponibles dans l'apoplasme des tissus infectés, *U. maidis* reprogramme les différents transporteurs de sucre (Sosso et al., 2019).

En effet, les gènes codant pour les transporteurs SWEETs, impliqués dans l'efflux des sucres vers l'apoplaste (Chen et al., 2012), sont sur-exprimés tandis que les gènes codant pour les transporteurs SUT et STP (Slewinski, 2011; Hu et al., 2021), impliqués dans l'influx des sucres dans les cellules, sont sous-exprimés au cours de l'infection (Sosso et al., 2019). Cette reprogrammation du transport des sucres permet de créer un environnement riche en sucres à l'interface biotrophe entre le maïs et *U. maidis*, ce dernier disposant de ses propres transporteurs pour acquérir la ressource (Wahl et al., 2010). Cependant, contrairement aux agents pathogènes bactériens, dont des effecteurs TALEs ciblant les gènes SWEETs ont été validés (Gupta et al., 2021), aucun effecteur fongique impliqué dans la reprogrammation des transporteurs de sucre ou du métabolisme primaire n'a été identifié à ce jour.

2. La manipulation parasitaire du blé par *F. graminearum* au cours du FHB est caractéristique des agents pathogènes hémibiotrophes

Lors de l'infection du blé, *F. graminearum* forme, à partir des hyphes de croissance épiphytque, des structures complexes semblables aux apressoria, les « infection cushions » (ICs) qui permettent la pénétration de la cuticule de la plante en de multiples sites (Boenisch and Schäfer, 2011; Bormann et al., 2014). Les ICs sont notamment le siège de la synthèse de nombreux facteurs de virulence validés et prédits comme facilitant l'infection de l'hôte dont de nombreux effecteurs (Mentges et al., 2020). Le mode de vie hémibiotrophe de *F. graminearum* suggère l'existence d'un large panel de fonctions remplies par ses effecteurs et implique de les replacer dans le contexte temporel de leur sécrétion, c'est-à-dire sécrétion lors de la phase biotrophe, de la phase nécrotrophe ou encore lors de la transition entre les deux phases.

a. Phase biotrophe : la mise en place de l'infection

Le succès de l'infection est en premier lieu déterminé par la capacité des spores de *F. graminearum* à adhérer à la surface des épillets et à la capacité des hyphes de croissance épiphytiques à pénétrer les tissus. Ces deux étapes sont en parties déterminées par deux hydrophobines, Fghyd2 et Fghyd3, sécrétées par *F. graminearum* au début de l'infection et qui sont responsables à la fois de l'adhésion des conidies sur les surface des épillets et de la pénétration de l'hyphe à travers les interfaces eau-air (Quarantin et al., 2019b).

La sécrétion de deux arabinases, Arb93A et Arb93B, semblent également associées à la capacité de pénétration de *F. graminearum*. En effet, de par leur forte expression au cours des stades précoces de l'infection (pic d'expression à 1 jour après inoculation pour Arb93A) et de par leur fonction de dégradation de l'arabinan, un polysaccharide impliqué dans la flexibilité des parois cellulaires et essentiel au bon fonctionnement des cellules de gardes des stomates, ces deux arabinases pourraient jouer un rôle important dans la pénétration des hyphes par les stomates (Hao et al., 2019). De plus, le succès de l'infection de l'épi du blé par *F. graminearum* est dépendante de réponses chémotropiques associées à la détection de peroxydases sécrétées par l'hôte grâce au récepteur senseur des phéromones couplé à la protéine G, Ste2. Cette chémoperception semble être le résultat d'une véritable stratégie de manipulation de l'hôte par *F. graminearum*. En effet, la sécrétion de CWDEs par *F. graminearum* lors du contact avec son hôte stimulerait la sécrétion des peroxydases par le blé pour renforcer la paroi au niveau du site d'infection (Sridhar et al., 2020).

Outre ce rôle supposé dans la pénétration, l'expression transitoire d'Arb93B dans les feuilles de *Nicotiana benthamiana* est responsable de la suppression de la mort cellulaire induite par la protéine Bax mais aussi par la reconnaissance des IPs conservés, la chitine et flagelline 22, se manifestant par une réduction de la production de ROS. Ainsi, Arb93B intervient également dans la suppression des mécanismes de défense, un processus caractéristique de la phase biotrophe et nécessaire au succès de l'infection qui permet au champignon de s'installer durablement dans son hôte (Hao et al., 2019). D'autres effecteurs de *F. graminearum* impliqués dans la suppression des défenses de l'hôte et ce avec des modes d'action très diversifiés ont par ailleurs été validés. De la même manière que l'arabinase Arb93B, de nombreux effecteurs interviennent dans l'inhibition des mécanismes de défense et de mort cellulaire. C'est notamment le cas de trois effecteurs, FGSG_01831, FGSG_03599 et FGSG_12160, qui limitent l'accumulation de ROS induite par la reconnaissance de la chitine. Deux d'entre eux, FGSG_03599 et FGSG_12160, sont également associés à la suppression de la mort cellulaire induite par la protéine Bax (Hao et al., 2020). La protéine orpheline OSP24, spécifiquement sécrétée dans le cytoplasme des cellules du blé au cours de l'infection, joue un rôle clef dans la croissance des hyphes au niveau du rachis. OSP24 agit comme compétiteur de la protéine du blé TaFROG du blé pour la liaison avec la kinase TaSnRK1 α .

La liaison entre OSP24 et TaSnRK1 α entraîne une accélération de la dégradation de la kinase par ubiquitination et permet l'inhibition de la mort cellulaire induite par la protéine Bax ou l'éliciteur INF1 (Jiang et al., 2020). L'interaction entre une « fungalysin metallopeptidase » sécrétée, FgFly1, et le facteur de transcription (TF) TaCAMTA (calmodulin-binding transcription activator) du blé a également été validée. La délétion du TF CAMTA chez *A. thaliana* est associée à une augmentation de l'expression des gènes de défense NPR1 et permet d'augmenter la résistance au FHB. Ainsi, TaCAMTA est un régulateur négatif des défenses de la plante dont l'expression est manipulée par FgFly1 pour supprimer la mise en place des défenses (Wang et al., 2022). De plus, de nombreux FS du blé au FHB sont associés à la régulation des voies de signalisation hormonale et ont un impact direct sur l'inhibition des mécanismes de défense (Nalam et al., 2015; Gordon et al., 2016; Hu et al., 2018; Su et al., 2021). *F. graminearum* semble notamment manipuler l'équilibre des voies hormonales au détriment de celle du SA inductrice des réponses de défense. Chez *A. thaliana*, les gènes LOX1 et LOX5, codant pour des 9-LOXs et dont l'inactivation et l'inactivation de leur homologue TaLpx-1 chez le blé augmente la résistance au FHB, sont fortement exprimés en réponse à *F. graminearum*. L'inactivation de ces gènes conduit à une augmentation de la synthèse de SA et à une réduction de celle du JA. Ainsi, *F. graminearum* semble manipuler l'équilibre des voies hormonales antagonistes du SA et du JA pour inhiber la mise en place des réponses de défense (Nalam et al., 2015). De plus, le gène TaSSI2, facilite l'infection du blé par *F. graminearum* et *B. graminis* par l'inhibition de la voie du SA et des gènes de réponses en aval TaPR4 et TaPR5. L'expression de TaSSI2 étant négativement régulée par le méthyl-jasmonate (dérivé du JA) et positivement régulée par le SA et l'ABA, il serait impliqué dans le dialogue entre les différentes voies hormonales. Ce gène joue également un rôle dans le transport extracellulaire des lipides et participe à la tolérance des plantes au stress hydrique (Hu et al., 2018). L'inactivation d'un récepteur de l'ABA Ta_PYL4AS_A (Gordon et al., 2016), et d'un récepteur de l'auxine TaTIR1 (Su et al., 2021), limitant la progression du champignon et la synthèse de DON, les voies de l'ABA et de l'IAA semblent également favoriser la mise en place de l'infection du blé par *F. graminearum*. Cependant la manière dont les voies de l'IAA et de l'ABA facilitent l'infection reste encore à élucider (De Vleeschauwer et al., 2014).

Enfin, d'autres effecteurs exercent quant à eux un rôle de protection du champignon contre différents mécanismes de défenses de la plante. C'est notamment le cas des ceratoplatanines FgCPP1 et FgCPP2 (Quarantin et al., 2016) et de la protéine associée à la pathogénicité de groupe 1 FgPR-1L-4 (Lu and Edwards, 2018) dont les fonctions respectives semblent associées à la protection des hyphes de *F. graminearum* contre les chitinases et certaines protéases sécrétées par le blé en réponse à l'infection. *F. graminearum* sécrète également une enzyme de type tomatinase, FgTom1, dont le rôle supposé est de dégrader des métabolites spécialisés antifongiques de la classe des saponines similaires à la tomatine (Carere et al., 2017).

b. Phase biotrophe : l'acquisition de nutriments

Bien qu'à ce jour aucun effecteur impliqué dans le détournement des ressources de l'hôte (eau et nutriments) n'ait été validé chez *F. graminearum*, l'analyse des données de réponse de l'hôte suggèrent leur existence. En effet, la synthèse d'amidon semble altérée au cours de l'infection au contraire de la glycolyse qui est quant à elle activée, ce qui indiquerait une manipulation du métabolisme primaire de l'hôte et des transporteurs des sucres (Chetouhi et al., 2016; Fabre, 2020).

c. Transition entre les phases biotrophe et nécrotrophe: la propagation de *F. graminearum* dans l'épi

Lors de la transition entre les phases biotrophe et nécrotrophe, *F. graminearum* sécrète de nombreuses CWDEs permettant la propagation du champignon dans l'épi (Voigt et al., 2005; Pollet et al., 2009; Paccanaro et al., 2017; Quarantin et al., 2019a). Outre l'impact direct sur la dégradation de la paroi de la plante, certaines de ces CWDEs jouent également un rôle dans l'activation de la mort cellulaire, illustrant leur importance dans la transition vers la nécrotrophie. En effet, la lipase Fgl1, dont l'activité de dégradation de la paroi est fortement induite au cours de l'infection, exerce également d'autres fonctions requises pour le passage de *F. graminearum* d'un épillet à un autre (Voigt et al., 2005; Niu et al., 2013; Blümke et al., 2014). Dans un premier temps, la libération d'acides gras libres dans l'apoplasme suite à l'activité lipolytique de Fgl1 entraîne une inhibition des dépôts de callose au niveau des faisceaux vasculaires des épillets infectés, permettant ainsi à *F. graminearum* de pouvoir se

propager dans l'épi (Blümke et al., 2014). Fgl1 est également capable d'interagir directement avec une protéine immunophiline du blé, FKBP12. Cette interaction altère la formation du complexe entre FKBP12 et une protéine de réponse aux éliciteurs ERG possédant un domaine de fixation du Ca^{2+} , causant l'instabilité ou la sous expression d'ERG et une potentielle altération de la signalisation calcique. Dans le tabac, l'expression transitoire de Fgl1 induit la mort cellulaire. Ainsi, avec son rôle dans la manipulation de la signalisation calcique de l'hôte, Fgl1 semble directement impliqué dans le passage vers la nécrotrophie au sein de l'épillet infecté (Niu et al., 2013). *F. graminearum* dispose également d'un arsenal de xylanases sécrétées permettant la dégradation de l'hemicellulose, un composé essentiel de la structure de la paroi de l'hôte (Pollet et al., 2009). De plus, certaines xylanases sont également des facteurs d'induction des réponses hypersensitives de l'hôte, favorisant ainsi le passage vers la nécrotrophie (Sella et al., 2013; Tundo et al., 2015).

L'ensemble des effecteurs et FS validés au sein du système blé-*F. graminearum* illustre la complexité de l'interaction avec le blé ainsi que l'importance de la manipulation des mécanismes de défense dans l'établissement et le succès de l'infection. L'étude des déterminants moléculaires de l'interaction a fait l'objet d'une revue écrite en co-premier auteur et publiée dans *Frontiers in Plant Science* (Fabre et al., 2020).

Cependant, au regard du pan secretome de l'espèce composé de 893 protéines (Alouane et al., 2021), les effecteurs validés actuellement ne représentent qu'une infime proportion des effecteurs putatifs et ne couvrent ainsi qu'une partie des processus impliqués dans la sensibilité du blé à *F. graminearum*. La validation des effecteurs et de leurs cibles dans l'hôte est un processus long et complexe, limité par la capacité à obtenir des mutants du champignon et par la redondance des fonctions exercées par les effecteurs entraînant l'absence de phénotype associé au mutant. Malgré le développement de nombreuses méthodes haut débit telles que l'utilisation de systèmes hétérologues, l'inactivation de gène induite par l'hôte (Host-induced gene silencing, HIGS), la co-immunoprécipitation ou encore les techniques levure double hybride (Varden et al., 2017), il reste nécessaire de réduire le nombre d'effecteurs candidats prédits à l'échelle génomique et de les hiérarchiser.

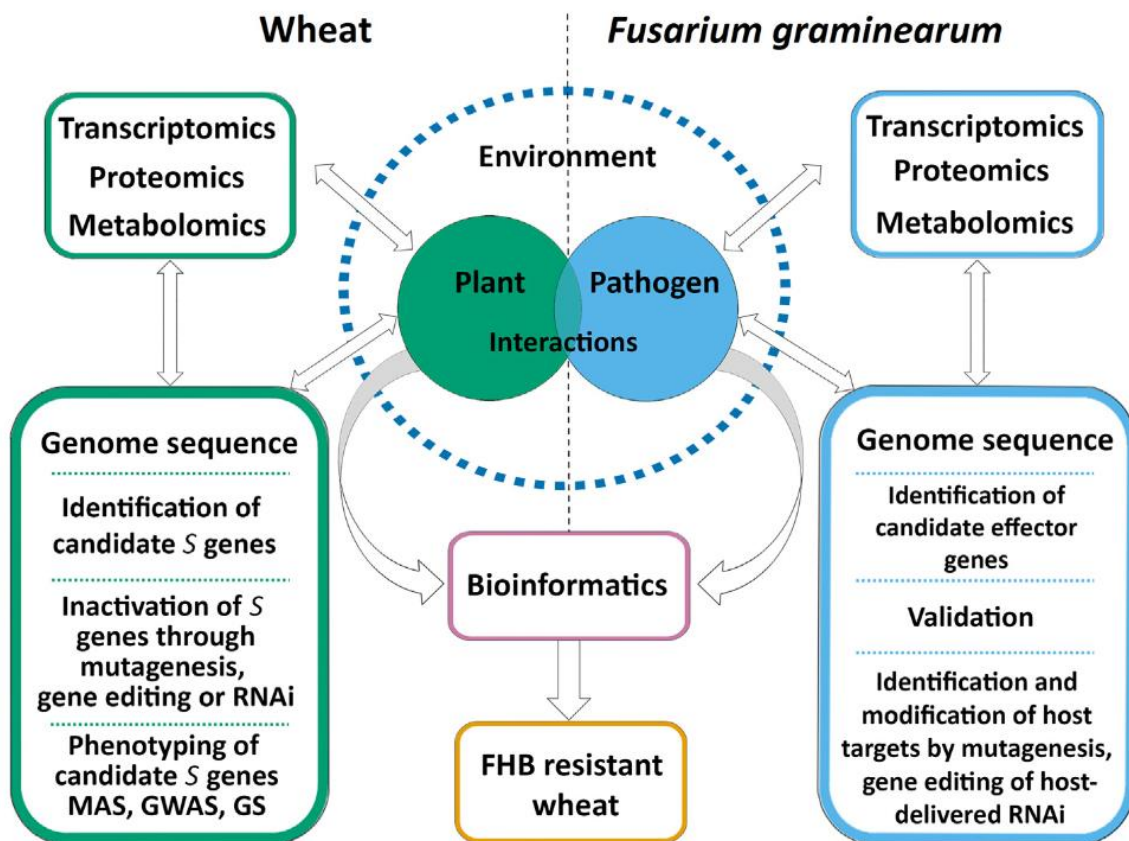


Figure 5. Méthodologie permettant l'identification des acteurs clefs du dialogue moléculaire gouvernant la sensibilité du blé à *F. graminearum*, représentant des ressources d'intérêt pour améliorer la résistance du blé au FHB. (Gorash et al., *Crop J.*, 2021)

III. L'analyse du dialogue moléculaire, un levier pour identifier les acteurs clefs de l'interaction

L'utilisation de méthodes exploratoires *in planta*, transcriptomiques et protéomiques, sont particulièrement utiles pour déchiffrer le dialogue moléculaire mis en place au cours de l'interaction et caractériser en parallèle l'effectome effectif de l'agent pathogène et les réponses de l'hôte (*Figure 5*) (Beckerson et al., 2019; Lorrain et al., 2019; Jaswal et al., 2020; Depotter and Doehlemann, 2020; Gorash et al., 2021). L'identification de l'effectome effectif de *F. graminearum* dans l'épi du blé est d'autant plus importante qu'il s'agit d'un agent pathogène généraliste susceptible d'adapter en partie sa stratégie d'infection à l'espèce ou au tissu infecté (Thordal-Christensen et al., 2018; Petre et al., 2020).

De plus, ces méthodes permettent de hiérarchiser les effecteurs en fonction différents critères tels que l'intensité ou le timing d'expression (Depotter and Doehlemann, 2020). Enfin, comme vu précédemment les réponses des plantes aux stress biotiques reposent sur des réseaux de régulation particulièrement complexes interconnectant l'ensemble des processus biologiques de la plante nécessitant ainsi une vision d'ensemble des fonctions impactées lors de l'infection, vision permise par les analyses exploratoires *in planta*.

1. Déterminer l'importance d'un effecteur dans le devenir de l'interaction

a. Limites de l'identification *in silico* d'effecteurs fongique

Bien que nécessaires et très utiles pour identifier le catalogue d'effecteurs putatifs des champignons pathogènes, les méthodes d'identification *in silico* font face à de nombreuses limitations. Contrairement aux effecteurs des bactéries gram négatives et des oomycètes qui possèdent des structures spécifiques, les signaux de sécrétion de type III (Deng et al., 2017) et les motifs RXLR ou LxLFLAK (McGowan and Fitzpatrick, 2020) respectivement, les effecteurs fongiques ne présentent pas de similarité de séquence ou de motifs structuraux conservés (Sperschneider et al., 2015). Aujourd'hui les méthodes de prédiction sont basées sur plusieurs critères dont la présence d'un peptide signal, l'absence de domaines transmembranaires, la charge nette, et le contenu en cystéine, serine et tryptophane (Sperschneider et al., 2016).

Cependant, ces critères sont restrictifs. Certains effecteurs ne possèdent pas de signaux de sécrétion dits conventionnels et des différences en terme de taille et de contenu en cystéine sont observées entre effecteurs apoplasmiques et effecteurs intracellulaires (Sperschneider et al., 2015, 2018; Jaswal et al., 2020; Sperschneider and Dodds, 2022). C'est par exemple le cas de l'effecteur Vdls1 de *V. dahliae* qui supprime l'immunité induite par le SA mais qui ne présente pas de peptide signal (T. Liu et al., 2014). De plus, des effecteurs bactériens, exerçant leur fonction au niveau des membranes de la cellule hôte, possèdent des domaines transmembranaires, ce critère de sélection doit donc être utilisé avec précaution et pourrait exclure des effecteurs fongiques importants pour le devenir de l'interaction (Weigle et al., 2017; Depotter and Doehlemann, 2020). Ainsi seuls deux critères sont universels pour les effecteurs fongiques, d'une part la sécrétion de manière conventionnelle ou non et d'autre part la fine régulation de leur synthèse au cours de l'infection. Cela rend ainsi nécessaire de combiner les approches de prédiction *in silico* avec des analyses *in planta* exploratoires (Sperschneider et al., 2015; Gervais et al., 2017; Uhse and Djamei, 2018; Sperschneider and Dodds, 2022).

b. Le rôle d'un effecteur est déterminé par la chronologie de sa synthèse

Comme vu précédemment, un effecteur exerce son activité à un moment précis de l'interaction (Giraldo and Valent, 2013). L'analyse *in planta* de l'interaction sur une cinétique d'infection représente ainsi un levier important pour l'identification des effecteurs et permet de les replacer au sein de leur contexte biologique (Kleemann et al., 2012; Gervais et al., 2017; Lanver et al., 2018). L'analyse du transcriptome de *Leptosphaeria maculans* a mis en évidence cette synthèse par vague des effecteurs du champignon aux différents stades de l'infection de *Brassica napus* avec des effecteurs précoces fortement exprimés lors de l'infection des cotylédons et des effecteurs tardifs, sous exprimés au début de l'infection, sur-exprimés lors de la colonisation systémique de la tige (Gervais et al., 2017).

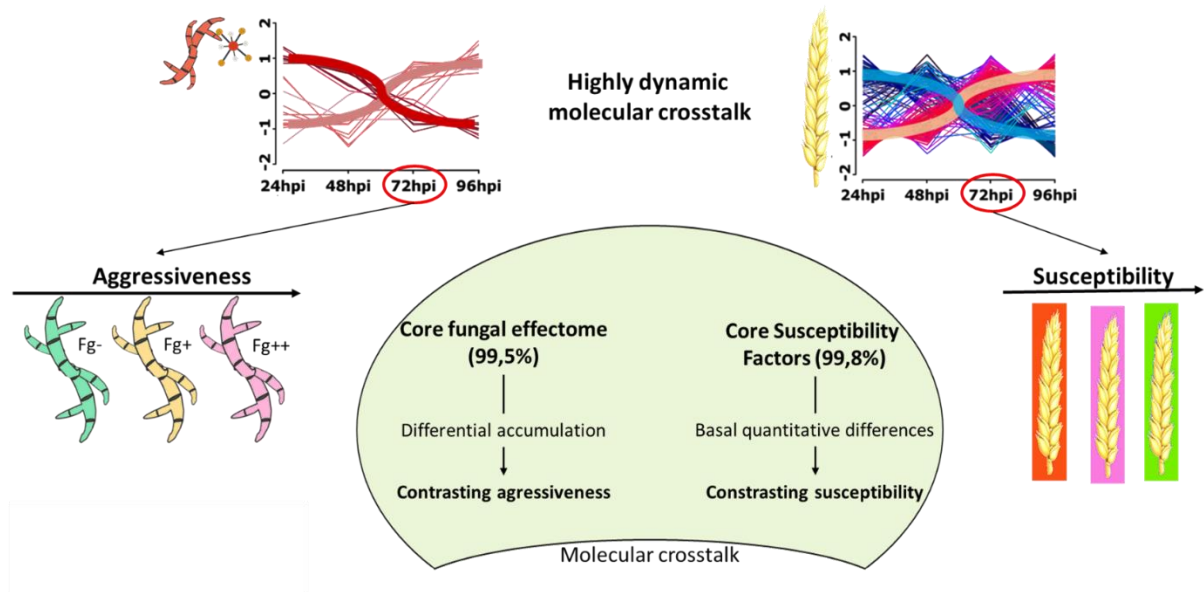


Figure 6. Représentation simplifiée de la dynamique et de la structure du dialogue moléculaire établi au cours de l'interaction entre le blé et *F. graminearum*. La nature dynamique du dialogue moléculaire se traduit par la synthèse de manière séquentielle des effecteurs associée à un ajustement coordonné des réponses du blé de manière. Ce dialogue moléculaire se structure autour de déterminants très conservés quel que soit la souche et son niveau d'agressivité et quel que soit le cultivar de blé et son niveau de sensibilité, les différences d'agressivité et de sensibilité s'expliquant par des différences d'accumulation des protéines. Cette figure a été construite à partir des travaux conduits par Francis Fabre à l'échelle du dual-protéome. (Fabre et al., *Plant Cell Environ.*, 2019b ; Fabre et al., *Front. Microbiol.*, 2019a ; Fabre et al., *Front. Plant. Sci.*, 2021

Dans le cadre du FHB, deux études *in planta* ont démontré la nature dynamique de l'effectome putatif de *F. graminearum* (Brown et al., 2017; Fabre et al., 2019b). Lors de l'analyse du transcriptome de *F. graminearum* lors des phases asymptomatiques et symptomatiques, Brown et collaborateurs, ont identifié 40 gènes codant pour des effecteurs putatifs dont l'expression est régulée de manière très précise au cours de l'infection, avec 18 gènes fortement induits dans les tissus asymptomatiques et 22 dans les tissus symptomatiques (Brown et al., 2017). De plus l'analyse protéomique des stades précoces de la maladie (24, 48, 72 et 96 heures post infection, hpi) par Fabre et collaborateurs, a mis en évidence une synthèse par vague de 63 effecteurs putatifs de *F. graminearum*, 25 étant fortement synthétisés dès le premier point de cinétique. Cette synthèse séquentielle des effecteurs est également marquée par une importante phase de transition entre 48hpi et 72hpi, correspondant au début de la cellularisation du grain (Figure 6). De plus, au cours de la cinétique d'infection, les ajustements d'une partie du protéome du blé se font de manière concomitante aux variations de l'abondance des effecteurs démontrant ainsi l'importance de la chronologie dans le processus de manipulation parasitaire (Fabre et al., 2019b). Ces deux études illustrent ainsi la fine régulation de la synthèse des effecteurs de *F. graminearum* et le rôle précis joué par ces derniers dans la manipulation de l'hôte et l'adaptation au contexte moléculaire de l'hôte.

c. L'importance d'un effecteur est déterminée par sa conservation

Au-delà de la chronologie de sécrétion, le rôle d'un effecteur au sein du dialogue moléculaire dépend en grande partie de sa conservation entre les espèces et entre les souches d'une même espèce (Depotter and Doehlemann, 2020). De plus, puisque l'environnement des agents pathogènes correspond aux structures et molécules de leurs hôtes, la conservation des effecteurs est également en partie dépendante de la gamme d'hôte de l'agent pathogène ainsi que des processus de coévolution avec ses hôtes et cibles *in planta* (Arroyo-Velez et al., 2020; Petre et al., 2020). Ainsi, les effecteurs conservés ciblent des processus conservés chez les plantes et sont indispensables au succès de l'infection tandis que les effecteurs spécifiques sont impliqués dans l'adaptation à un hôte particulier ou sont associés à l'agressivité des souches (Arroyo-Velez et al., 2020; Depotter and Doehlemann, 2020; Petre et al., 2020).

C'est par exemple le cas de Pep1, un effecteur conservé chez les champignons pathogènes de la famille des *Ustilaginaceae* qui infectent des plantes monocotylédones ou des dicotylédones et dont le rôle d'inhibition des peroxydases apoplasmiques de la plante pour supprimer l'accumulation des ROS est indispensable au succès de l'infection (Hemetsberger et al., 2015). La diversité des deux partenaires de l'interaction doit donc être prise en compte pour déchiffrer la complexité et la diversité des composantes moléculaires qui déterminent la sensibilité et l'agressivité. Cette prise en compte de la diversité permet également d'identifier de manière pertinente l'effectome d'une espèce et de hiérarchiser les effecteurs en fonction leur importance dans le dialogue moléculaire.

Bien que de nombreuses données concernant la variabilité génomique au sein des espèces de champignons pathogènes s'accumulent notamment par l'intermédiaire de la construction de pangénomes (Badet and Croll, 2020; Alouane et al., 2021), les analyses *in planta* de l'impact de cette diversité restent relativement rares mais améliorent notre compréhension des liens entre stratégie d'infection et degré de spécialisation des agents pathogènes (Bruce et al., 2014; Palma-Guerrero et al., 2016; Rutter et al., 2017; Beckerson et al., 2019; Haueisen et al., 2019). Il en va de même pour l'analyse *in planta* des effecteurs sécrétés dans différents hôtes ou cultivars d'une même espèce (Plett et al., 2014; Poloni and Schirawski, 2016; Lorrain et al., 2018; Kamel et al., 2017). En effet, l'analyse du sécrétome de *Melampsora larici-populina* exprimé dans les deux hôtes nécessaires pour compléter son cycle biologique, le peuplier et le mélèze, a permis d'identifier un large corpus d'effecteurs conservés chez les deux hôtes ainsi que les effecteurs potentiellement impliqués dans l'adaptation aux spécificités de l'espèce hôte (Lorrain et al., 2018).

Dans le cadre du pathosystème blé-*F. graminearum*, l'analyse génomique de 8 souches d'agressivité contrastée a mis en évidence l'existence d'un pangéome dit ouvert avec 45% de gènes appartenant au « core génome », c'est-à-dire partagés par l'ensemble des souches. Concernant le pan-sécrétome, composé de 893 protéines, 53% d'entre elles sont conservées chez l'ensemble des 8 souches. Malgré d'importantes différences de symptômes causés par ces 8 souches, aucun lien clair n'a pu être établi entre l'agressivité des souches et leur contenu en protéines sécrétées (Alouane et al., 2021), illustrant ainsi la nécessité de caractériser l'impact de la diversité génomique *in planta*.

Cependant, l'impact de la diversité génétique de du blé et de *F. graminearum* sur la stratégie d'infection mise en place n'a été qu'effleurée. L'étude du transcriptome d'une souche de *F. graminearum* infectant le blé, l'orge et le maïs a mis en évidence l'existence d'un « core transcriptome » représentant seulement 44% de l'ensemble des gènes exprimés et l'expression hôte-spécifique d'un grand nombre de gènes en particulier chez le blé (1650 gènes), indiquant ainsi une adaptation de sa stratégie d'infection à l'espèce hôte (Harris et al., 2016). L'infection de quatre génotypes de blé de sensibilité contrastée par une souche de *F. graminearum* a permis de montrer que très peu de gènes sont exprimés de manière cultivar-spécifique indiquant que la stratégie d'infection ne semble pas dépendante du fond génétique du blé. De plus, malgré des profils d'expression globalement homogènes chez l'ensemble des cultivars au cours du temps, les gènes sont plus fortement exprimés lors de l'infection du cultivar le plus sensible (Pan et al., 2018). Cependant, dans ces deux études, la question de la diversité de l'effectome mis en place n'a pas été posée. L'impact de la diversité de *F. graminearum* et du blé sur l'effectome n'a été adressé clairement qu'à une seule reprise en 2019 via l'analyse du protéome *in planta* de trois souches d'agressivité contrastée infectant trois cultivars de blé de sensibilité contrastée (Fabre et al., 2019a). Avec 99.5% des effecteurs putatifs exprimés dans l'ensemble des combinaisons hôte-souche, l'infection du blé par *F. graminearum* repose sur une stratégie d'infection largement conservée et le corpus d'effecteurs mis en place ne semble pas déterminer l'agressivité des souches ou la sensibilité des cultivars (Figure 6). De plus, l'absence d'effets spécifiques des cultivars sur l'abondance des effecteurs des trois souches renforce l'idée d'une stratégie d'infection conservée. L'analyse de l'abondance des effecteurs putatifs a cependant permis de mettre en évidence une corrélation très forte entre l'intensité des symptômes induite par les souches et la quantité des effecteurs synthétisés par ces dernières, la souche la plus agressive présentant des niveaux d'abondance bien supérieurs aux deux autres (Fabre et al., 2019a). Ainsi, l'ensemble des trois études réalisées *in planta* a permis de montrer que *F. graminearum* semble globalement adapter sa stratégie d'infection en fonction de l'espèce infectée mais qu'au sein d'une même espèce hôte sa stratégie d'infection est fortement conservée et ne dépend pas du fond génétique utilisé (Harris et al., 2016; Pan et al., 2018; Fabre et al., 2019a).

2. Identifier et déterminer l'importance des facteurs de sensibilité dans le dialogue moléculaire

a. La sensibilité du blé semble reposer sur un socle de réponses communes

De par la nature strictement quantitative et l'efficacité relativement faible des QTLs de résistance, l'infection du blé par *F. graminearum* se produit quel que soit le niveau de résistance du cultivar infecté (G. Bai et al., 2018; T. Li et al., 2019). Cela suppose qu'il existe chez le blé un corpus de FS partagés. Cette hypothèse est notamment renforcée par la forte conservation de la stratégie d'infection de *F. graminearum* quel que soit le cultivar de blé infecté (Fabre et al., 2019a). La conservation des réponses à *F. graminearum* de différents cultivars de blé de sensibilité contrastée a été mise en évidence une première fois à l'échelle transcriptomique par Wang et collaborateurs en 2018 (Wang et al., 2018). En effet, malgré des différences de niveau d'expression entre cultivars, les mêmes gènes différentiellement exprimés (DEGs) en réponse au FHB ont été identifiés chez les quatre cultivars utilisés dans cette étude, 'Sumai 3' et trois cultivars canadiens (Wang et al., 2018). En 2021, les travaux de Fabre et collaborateurs réalisés à l'échelle du protéome ont montré la forte conservation des réponses de trois cultivars de blé de sensibilité contrastée à trois souches de *F. graminearum* d'agressivité contrastée (Fabre et al., 2021). En effet, la quasi-totalité des protéines détectées sont communes aux trois cultivars (sauf 6 protéines) et 99.8% de l'ensemble des protéines ont été détectées dans l'ensemble des échantillons infectés, illustrant l'existence d'un « core-protéome » en réponse à *F. graminearum* (Figure 6). De plus, 95% des réponses à l'infection ne présentent pas de spécificités associées au cultivar et aucun lien clair n'a pu être établi entre la sensibilité des cultivars et les réponses cultivar-spécifiques. Ceci illustre ainsi une forte généralité des réponses du blé au FHB. Cependant, 25% des réponses au FHB sont également associées à des différences basales entre cultivars, c'est-à-dire des différences observées entre les cultivars en conditions optimales qui sont conservées lors de l'infection. Les différences de sensibilité observée entre les cultivars pourraient donc être expliquées par leurs caractéristiques intrinsèques plutôt que par des réponses spécifiques (Fabre et al., 2021).

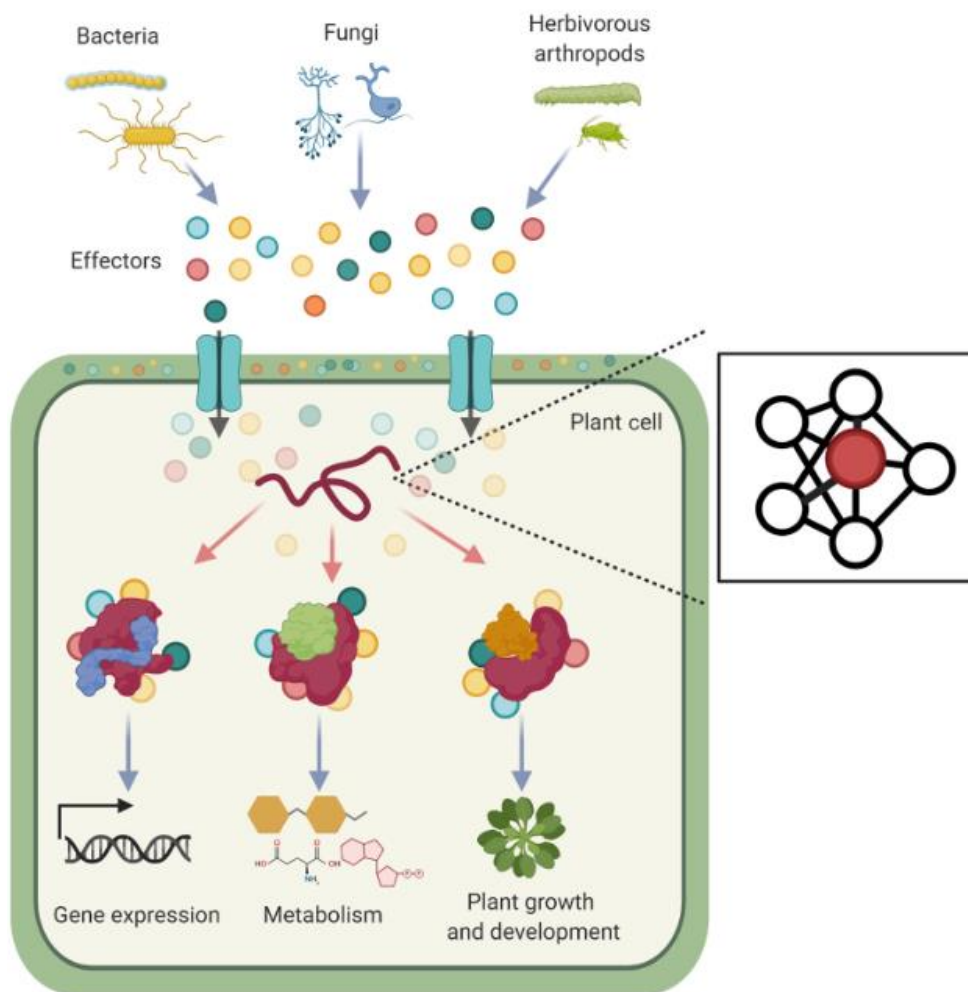


Figure 7. Place des hubs biologiques au sein des interactions plantes-pathogènes. Les hubs biologiques des plantes sont la cible d'effecteurs (ponts de couleur) sécrétés par des pathogènes appartenant à des espèces, des genres et des règnes différents. Ces hubs biologiques (forme rouge) sont des éléments très connectés et occupant une position centrale dans la fonctionnement de la plante (nœud rouge du réseau) et dont la manipulation par les effecteurs permet de contrôler un grand nombre de processus biologiques de l'hôte. (Ceulemans et al., Trends Plant Sci., 2021)

Mis en lien avec les résultats concernant la stratégie d'infection de *F. graminearum* présentés dans le III.1.c, les travaux de Fabre et collaborateurs ont démontré que le dialogue moléculaire entre le blé et *F. graminearum* repose sur un « core-dual protéome » impliquant une manipulation d'un corpus de FS conservés chez le blé. Cette hypothèse est notamment renforcée par la conservation au sein du blé et des espèces apparentées du facteur de sensibilité Sf-Fhb-7AS validé en 2021 par Chhabra et collaborateurs (Chhabra et al., 2021).

b. Reconstruire les réseaux de régulation des réponses du blé au FHB

Les interactions entre les plantes et les agents pathogènes s'accompagnent d'une vaste reprogrammation des gènes permettant la mise en place de réponses coordonnées (Wilkins et al., 2016). Bien que les analyses de génomiques fonctionnelles permettent d'identifier les gènes dérégulés en réponse à l'infection et d'extraire un grand nombre d'informations sur les processus impactés, la compréhension de la sensibilité ne peut se limiter à l'étude des composantes de manière indépendante et requiert d'acquérir des connaissances sur les connexions entre les différents processus impactés et les mécanismes permettant leur coordination (Dong et al., 2015; Peyraud et al., 2017). Les réponses aux stress reposent sur de vastes réseaux de régulation des gènes connectant l'ensemble des processus biologiques de la plante et permettant de coordonner dans le temps et l'espace l'expression des gènes impliqués (Tsuda and Somssich, 2015; Couto and Zipfel, 2016; Wilkins et al., 2016; Ning et al., 2017). La reconstruction des réseaux de régulation des réponses est ainsi une étape clef de l'étude des pathosystèmes permettant de replacer les réponses de la plante dans leur contexte biologique et facilitant l'interprétation des propriétés d'un phénomène biologique complexe et dynamique tel que la sensibilité (Mine et al., 2014; Mochida et al., 2018).

Au regard de la quantité d'information générée, des milliers de gènes dérégulés, il est également nécessaire de trier et de hiérarchiser les gènes candidats réellement ciblés par l'agent pathogène (Engelhardt et al., 2018; Mochida et al., 2018). En effet, les protéines jouant un rôle central dans les réseaux de régulation sont des hubs biologiques clefs et représentent des cibles potentielles importantes des effecteurs (*Figure 7*) (Mukhtar et al., 2011; Carella et al., 2018; Engelhardt et al., 2018; Ceulemans et al., 2021). Le rôle central joué par les hubs de signalisation au sein des interactions plantes-pathogènes a été particulièrement bien illustré par Ceulemans et collaborateurs qui ont montré que 29% des cibles chez *A. thaliana* partagés

par au moins deux effecteurs interagissent avec au moins 50 protéines *in planta* (Ceulemans et al., 2021). Enfin, les FS sont non seulement impliqués dans la mise en place d'une interaction compatible mais aussi par définition dans des processus fondamentaux des plantes (Lapin and Van den Ackerveken, 2013; van Schie and Takken, 2014; Engelhardt et al., 2018; Garcia-Ruiz et al., 2021). Ainsi, au-delà de l'identification du catalogue de FS ciblés par un agent pathogène, il est nécessaire de comprendre les fonctions exercées par ces FS et de replacer ces derniers au sein de leur contexte biologique.

En combinant les informations provenant de l'ensemble des analyses *in planta* de l'interaction, des processus associés à la sensibilité ont pu être identifiés (Fabre et al., 2020). Ces derniers, cohérents avec les informations générées par la validation des gènes de sensibilité décrits précédemment, sont essentiellement impliqués dans les réseaux de régulation des plantes. Cela concerne notamment les voies de signalisation hormonales, avec les voies de l'IAA (Biselli et al., 2018; Wang et al., 2018; Brauer et al., 2019), de l'ABA (Wang et al., 2018) et de l'ET (Pan et al., 2018; Xiao et al., 2013) associées à la sensibilité ainsi que la perturbation de la coordination entre les voies antagonistes du SA et du JA (Ding et al., 2011; Fabre et al., 2019b). D'autres composantes des voies de signalisation des plantes ont également été associées à la sensibilité du blé au FHB. C'est le cas de certaines protéines kinases et de TFs des familles WRKY, MYB, bZIP et NAC (Pan et al., 2018). Enfin, le dialogue moléculaire établi entre *F. graminearum* et le blé se superpose à l'embryogénèse (Chetouhi et al., 2016; Fabre et al., 2019b), un processus biologique impliquant une reprogrammation coordonnée d'un grand nombre de gènes et donc un rôle prépondérant joué par les protéines hubs de signalisation (Chi et al., 2019; Xiang et al., 2019) qui représentent ainsi des cibles potentielles des effecteurs de *F. graminearum* pour manipuler le développement du grain (Fabre, 2020).

Cependant, la grande majorité des études *in planta* de l'interaction blé-*F. graminearum* ont principalement été adressées par le prisme des processus impliqués dans la résistance (Kazan and Gardiner, 2018) et ne permettent pas de clairement discuter des déterminants du blé responsables de la sensibilité au FHB (Fabre et al., 2020). De plus, à ce jour, les mécanismes de régulation structurant les réponses du blé à *F. graminearum* restent largement méconnus et nécessitent d'être explorés.

c. Appréhender les interactions plantes-pathogènes à l'échelle du pathosystème

L'étude du dialogue moléculaire conduit à l'identification de l'effectome de l'agent pathogène et au corpus complet des réponses de l'hôte. Or, la fonction d'un effecteur ne peut être dissociée de sa ou ses cibles dans l'organisme hôte (Arroyo-Velez et al., 2020; He et al., 2020). De plus, parmi le catalogue de réponses, seules certaines sont la conséquence directe de la manipulation par les effecteurs et correspondent aux FS. Ainsi comprendre et identifier les composantes du dialogue moléculaire nécessite de connecter les informations des deux organismes, la plante et l'agent pathogène étant deux parties indissociables d'un même système biologique. Ainsi, les informations obtenues pour chacun des protagonistes ne peuvent être uniquement considérées de manière indépendante et ne prennent véritablement leur sens que lorsqu'elles sont replacées dans le contexte de l'interaction (Peyraud et al., 2017; Nobori et al., 2018; Fabre et al., 2020). Paradoxalement à ce jour les réponses des plantes et les effecteurs des agents pathogènes n'ont que très rarement été étudiés comme un système en interaction (Peyraud et al., 2017).

Dans le cadre de l'interaction entre le blé et *F. graminearum*, les études « dual-protéomiques » réalisées par Fabre et collaborateurs (Fabre et al., 2019a, 2019b, 2021) se sont attachées à connecter les informations provenant des deux organismes. L'analyse couplée de la localisation des effecteurs de *F. graminearum* chez son hôte et de la localisation sous-cellulaire des protéines dérégulées du blé a notamment permis de montrer que les fonctions chloroplastiques semblent jouer un rôle pivot au sein de l'interaction avec les effecteurs chloroplastiques représentant 8.4% des protéines fongiques présentant des changements d'abondance et les protéines chloroplastiques représentant 40% des protéines dérégulées du blé. De plus, les profils d'abondance des effecteurs adressés au chloroplaste et les protéines chloroplastiques du blé sont fortement corrélés (Fabre et al., 2019b). Enfin, l'intégration des profils des protéines du blé présentant des spécificités associées au cultivar (Fabre et al., 2021) avec les profils des effecteurs identifiés dans les mêmes échantillons (Fabre et al., 2019a) a permis d'identifier des motifs de corrélation supérieurs à 95% entre 10 effecteurs putatifs et 13 protéines du blé qui représentent ainsi des cibles putatives robustes de *F. graminearum* (Fabre et al., 2021).

Ces deux exemples démontrent clairement l'intérêt de considérer l'interaction entre la plante et l'agent pathogène à l'échelle du pathosystème pour déchiffrer les déterminants clefs du dialogue moléculaire.

**Article de Review - Searching for FHB Resistances in
Bread Wheat: Susceptibility at the Crossroad**



Searching for FHB Resistances in Bread Wheat: Susceptibility at the Crossroad

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OPEN ACCESS

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Specialty section:

This article was submitted to
Plant Microbe Interactions,
a section of the journal
Frontiers in Plant Science

Received: 29 March 2020

Accepted: 07 May 2020

Published: 11 June 2020

Citation:

Fabre F, Rocher F, Alouane T,
Langin T and Bonhomme L (2020)
Searching for FHB Resistances
in Bread Wheat: Susceptibility
at the Crossroad.
Front. Plant Sci. 11:731.
doi: 10.3389/fpls.2020.00731

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum*, is one of the most devastating fungal wheat diseases. During the past decades, many efforts have been deployed to dissect FHB resistance, investigating both the wheat responses to infection and, more recently, the fungal determinants of pathogenicity. Although no total resistance has been identified so far, they demonstrated that some plant functions and the expression of specific genes are needed to promote FHB. Associated with the increasing list of *F. graminearum* effectors able to divert plant molecular processes, this fact strongly argues for a functional link between susceptibility-related factors and the fate of this disease in wheat. In this review, we gather more recent data concerning the involvement of plant and fungal genes and the functions and mechanisms in the development of FHB susceptibility, and we discuss the possibility to use them to diversify the current sources of FHB resistance.

Keywords: *Triticum aestivum*, *Fusarium graminearum*, scab, susceptibility factors, S genes, fungal effectors, new resistance sources

INTRODUCTION

Fusarium head blight (FHB) is a cereal fungal disease primarily induced by *Fusarium graminearum* (Goswami and Kistler, 2004; Xu and Nicholson, 2009). In wheat, FHB has a direct impact on yield and grain quality, reducing grain weight as well as changing protein accumulation. FHB also causes serious health concerns through the contamination of grains by mycotoxins (e.g., deoxynivalenol, DON, a group 3 carcinogenic toxin), which are resilient to most transformation processes (Li et al., 2014). FHB has become a major threat for wheat crops since the early 1990s, especially in the main producing areas, such as North America, Europe, and China (Zhang et al., 2012). For example, economic losses have been estimated to a total of \$1.176 billion over 2015 and 2016 in the United States (Wilson et al., 2018). Such losses are expected to increase as a result of an amplification of the frequency and the intensity of FHB outbreaks due to rises in temperatures and occasional increases in air humidity expected with the climate change (Luck et al., 2011; Shah et al., 2014).

Although the combined use of tolerant wheat cultivars, fungicides, and specific management practices (e.g., tillage and crop rotation) can reduce part of the losses due to the disease (Haidukowski et al., 2005; Hollingsworth et al., 2008; Salgado et al., 2014; Dahl and Wilson, 2018), no efficient strategy can fully control FHB epidemics so far (Mesterházy et al., 2005;

Tóth et al., 2008). Primarily addressed through the search for genetic resistance, the last two decades of prolific FHB researches turn out with more than 550 quantitative trait loci (QTLs) (Steiner et al., 2017; Venske et al., 2019), covering the whole genome of wheat but with little effect on resistance improvement and failing in identifying regular resistant genes. Twenty years after the identification of *Fhb1*, the most stable and efficient locus for wheat resistance to FHB (Bai et al., 1999), it was recently shown that a deletion spanning the start codon or an N-terminal mutation of the *TaHRC* gene encoding a putative histidine-rich calcium-binding protein explains part of the *Fhb1*-mediated resistance (Li et al., 2019; Su et al., 2019). Although still in dispute, *TaHRC* constitutes the first susceptibility (S) gene to FHB in wheat and directly questions the involvement of susceptibility factors in the disease progress. With this in mind, the purpose of this review is to discuss the growing interest about the determinism of susceptibility to FHB by gathering information from both interacting partners and by emphasizing on its benefits in diversifying the current sources of FHB resistance.

FHB INFECTION PROCESS: IS SUSCEPTIBILITY BEHIND THE MIRROR?

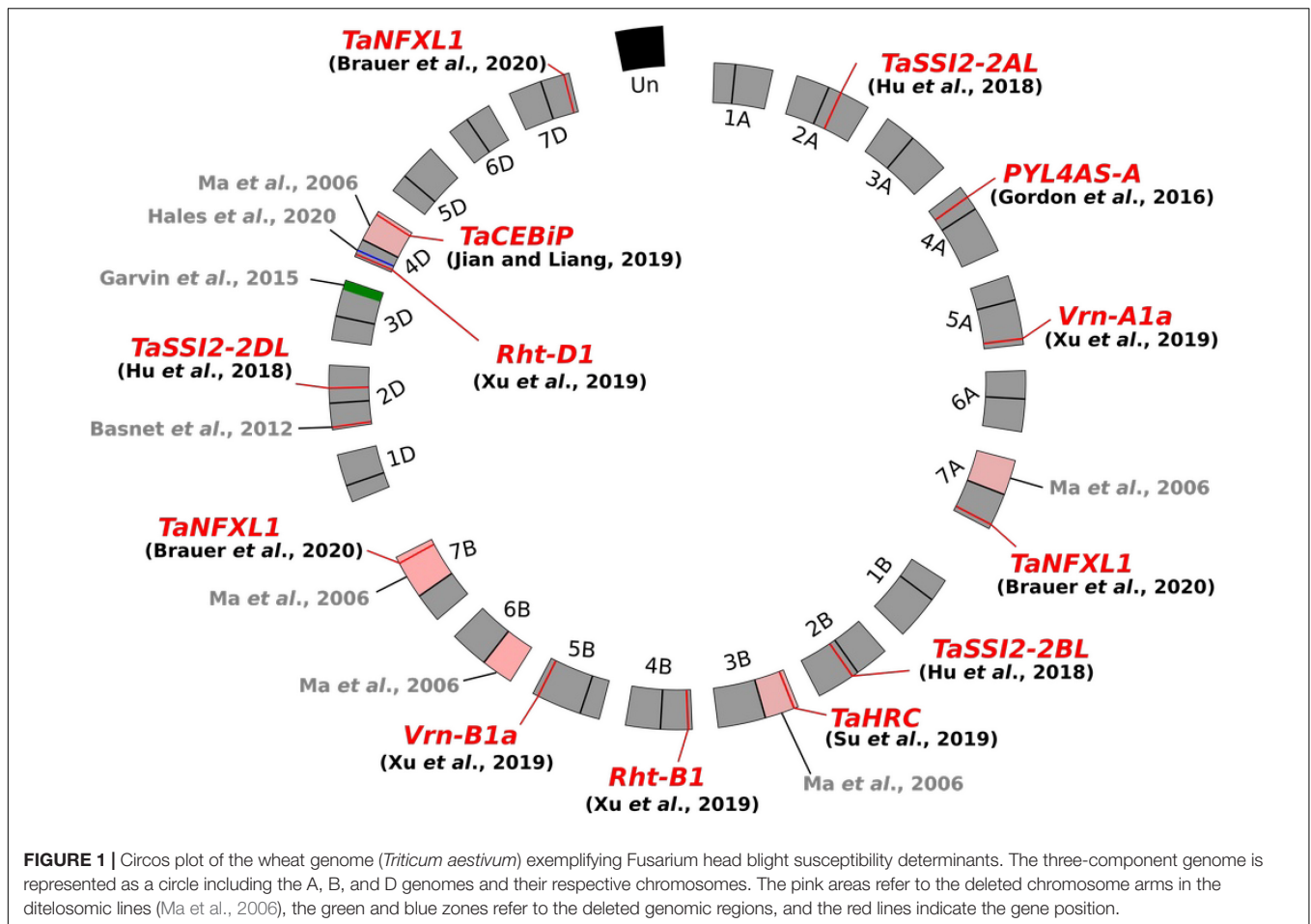
Although the recessive nature of some plant resistances has been established for decades, the concept of susceptibility factors, encoded by the so-called susceptibility genes (S genes), has been clearly defined in 2002 by describing the function of the *pmr6* plant gene that promotes the infection process and supports the pathogen's growth and development (Vogel et al., 2002). Many S genes are now described in plants [reviewed in van Schie and Takken (2014)]. Albeit relatively few, those controlling the wheat/pathogens interactions fit well with this model. For example, the monodehydroascorbate reductase gene, *TaMDHAR4*, has been demonstrated to promote wheat stripe rust infection (Feng et al., 2014). Its mutation results in reducing the hyphae growth of the biotrophic pathogen *Puccinia striiformis*, thus inhibiting its sporulation and enhancing necrosis at the infection site. Further studies in the same interaction evidenced two other S genes, *TaMDAR6* and *TaSTP13* (Abou-Attia et al., 2016; Huai et al., 2020), emphasizing the existence of several S genes in the wheat genome and suggesting that some of them might be implicated in wheat susceptibility-related mechanisms to FHB. If most studies have focused on the genetic determinants of wheat resistance to FHB so far, an interesting alternative is to consider the molecular and the physiological processes that make the host plant susceptible to *F. graminearum*. An extensive literature dealing with large-scale analyses has already shown that, compared to resistant cultivars, the most susceptible ones are characterized by a specific deregulation of genes involved in a wide range of molecular processes (e.g., transcription factors, enzymes involved in primary and secondary metabolism, and defense-related genes), suggesting the intricate participation of a wealth of potential susceptibility factors (Ding et al., 2011; Gottwald et al., 2012; Erayman et al., 2015; Pan et al., 2018; Wang et al., 2018; Brauer et al., 2019).

Genetics Demonstration of the Existence of Wheat Susceptibility Factors to FHB

The involvement of putative susceptibility determinants during FHB development has been primarily suggested by studies using wheat aneuploid lines (Figure 1). Ma et al. (2006) first evidenced that ditelosomic lines lacking in specific chromosome arms displayed an enhanced resistance to *F. graminearum* infection, suggesting the removal of pivotal susceptibility factors along with chromosome fragment deletion. Likewise, Garvin et al. (2015), in an attempt to introgress a new FHB resistance locus from the cultivar (cv.) "Freedom" into the susceptible cv. "Apogee," have shown that the most resistant line was characterized by the deletion of a chromosome segment of about 19% of the length of the 3DL arm in comparison with the cv. "Apogee" (Figure 1). The wheat line missing this genomic interval resulted in up to 59% decrease of FHB severity as compared to cv. "Apogee" and displayed a significant reduction of DON accumulation (Garvin et al., 2015). Similarly, another chromosomal fragment of 31.7 Mbp on the short arm of chromosome 4D was demonstrated to contain potential wheat susceptibility factors to FHB (Hales et al., 2020). Its deletion leads to a significant decrease of *F. graminearum* spreading in wheat spikes. Evidence of susceptibility factors to FHB has also been provided through allele mining studies. The dwarfing allele at the locus *Rht-D1* (*Rht-D1b*, formerly termed *Rht2*) has not been associated to FHB susceptibility by a direct effect of the plant height but rather through a pleiotropic or linkage effect (Draeger et al., 2007). Further experiments demonstrated that, in the cv. "Spark," FHB resistance was largely conferred by the wild allele of the *Rht-D1* gene, while the *Rht-D1b* allele found in the susceptible lines was responsible for approximately 50% of the phenotypic variance associated with the magnitude of initial infection (Srinivasachary et al., 2009). A similar increase of FHB infection has been described for the two particular alleles of the vernalization-related genes *Vrn-A1* and *Vrn-B1* (Xu et al., 2020). An FHB susceptibility source has also been identified in the "Sumai 3" *Qfhs.kibr-2DS* QTL (Figure 1), in which a specific allele encoding a multidrug resistance-associated protein was identified in the susceptible "Sumai 3"-derived cv. named "Gamenya," unveiling that the FHB susceptibility determinants could be fortuitously inherited from resistant cultivars (Handa et al., 2008; Basnet et al., 2012; Niwa et al., 2014). Such examples suggest that a substantial subset of S genes/factors could be present in the wheat genome and highly conserved among the wheat cultivars. Although most of these studies provide only indirect evidences about the molecular determinism of FHB susceptibility, these results emphasize the relevance of considering the diversity of S genes as a complementary and promising approach to improve wheat resistance to FHB.

Role of Phytohormones in FHB Development

Several works have already suggested that wheat hormonal pathways play a favorable role in FHB development. For instance, reducing EIN2 expression in wheat, one of the major components of ethylene signaling, decreased the disease symptoms and DON



accumulation in the grains (Chen et al., 2009). Further large-scale transcriptomics showed that the ethylene pathway was specifically induced in the FHB-susceptible NAUH117 line, as compared to the resistant Wangshuibai landrace (Xiao et al., 2013). The abscisic acid (ABA) signaling pathway has also been demonstrated to favor *F. graminearum* infection in wheat spikes (Gordon et al., 2016; Wang et al., 2018). A virus-induced gene silencing approach demonstrated a role of the wheat ABA receptor *Ta_PYL4AS_A* (Figure 1), and its close homologs, in mediating FHB susceptibility and in decreasing mycotoxin accumulation (Gordon et al., 2016). Likewise, transcriptional and hormonal profiling showed that wheat genes involved in auxin biosynthesis were highly up-regulated, along with auxin accumulation, in the susceptible cultivars during the *F. graminearum* infection process as compared to the resistant ones (Biselli et al., 2018; Wang et al., 2018; Brauer et al., 2019). Salicylic and jasmonic acids are also widely described for their role in modulating FHB responses and in discriminating resistant vs. susceptible cultivars as well (Ding et al., 2011; Gottwald et al., 2012; Sun et al., 2016; Wang et al., 2018). Upon *F. graminearum* infection, their respective actions occur in two phases, an initial induction of salicylic acid happens at the early stages followed by the synthesis of jasmonic acid at the later stages (Ding et al., 2011). In addition, the silencing of the wheat *TaSSI2* gene has

been shown to increase FHB resistance, promoting salicylic acid signaling (Hu et al., 2018) and potentially altering the jasmonic acid pathway as demonstrated in *Arabidopsis ssi2* mutant lines (Kachroo et al., 2004). This illustrates further the involvement of these two antagonist hormones in the FHB progress and suggests that susceptibility may involve systemic signals capable of deeply reshaping the plant physiology.

Shaping FHB Susceptibility in the Course of Grain Development

With a period of maximal susceptibility occurring within 3 days after anthesis (Beccari et al., 2019), FHB develops concomitantly with the grain filling period, during which a large number of plant physiological processes allow a massive accumulation of sugars, lipids, and proteins (Nadaud et al., 2010), resulting in a possible nutrient reservoir in the infection area. Spike ontogeny could thus indirectly and sequentially set up a range of susceptibility factors that can partly explain the dynamics of fungal development during the infection course (Chetouhi et al., 2015, 2016). Extensively boosted during the endosperm expansion, *in planta F. graminearum* growth is associated with massive protein abundance adjustments detectable simultaneously in both plant and fungal proteomes at 48–72 h post-inoculation at

anthesis transition (Fabre et al., 2019b). At this stage, extensive metabolic changes in wheat rachis nodes have been reported, including a strong increase of gibberellic acid amount as well as glycolysis intermediates, suggesting that a release of wheat storage carbohydrates could possibly be used for fungal metabolic requirements (Bönnighausen et al., 2018). This is also supported by the converging evidences of large decreases in the expression of genes involved in sucrose and starch metabolism (Erayman et al., 2015; Chetouhi et al., 2016). Starch components, such as amylopectin and amylose, are known to be difficult for the fungus to recycle as a carbon source and for DON production (Oh et al., 2016), unlike sucrose (Jiao et al., 2008; Kawakami et al., 2014), suggesting that the deregulation of the host energy processes at the early stage of the disease could be one of the key factors that determine wheat susceptibility. However, the links are not obvious since many other studies have shown that primary metabolism and especially photosynthesis are extensively rearranged (Long et al., 2015; Biselli et al., 2018; Li et al., 2018; Fabre et al., 2019b). Although this can substantially limit the accumulation of sugars, this could also be seen as a means of constraining the energy requirements necessary to trigger defense mechanisms (Bolton, 2009). In agreement with many previous studies suggesting a key role of chloroplast in plant susceptibility (Lo Presti et al., 2015; Sowden et al., 2018; Han and Kahmann, 2019; Kretschmer et al., 2020), the remodeling of its functioning in wheat spikes during FHB is suspected to be a link between the plant defense responses and the adjustments of primary metabolism. This raises direct questions about the mechanisms used by the fungus to achieve such effects (Fabre et al., 2019b).

***Fusarium graminearum* EFFECTORS: KNOCKING AT THE WHEAT CELL DOOR TO TRIGGER SUSCEPTIBILITY?**

Several studies have already demonstrated that plant susceptibility factors could be diverted by a range of pathogen effectors. Consisting of proteins, RNA, and metabolites, effectors are molecules synthesized by the pathogen, delivered in host tissues, and able to alter the structure and the function of the host cell (Hogenhout et al., 2009; Lo Presti et al., 2015). Compared to bacteria, knowledge about fungal effectors remains relatively limited (Niu et al., 2013). However, a number of studies have provided essential information on the ability of *F. graminearum* in interfering with wheat molecular processes. Identifying *F. graminearum* effectors and understanding their roles in the infectious process could be a relevant strategy for identifying wheat susceptibility factors.

Breaking Wheat's Defenses

One of the first characterized effectors of *F. graminearum* is the secreted DON mycotoxin (Miller and Young, 1985). Although its synthesis is not necessary for the penetration phase, its role in fungal spreading within the spike has been reported (Bai et al., 2001). DON acts as an inhibitor of protein and nucleotide synthesis in the host cell (Audenaert et al., 2013). Through such

an effect, DON is supposed to alter the mitochondrial functions of many eukaryotes, and its role in inhibiting programmed cell death as well as in the expression of defense compounds (chitinases, peroxidases, and pathogen-related proteins) has already been described (Brown et al., 2011; Audenaert et al., 2013; Diamond et al., 2013). A recent report has shown that DON promotes the *TaNFXL1* transcription factor in wheat, leading to FHB susceptibility through uncharacterized mechanisms (Brauer et al., 2020). Other *F. graminearum* effectors have been reported so far, revealing that proteins belonging to the cell-wall-degrading enzymes (CWDEs) are important promoters of wheat susceptibility to FHB (Quarantin et al., 2016, 2019; Paccanaro et al., 2017; Lu and Faris, 2019). For instance, several studies identified *F. graminearum* xylanases with a direct impact on cell wall weakening and an indirect role in enhancing hypersensitive-like symptoms in plant tissues (Paper et al., 2007; Pollet et al., 2009; Sella et al., 2013; Tundo et al., 2015). The FGL1 lipase, another CWDE effector (Voigt et al., 2005), was shown to physically interact with the wheat immunophilin protein FKBP12, altering the establishment of the FKBP12/ERG complex, which finally triggers cell death (Niu et al., 2013). In addition, by degrading the plant cell wall, the FGL1 effector promotes the release of free fatty acids that inhibit the callose deposits associated with the immune responses (Blümke et al., 2014). Similarly, the arabinanase Arb93b, induced during the early stage of FHB, was shown to suppress ROS-activated defense along with its arabinan-degrading activity (Hao et al., 2019). Besides protein effectors, sRNA products have also been reported to control plant responses. The 18-nt-length sRNA (Fg-sRNA1) targets a wheat chitin elicitor-binding protein, which is likely to function in wheat disease resistance signaling pathways (Jian and Liang, 2019). The identification of non-targeted allelic variants could thus guide future research toward “loss-of-susceptibility” forms of resistances.

Predicted Effector Searches Reveal an Increasingly Complex Arsenal

Although the catalog of characterized *F. graminearum* effectors remains limited, substantial efforts using genomics approaches have provided a large set of new candidates. Using the reference genome sequence, Brown et al. (2012) established a predicted secretome of 574 proteins sharing the structural features of secreted proteins (small size, cysteine-rich proteins, and signal peptides). This revealed a diverse hydrolytic arsenal and a range of putative effectors that could be potentially delivered in the wheat tissues. Secretome was further investigated by focusing on the 190 small secreted cystein-rich proteins (SS): the extracellular localization was confirmed for 25 of them, and the expression of 34 of them was demonstrated as regulated during the FHB progress (Lu and Edwards, 2015). The sequence analysis suggested that 17 SS harbor conserved functional domains such as glycosyl-hydrolase or pathogenesis-related domains, and two of them were homologous to Ecp2, a well-known effector produced by the tomato pathogen *Cladosporium fulvum* (Van den Ackerveken et al., 1993). Other studies dealing with *in vitro* or *in planta* approaches have also been successful in enlarging the list of candidate effectors (Paper et al., 2007;

Yang et al., 2012; Ji et al., 2013). By evidencing the protein repertoire found specifically in the extracellular part of the plant tissues or identified in liquid culture and confirmed *in planta* using qRT-PCR, these extended the range of putative function including a number of proteases, esterase, and nucleases. Based on the few structural information available about fungal effectors (Sperschneider et al., 2018), several exploratory reports provided novel insights in their diversity and dynamics (Lysøe et al., 2011; Brown et al., 2017; Fabre et al., 2019a,b). By dissecting the asymptomatic and the symptomatic stages of the FHB infection, Brown et al. (2017) revealed particular gene groups with specific abundance patterns illustrating the early expression of genes involved in the transport of amino acids, in polyamine synthesis and ABC transporters, while hydrolytic carbohydrate-active enzymes and lipases were found at later stages. The delivery of putative effectors by waves at specific stages of the infection has also been confirmed at the protein level using an *in planta* dual-proteome approach (Fabre et al., 2019b). This study further demonstrated that putative effectors could be already accumulated in spores or synthesized within hours, and extensive co-variations were evidenced between abundance changes of effectors and the regulation of plant chloroplast proteins, especially at the beginning of grain cellularization. In addition, strong links were evidenced between the abundance of candidate effectors and strain aggressiveness (Fabre et al., 2019a), emphasizing that increased knowledge of the fungal component could lead to a better understanding of the processes involved in host susceptibility.

CONCLUDING REMARKS

The past decades of researches on FHB in wheat have provided a wealth of information on the genetic and the molecular determinants of the disease progress in spikes, mostly focused on resistance mechanisms. Although still marginally

investigated, wheat susceptibility factors to FHB are emerging as key components that determine the fate of the disease, involving a complex molecular dialogue based on the interplay of fungal effectors and their plant targets. Understanding wheat susceptibility still requires many efforts on both partners and needs to fill the gap between wheat and fungal studies. This knowledge will open new strategies in order to control this complex plant/fungus interaction, providing alternative forms of resistance that are potentially more sustainable. While still a challenge, such loss-of-susceptibility forms have already demonstrated their potential to provide efficient and durable sources of disease resistance in crops (Pavan et al., 2010). They represent a promising strategy to control FHB epidemics and may provide a complementary approach to the introgression of gain-of-function resistance genes.

AUTHOR CONTRIBUTIONS

FF, FR, and TA organized, prepared, and drafted the manuscript. TL and LB designed, reviewed, and finalized the manuscript.

FUNDING

This work was supported by the Region Auvergne-Rhône-Alpes, the Fonds Européen de Développement Régional (FEDER support), and by the Agence Nationale de la Recherche (NewMyco project, ANR-15-CE21-0010). The present work falls within the thematic area of the French Government IDEX-ISITE initiative 16-IDEX-0001 (CAP 20-25).

ACKNOWLEDGMENTS

We thank Dr. C. Saintenac for his critical comments on the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Problématique et objectifs de Thèse

Au sein de l'équipe Maladies Des Céréales de l'Unité Mixte de Recherche Génétique, Diversité, Écophysiologie des Céréales (UMR 1095 GDEC, INRAE/UCA), les projets ont pour objectifs (1) d'apporter des éléments d'information originaux sur les mécanismes moléculaires et physiologiques impliqués dans le contrôle et le développement de la septoriose (STB) et de la fusariose de l'épi et (2) de mettre à la disposition de la sélection de nouveaux gènes, de nouvelles combinaisons génétiques et des mécanismes originaux de façon à élargir les spectres et les niveaux de résistance du blé à ces deux maladies fongiques et ainsi améliorer la durabilité des résistances. Pour répondre à ces deux objectifs, les travaux de recherche s'articulent autour de trois axes. Le premier axe, animé par Cyrille Saintenac, cherche à comprendre le déterminisme génétique et le fonctionnement des résistances du blé à la septoriose. Le deuxième axe, piloté par Ludovic Bonhomme, vise à identifier les facteurs de sensibilité du blé au FHB. Le troisième axe, animé par Thierry Langin, s'intéresse aux impacts de différents facteurs environnementaux, biotiques et abiotiques, sur la plasticité et la stabilité des réponses du blé à la septoriose et la fusariose de l'épi. Ce travail de thèse s'inscrit dans le deuxième axe de l'équipe et repose sur des approches de physiologie moléculaire intégrative de l'interaction entre le blé et *F. graminearum* afin d'identifier les déterminants fongiques et hôtes gouvernant la sensibilité du blé au FHB.

L'étude du pathosystème blé - *F. graminearum* dans l'équipe a débuté avec les travaux de Thèse de Chérif Chetouhi visant à caractériser les réponses d'un cultivar de blé sensible au FHB lors des principaux stades du développement du grain, allant de l'étape de division cellulaire à la fin de la phase de remplissage du grain (Chetouhi, 2015). Sur la base d'analyses protéomique et transcriptomique, les travaux de Chérif Chetouhi ont montré que le développement du grain se poursuit au cours de l'infection et ont mis en évidence des processus potentiellement associés à une manipulation parasitaire dont notamment la suppression des mécanismes de défense et de mort cellulaire ainsi que le détournement primaire lors de la phase du remplissage du grain (Chetouhi et al., 2015, 2016).



*Figure 8. Suivi du développement d'une souche de *F. graminearum* marquée à la GFP suite à l'inoculation d'un épillet directement dans la cavité florale. Les points choisis permettent de caractériser les stades précoces de l'interaction correspondant à la germination des spores au niveau de l'apex de l'ovaire (24hpi), le développement du mycélium au niveau de l'apex de l'ovaire (48hpi) et la colonisation de la glumelle par le mycélium (72hpi).*

Le suivi du développement d'une souche de *F. graminearum* marquée à la GFP au sein d'un épillet inoculé directement dans la cavité florale, par Rachel Abou-Sleiman stagiaire de Master II en 2014, a permis de clairement définir les stades précoces de l'interaction (Figure 8) étudiés à l'échelle protéomique au cours de la Thèse de Francis Fabre (Fabre, 2020). L'infection débute dès 24hpi par la germination des spores au niveau de l'apex de l'ovaire. A 48hpi, le mycélium se développe au niveau de l'apex de l'ovaire avant de coloniser la glumelle à 72hpi. Dans un premier temps, les travaux menés au cours de la Thèse de Francis Fabre ont permis la caractérisation fine, à l'échelle protéomique, des déterminants moléculaires de l'agent pathogène et de l'hôte mis en place lors des stades précoces de l'interaction entre le blé et *F. graminearum*. Au cours des 96 premières heures de l'interaction, le dialogue moléculaire établi entre le blé et *F. graminearum* repose sur la synthèse de manière séquentielle des effecteurs putatifs associée à un ajustement précis et coordonné des réponses de la plante, impliquant notamment de nombreux processus chloroplastiques. Ces travaux ont également mis en évidence l'existence d'une phase de transition marquée entre 72hpi et 96hpi qui se manifeste par d'importants changements d'abondance des protéines de *F. graminearum* et du blé (Fabre et al., 2019b). Dans un second temps, l'impact de différents fonds génétiques de *F. graminearum* et du blé sur le dialogue moléculaire de l'interaction a été évalué au point de transition 72hpi. Les résultats obtenus ont montré l'absence de spécificités souche-cultivar et suggèrent ainsi que le dialogue moléculaire repose sur des déterminants moléculaires de l'agent pathogène et de l'hôte très conservés (Fabre et al., 2019a, 2021)

En parallèle de la caractérisation de l'impact de la variabilité génétique sur le dialogue moléculaire établi *in planta*, les travaux réalisés au cours de la thèse de Tarek Alouane se sont attachés à décrire et comprendre la diversité au sein de l'espèce *F. graminearum* à l'échelle génomique (Alouane, 2022). Dans un premier temps, ces travaux ont permis de définir le premier pangénome de l'espèce caractérisé par une forte variabilité du nombre de gènes entre les souches étudiées et a établi l'existence d'un core-secretome putatif rassemblant 53% des protéines sécrétées identifiées dans l'ensemble des génomes des souches étudiées (Alouane et al., 2021).

Les travaux réalisés au cours de cette Thèse s'intègrent directement dans ce contexte scientifique et cherchent à clarifier différents aspects du dialogue moléculaire gouvernant la sensibilité du blé à *F. graminearum* : (1) Quels sont les effecteurs et facteurs de sensibilité conservés et nécessaires au succès de l'infection ? (2) Quelle est la nature et la place des facteurs de sensibilité ciblés par *F. graminearum* au sein des processus biologiques du blé ? (3) Quelles sont les conséquences du dialogue moléculaire établi au cours de l'infection sur le fonctionnement de l'épi ?

Ainsi, pour répondre à ces questions, les travaux réalisés au cours de cette Thèse s'organisent autour de trois objectifs principaux :

Objectif 1 : Analyse *in planta* de la stratégie d'infection de *F. graminearum* : diversité et dynamique d'expression des gènes codant les effecteurs putatifs.

Au cours de ce premier objectif, nous avons mis en place un pipeline de transcriptomique "large échelle", RNA-seq, pour caractériser les gènes codant les effecteurs putatifs de *F. graminearum* au cours des stades précoces de l'infection, de 48hpi à 96hpi, identifiés comme étant des étapes clefs du cycle infectieux au cours de la Thèse de Francis Fabre. Sur la base de deux expérimentations indépendantes, nous avons tiré avantage de plusieurs fonds génétiques de *F. graminearum* et du blé pour identifier les effecteurs clefs du dialogue moléculaire. Au cours d'une première expérimentation, l'infection du cultivar de blé sensible 'Récital' par les trois souches de *F. graminearum* d'agressivité contrastée étudiées au cours des travaux de Francis Fabre, a permis d'évaluer l'impact de la variabilité de l'agent pathogène sur sa stratégie d'infection. Lors de la deuxième expérimentation, l'infection de cinq cultivars de blé de sensibilité contrastée par une souche agressive, a permis d'évaluer l'impact de la variabilité génétique de l'hôte sur la stratégie d'infection de *F. graminearum*.

Cet objectif, correspondant au premier chapitre de la Thèse, a permis la rédaction d'un premier article intitulé « *Fusarium graminearum* Infection Strategy in Wheat Involves a Highly Conserved Genetic Program That Controls the Expression of a Core Effectome » publié en février 2022 dans la revue *International Journal of Molecular Science*.

Objectif 2 : Approche systémique et intégrative de l'interaction blé-*F. graminearum* pour identifier des facteurs de sensibilité candidats au FHB et définir leur rôle au sein de l'hôte.

Ce deuxième objectif repose sur l'analyse des données du blé et de *F. graminearum* acquises au cours des deux expérimentations présentées dans l'objectif 1. Dans un premier temps, le réseau de régulation des réponses du cultivar sensible 'Récital' infecté par les trois souches d'agressivité contrastée a été reconstruit *in silico* permettant ainsi d'en déterminer la structure et les régulateurs principaux. Dans un second temps, l'expression des gènes codant les effecteurs conservés de *F. graminearum* et des gènes de réponses de 'Récital' ont été intégrés permettant l'identification de facteurs de sensibilité putatifs. Enfin, l'analyse des réponses des cinq cultivars de sensibilité contrastée a permis d'identifier un corpus de facteurs de sensibilité conservés quel que soit le cultivar ou la souche étudiés.

Les résultats acquis au cours de ce deuxième objectif sont décrits dans le deuxième chapitre de la thèse et font l'objet d'un second article intitulé « System-level transcriptomics of wheat responses to FHB identifies gene regulatory networks and conserved master regulators as reliable candidate susceptibility genes » en cours de soumission.

Objectif 3 : Caractérisation de l'impact du dialogue moléculaire gouvernant l'interaction sur le statut physiologique de l'épi.

A ce jour, les méthodes de phénotypage de l'épi de blé au cours de l'infection par *F. graminearum* ne permettent pas d'apporter d'informations pertinentes concernant son état de fonctionnement. Au cours de cet objectif, nous avons développé un protocole de phénotypage original du statut physiologique de l'épi reposant sur la mesure des échanges de gaz. Associé à l'approche transcriptomique, cette approche dite d'écophysiologie permet d'apporter des éléments originaux concernant l'impact du FHB sur le statut physiologique de l'épi au cours des premiers stades de l'infection. Les résultats obtenus au cours de cet objectif sont présentés dans le troisième chapitre de la Thèse.

Un quatrième chapitre permettra de discuter l'ensemble des résultats acquis et de les repositionner dans le contexte biologique des interactions plantes-pathogènes. Enfin, une conclusion générale terminera ce document et permettra de présenter les nouvelles questions biologiques que ces travaux soulèvent en précisant les perspectives de recherche qu'elles ouvrent.

Chapitre I - Analyse *in planta* de la stratégie d'infection de *F. graminearum* : diversité et dynamique d'expression des gènes codant les effecteurs putatifs

Contexte, enjeux et méthodologie

La capacité de l'agent pathogène à manipuler les processus biologiques de son hôte représente un des facteurs clefs de la sensibilité. Cette dernière est gouvernée par un dialogue moléculaire complexe impliquant notamment la sécrétion, par l'agent pathogène, d'effecteurs qui ciblent des fonctions spécifiques de l'hôte, les facteurs de sensibilité (O'Connell and Panstruga, 2006; Selin et al., 2016; Zeilinger et al., 2016; M. Khan et al., 2018; He et al., 2020; Jaswal et al., 2020). Identifier le catalogue d'effecteurs d'un agent pathogène, son effectome, représente donc un levier majeur pour améliorer notre compréhension de l'interaction et améliorer les stratégies de sélection de nouvelles résistance par l'identification de leurs cibles chez la plante, le targetome (Nejat et al., 2017; Prasad et al., 2019; Jaswal et al., 2020).

Cependant, l'absence de séquences ou de motifs structurels conservés entre les effecteurs fongiques rend leur recherche *in silico* particulièrement complexe (Sperschneider et al., 2015). Les seules caractéristiques universelles des effecteurs fongiques sont leur synthèse finement régulée au cours de l'interaction et leur sécrétion dans l'hôte (Sperschneider et al., 2015; Uhse and Djamei, 2018), rendant nécessaire l'utilisation de méthodes d'analyses exploratoires *in planta* pour identifier l'effectome réel de l'agent pathogène au sein de la plante hôte (Lorrain et al., 2019; Prasad et al., 2019; Depotter and Doehlemann, 2020).

Outre sa chronologie de sécrétion, le rôle d'un effecteur dans l'issue de l'interaction est également dépendant de sa conservation entre les souches d'une espèce et entre les espèces d'agents pathogènes, les effecteurs conservés seraient ainsi nécessaire au succès de l'infection tandis les effecteurs spécifiques seraient impliqués dans les processus d'adaptation à l'hôte et d'agressivité des souches (Arroyo-Velez et al., 2020; Badet and Croll, 2020; Depotter and Doehlemann, 2020; Petre et al., 2020). Cette conservation est également en partie déterminée par les processus de coévolution avec les hôtes et leurs targetome (Arroyo-Velez et al., 2020; Depotter and Doehlemann, 2020).

Par conséquent, il est nécessaire de prendre en compte la diversité génétique des deux partenaires de l'interaction pour identifier les acteurs clés de la stratégie d'infection d'un agent pathogène. Dans le cas de *F. graminearum*, bien que la variabilité à l'échelle génomique ait été décrite de manière approfondie avec la publication en 2021 du premier pangénome de l'espèce (Alouane et al., 2021), il existe un réel manque de connaissances concernant l'impact de la diversité génétique de l'hôte et de l'agent pathogène sur l'effectome mis en place lors de l'infection du blé.

De par sa puissance de détection élevée et du fait de l'important déséquilibre entre la quantité de transcrits du blé et de *F. graminearum*, la technologie RNA-seq est particulièrement adaptée pour identifier et caractériser l'effectome *in planta* de *F. graminearum* et ce même aux stades précoces de l'interaction. Dans le but de caractériser la stratégie d'infection de *F. graminearum*, tant en terme de la diversité des déterminants que de leurs dynamiques, le profil d'expression des gènes de *F. graminearum* a été réalisé au cours d'une cinétique d'infection, de 48hpi à 96hpi, déterminée au cours des travaux de Francis Fabre (Fabre et al., 2019b) et en utilisant une double approche : la première reposant sur l'infection de cinq cultivars de sensibilité contrastée par une souche très agressive et la deuxième reposant sur l'infection d'un cultivar très sensible par trois souches d'agressivité contrastée. Ces travaux se sont focalisés sur les gènes codant les effecteurs putatifs de *F. graminearum*, identifiés selon les deux critères suivants : (1) la sécrétion prédite de la protéine codée et (2) la modulation de l'expression du gène au cours de l'infection. De plus, la recherche des signaux d'adressage et des fonctions des effecteurs putatifs identifiés a permis d'identifier les compartiments cellulaires de l'hôte ciblés par *F. graminearum* et ainsi d'apporter des informations pertinentes concernant le rôle joué par ces effecteurs au cours des stades précoces de l'infection. L'association des résultats obtenus par l'analyse des deux expérimentations a permis de proposer la première description détaillée du répertoire d'effecteurs exprimés *in planta* par *F. graminearum* et d'identifier les déterminants clés du processus infectieux.

Article 1 - *Fusarium graminearum* Infection Strategy in Wheat Involves a
Highly Conserved Genetic Program That Controls the Expression of a Core
Effectome



Article

Fusarium graminearum Infection Strategy in Wheat Involves a Highly Conserved Genetic Program That Controls the Expression of a Core Effectome

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Abstract: *Fusarium graminearum*, the main causal agent of Fusarium Head Blight (FHB), is one of the most damaging pathogens in wheat. Because of the complex organization of wheat resistance to FHB, this pathosystem represents a relevant model to elucidate the molecular mechanisms underlying plant susceptibility and to identify their main drivers, the pathogen's effectors. Although the *F. graminearum* catalog of effectors has been well characterized at the genome scale, in planta studies are needed to confirm their effective accumulation in host tissues and to identify their role during the infection process. Taking advantage of the genetic variability from both species, a RNAseq-based profiling of gene expression was performed during an infection time course using an aggressive *F. graminearum* strain facing five wheat cultivars of contrasting susceptibility as well as using three strains of contrasting aggressiveness infecting a single susceptible host. Genes coding for secreted proteins and exhibiting significant expression changes along infection progress were selected to identify the effector gene candidates. During its interaction with the five wheat cultivars, 476 effector genes were expressed by the aggressive strain, among which 91% were found in all the infected hosts. Considering three different strains infecting a single susceptible host, 761 effector genes were identified, among which 90% were systematically expressed in the three strains. We revealed a robust *F. graminearum* core effectome of 357 genes expressed in all the hosts and by all the strains that exhibited conserved expression patterns over time. Several wheat compartments were predicted to be targeted by these putative effectors including apoplast, nucleus, chloroplast and mitochondria. Taken together, our results shed light on a highly conserved parasite strategy. They led to the identification of reliable key fungal genes putatively involved in wheat susceptibility to *F. graminearum*, and provided valuable information about their putative targets.

Keywords: *Fusarium graminearum*; *Triticum aestivum*; plant–fungus interaction; in planta; transcriptomics; effectoromics; susceptibility factors



Citation: Rocher, F.; Alouane, T.; Philippe, G.; Martin, M.-L.; Label, P.; Langin, T.; Bonhomme, L. *Fusarium graminearum* Infection Strategy in Wheat Involves a Highly Conserved Genetic Program That Controls the Expression of a Core Effectome. *Int. J. Mol. Sci.* **2022**, *23*, 1914. <https://doi.org/10.3390/ijms23031914>

Academic Editor: Andreas Burkovski

Received: 28 January 2022

Accepted: 4 February 2022

Published: 8 February 2022

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1. Introduction

Fusarium Head Blight (FHB), mainly caused by the Ascomycota fungus *Fusarium graminearum*, is one of the most prevalent diseases of small grain cereals, especially in wheat [1,2]. With direct impacts on yield, grain quality and through the accumulation of carcinogenic mycotoxins (e.g., deoxynivalenol, DON) [3–5], FHB is considered as a major

limiting factor for wheat production in Europe, North America and Asia [6–9], resulting in substantial economic losses that reached up to USD 1.176 billion over 2015 and 2016 in the USA for instance [10]. Because FHB is expected to be even more frequent and intense along with the rises of temperatures and the occasional increases in air humidity promoted through the climate change [11,12], further research is needed to develop better management strategies and sustainable control solutions [13]. FHB resistance trait is strictly quantitative and involves multiple Quantitative Trait Loci (QTLs) with relatively weak effects [14,15] that makes them insufficient when environmental conditions are favorable to the fungus. Thus, identifying sustainable solutions able to efficiently control FHB epidemics requires the search of alternative sources of resistance. For the last twenty years, the multiple evidences of the role of a plant's susceptibility factors in promoting pathogen infection have opened new opportunities to identify such pivotal determinants of plant diseases, and a number of studies already reported that mutation or loss of susceptibility genes can be used in resistance breeding [16,17]. With the increasing evidences of the role of wheat's susceptibility factors in FHB development [18–23], elucidating the mechanisms of wheat susceptibility to *F. graminearum* appears as a promising approach to improve FHB resistance [17,24–26].

A pathogen's ability to hijack a host's biological processes such as defense responses, physiology and primary metabolism to exploit host resources is assumed to be one of the key drivers of a plant's susceptibility. These interactions involve a complex molecular crosstalk between the two partners, including the delivery of effectors, which include small secreted proteins able to alter host cell structure and to target specific functions into host tissues, the so-called susceptibility factors [27–32]. The role of an effector is therefore determined by its in planta localization, i.e., the apoplast or host's intracellular compartments, and the targeted susceptibility factors [32–35]. Mining a robust catalog of pathogen effectors, i.e., the effectome, offers major opportunities to improve resistance breeding through the identification of the host's susceptibility factors, i.e., the targetome. This further could make possible the identification of functional markers to screen plant germplasm, as well as new targets for host-induced gene silencing [32,36,37]. However, their systematic search in silico is still challenging because most of them lack shared protein features or conserved domains within and across species, and very few are structurally characterized [28,32,35]. The only universal fungal effector's characteristics are their expected secretion and their fine-tuned synthesis along the infection progress [35,38], making in planta exploratory methods such as transcriptomics and proteomics necessary to narrow down the effector candidates and identify the active ones [37,39,40].

Numerous effectors are deployed by pathogens and their role within the molecular crosstalk and in the fate of the interaction is determined by their conservation among pathogen species or between the different strains of a particular species. This conservation is also partly driven by the coevolution with their hosts and their targetome [40,41]. Conserved effectors are thought to play an indispensable role to ensure compatibility by targeting conserved host's immune or metabolism functions, while specific effectors are thought to be involved in the host's adaptation and strain aggressiveness [40–43]. Thus, elucidating the complexity of such molecular crosstalk underlying plant–pathogen interactions and elaborating robust and relevant effectomes require consideration of the diversity from both partners of the interaction. The genomics variability of many fungal pathogen species is well characterized, but its impacts on the infection program remains to be addressed [44–48].

In *Fusarium graminearum*, genomics variability has been well characterized and the first pangenome of the species, built from 20 strains, was published in 2021 [49]. If the in silico characterization of *F. graminearum* secretome [49,50] is now available, our knowledge about the effective in planta effectome remains fragmented and needs to be clarified. Several in planta studies outlined a highly dynamic and complex molecular dialogue between wheat and *F. graminearum*, involving a stage-specific delivery of the effectors [19,25,51,52]. However, the impacts of wheat and *F. graminearum* genetic backgrounds are largely un-

known. In a previous proteomics study, *F. graminearum* infection strategy was described in three strains of contrasting aggressiveness facing three wheat cultivars of contrasting susceptibility at one time point, resulting in the identification of highly conserved fungal determinants of the infection [20]. Owing to its higher ability in detecting low-abundant molecules, as are the fungal molecules within host tissues, applying RNA-seq technology over infection progress appears as a promising approach to complete the picture of *F. graminearum* effectome during FHB and to identify its core components.

The presented work addresses qualitative and quantitative characterization of *F. graminearum* paneffectome using an RNAseq-based profiling of the effector coding genes during a time course infection in a two-pronged approach: one using a single aggressive strain facing five wheat cultivars of contrasting susceptibility and a second using three strains of contrasting aggressiveness infecting a single susceptible host. Our results led to the first detailed in planta description of the *F. graminearum* effector repertoire and identified the dynamics of key drivers of the infection process.

2. Results

Fusarium graminearum transcripts were detected in all infected plant samples with an increase of assigned read pairs along with the infection progress, as expected. In the Host Variability (HostV) experiment, the assignment to *F. graminearum* genome accounted for 1.7%, 8.3% and 16.2% of the assigned pairs at 48 h post-inoculation (hpi), 72 and 96 hpi, respectively (Supplementary Table S1A). In the Pathogen Variability (PathoV) experiment, it corresponded to 3.5%, 8.6% and 22.6% of the assigned pairs at 48, 72 and 96 hpi, respectively (Supplementary Table S1B).

2.1. In Planta Expression Signature of the *F. graminearum* Gene Set Coding Secreted Proteins

A total of 9544 and 9898 *F. graminearum* transcripts were reliably identified in the HostV and PathoV experiments, respectively (Figure S1A). These two expressed gene sets shared 93% of their accessions. They included 1105 and 1162 genes encoding putative secreted proteins (SP genes) in HostV and PathoV, respectively, accounting for 1183 unique gene accessions (Supplementary Table S2 and Figure S1B). Noticeably, the pangenome mapping approach allowed the identification of 151 new fungal transcripts, absent from the PH-1 reference genome, of which 16 genes were SP genes.

In the HostV data set, 974 SP genes (88.2%) among the 1105 expressed SP genes were expressed in all hosts independently of their susceptibility level, thus representing a host-independent SP gene set (Figure 1A). About 8.7% of the SP gene set (96 genes) was expressed only in some of the different hosts, of which 28% were not detected in the Asian wheat line 'Chinese Spring' (CS). A total of 35 genes were identified in only one host of which 43% were specific to the FHB susceptible wheat cultivar 'Recital' (REC). A total of six newly characterized gene accessions, not present in the PH-1 reference genome, were detected, including three genes expressed in all the hosts. In the PathoV data set, 1016 SP genes (87.4%) among the expressed SP genes were expressed in all strains independently of their aggressiveness; they form a strain-independent SP gene set (Figure 1B). About 5.5% of the PathoV SP gene set (64 genes) was expressed only by some of the different strains. A total of 82 genes were identified in only one strain and nearly 45% of them were found in the most aggressive strain MDC_Fg1. The PathoV data set resulted in 15 newly identified SP genes of which seven were specifically expressed by MDC_FgU1, four others by MDC_Fg1 and two by MDC_Fg13. SP genes expressed systematically in all the infected hosts and by all the different strains accounted for more than 80% of the whole SP detected genes in both HostV and PathoV experiments (Figure 1C).

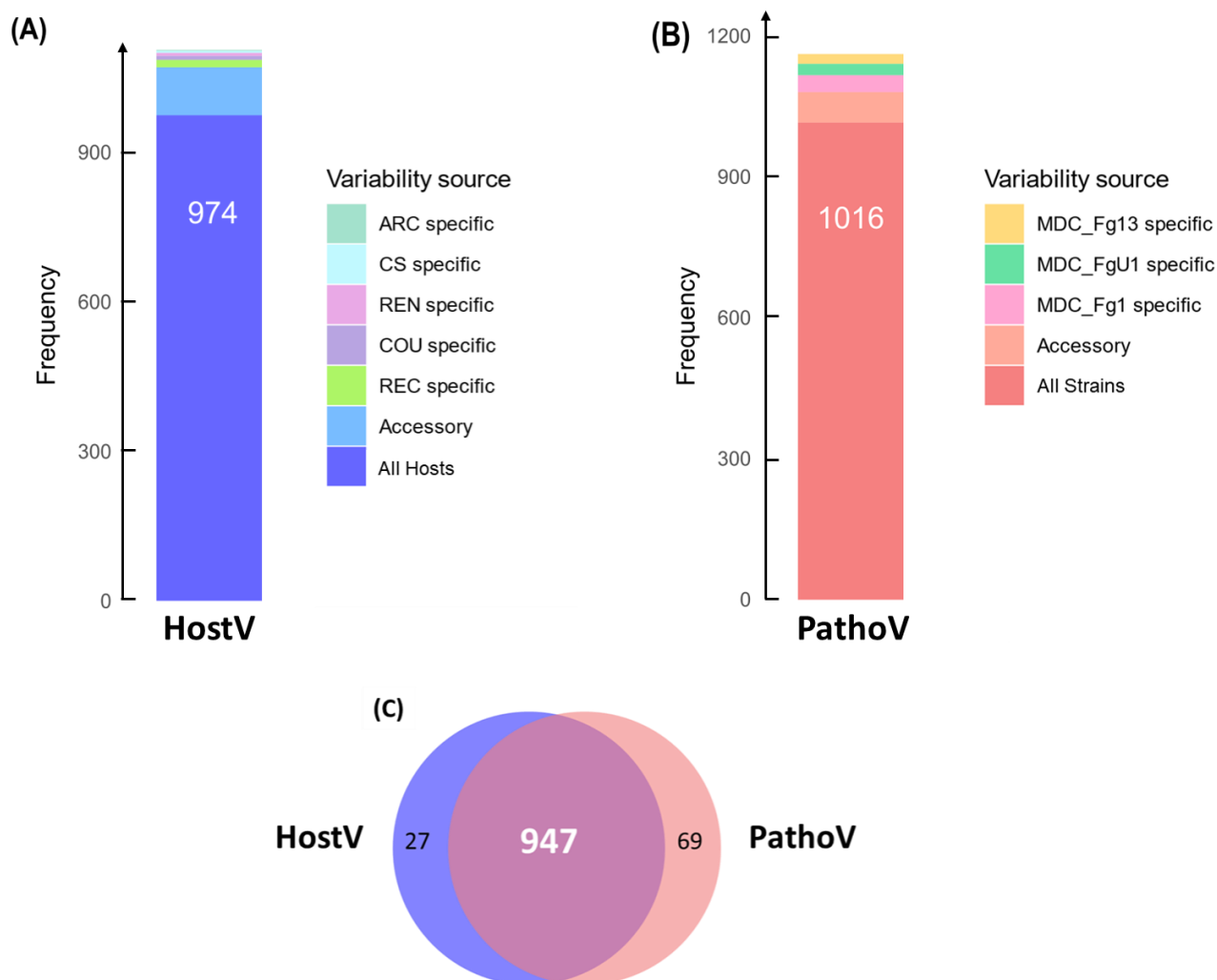


Figure 1. In planta expression signature of *F. graminearum* genes coding for putative secreted proteins. Barplots represent the structure of the gene sets coding for putative secreted proteins expressed in planta for the HostV (A) and the PathoV (B) experiments. HostV barplot displays the number of genes expressed by the strain MDC_Fg1 in all the infected hosts, in some hosts (Accessory) and in only a specific host: ‘Arche’ (ARC) specific, ‘Courtot’ (COU) specific, ‘Chinese Spring’ (CS) specific, ‘Recital’ (REC) specific or ‘Renan’ (REN) specific. PathoV barplot displays the number of genes expressed by the three strains MDC_Fg1, MDC_Fg13 and MDC_FgU1, by two strains only (Accessory) and by only one strain (MDC_Fg1 specific, MDC_Fg13 specific, MDC_FgU1 specific). The Venn diagram (C) represents the intersection of the gene sets expressed in all the hosts (HostV) and expressed in all the strains (PathoV).

2.2. Characterization of Differential Expression of SP Gene Sets at the Early Stages of Infection

2.2.1. Fungal Gene Expression in Different Host Cultivars

Among the 3180 HostV differentially expressed genes (DEGs) (Figure S2A), a significant enrichment of SP genes was found (p -value $< 1.13 \times 10^{-64}$), summing 626 accessions significantly impacted by at least one of the tested factors (host, infection progress or their interaction effect) (Figure 2A). They included 201 (32.1%) and 150 (24%) genes whose expression was only impacted by the infection progress (infection regulated genes; Infection-DEGs) and by the host genetic background (host regulated genes; Host-DEGs), respectively, while 204 (32.6%) genes exhibited basal expression differences depending on the infected host cultivar along with differential expression driven by the infection progress (Host + Infection regulated genes; H + I-DEGs). Only 11.3% of the SP DEGs (71 genes) displayed a significant interaction of the two main factors (Host \times Infection Progress regulated gene

set; HxI-DEGs), depicting differences in expression dynamics in the different host cultivars. Overall, 476 SP genes (76%) were significantly regulated during the infection progress.

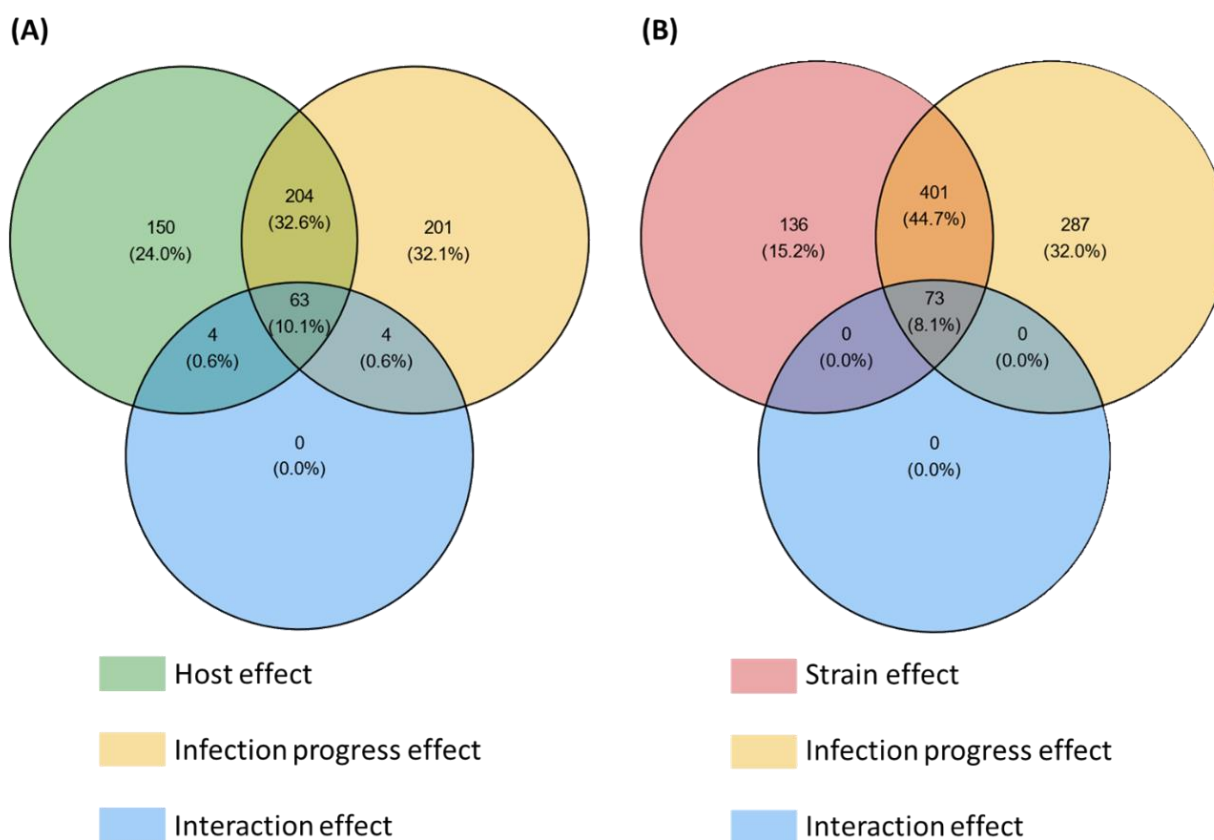


Figure 2. Number of secretome genes from the HostV (A) and the PathoV (B) experiments significantly impacted by the different effects tested in the differential expression (DE) analysis. For each factor of the DE analysis, the Venn diagrams indicate the number of genes displaying significant expression variations. Significance threshold: p -value corrected by Benjamini–Hochberg method < 0.001 .

2.2.2. Fungal Strain Specificities

Among the 5879 PathoV DEGs (Figure S2B), a significant enrichment of SP genes was found (p -value $< 3.85 \times 10^{-42}$), summing 897 accessions significantly impacted by at least one of the tested factors (strain, infection progress or their interaction effect) (Figure 2B). SP genes whose expression was only impacted by the infection progress (Infection-DEGs) were more than twice as numerous as those only regulated by the strain genetic background (Strain-DEGs) with, respectively, 287 (32%) and 136 (15.2%) genes, while 401 genes (44.7%) exhibited basal expression differences between the different strains along with differential expression driven by the infection progress (Strain + Infection regulated genes; S + I-DEGs). Only 8.1% of the SP DEGs (73 genes) displayed a significant interaction of the two main factors (Strain \times Infection Progress regulated genes; SxI-DEGs), depicting differences in expression dynamics depending on the strain. Overall, 761 SP genes (85%) were significantly regulated during the infection progress.

2.3. Parsing Secretome Gene Sets towards Effectome Gene Sets That Are Regulated along the Infection Progress

Among the DEGs that proved to be SP genes, those demonstrating fluctuating transcription during the interaction are supposed to be essential components of the infection progress (as effectors are defined [32,35]). With this regard, we established two effectomes in both HostV and PathoV data sets with, respectively, 476 and 761 accessions, by selecting

only SP genes with varying expression levels during the infection progress (Supplementary Table S3A,B).

In the HostV data set, the aggressive strain MDC_Fg1 displayed 91% (433 genes) of its effectome in all the infected wheat genotypes (Figure 3A). Genes found in only a subset of hosts accounted for 35 genes including two newly identified genes absent from the PH-1 genome while only eight effector genes were host-specific. To gain a better understanding on the impacts of host on fungal gene expression along with the infection progress, we computed a Partial Least Squares Discriminant Analysis (PLS-DA) on the genes expressed in all the hosts (Figure 4A). The first component, explaining 42% of total variance, clearly discriminated the infection stages with the 48 hpi time point on the right side and the 96 hpi time point on the left side of the PLS-DA. For all hosts except CS, the 72 hpi time point marked a transition between the 48 hpi and 96 hpi time-points. The second component, explaining 17% of the variance, discriminated the Asian and spring wheat line CS from the European and winter wheat lines.

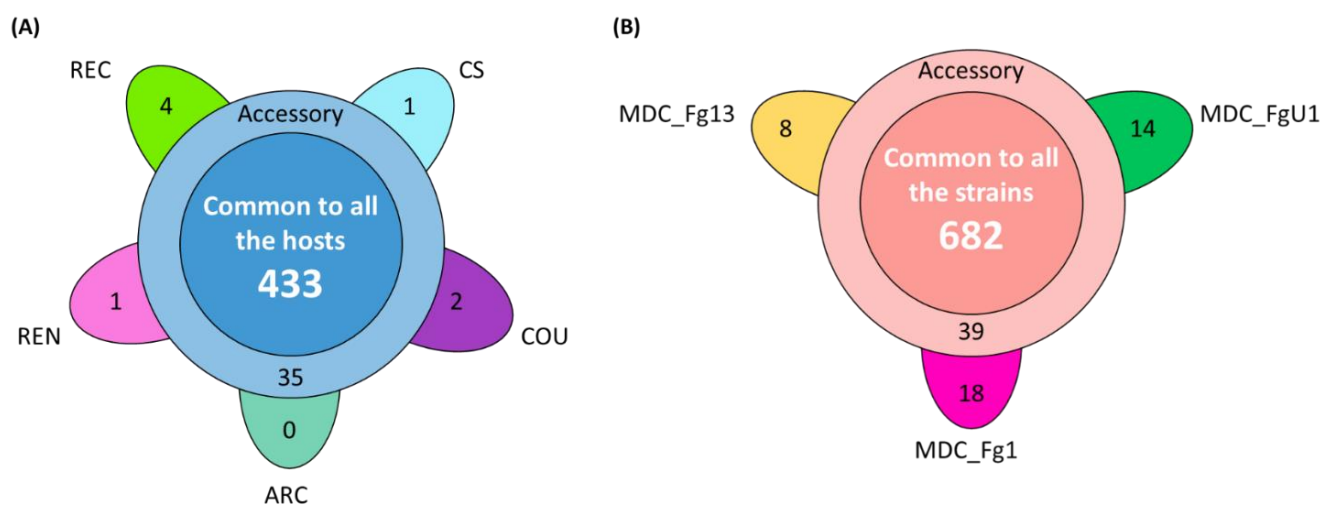


Figure 3. Structure of the HostV (A) and PathoV (B) effectome gene sets. These sets gather the genes significantly regulated along infection progress and coding for putative secreted proteins. (A) The flower plot displays the number of genes expressed by the strain MDC_Fg1 in all the infected hosts (center circle), in some hosts (annulus) and in only a specific host (petals). (B) The flower plot displays the number of genes expressed by the three strains MDC_Fg1, MDC_Fg13 and MDC_FgU1 (center circle), by two strains only (annulus) and by only one strain (petals).

In the PathoV data set, the three different *F. graminearum* strains also displayed a conserved effectome gene set, including 90% (682 genes) of the effectome (Figure 3B). As above, effectome genes identified in a subset of strains or in only one strain gathered 39 and 40 genes, respectively; eight of those were newly identified genes absent from the PH-1 genome. The PLS-DA on effectome genes expressed by the three strains showed that the first component, explaining 47% of the variance, also clearly discriminated the stages of infection with the 48 and 96 hpi time points located on the opposite sides of the factorial plane and the 72 hpi time point in between (Figure 4B). The second component of the PLS-DA, explaining 13% of variance, differentiated the most aggressive strain MDC_Fg1 from the two other strains (MDC_Fg13 and MDC_FgU1).

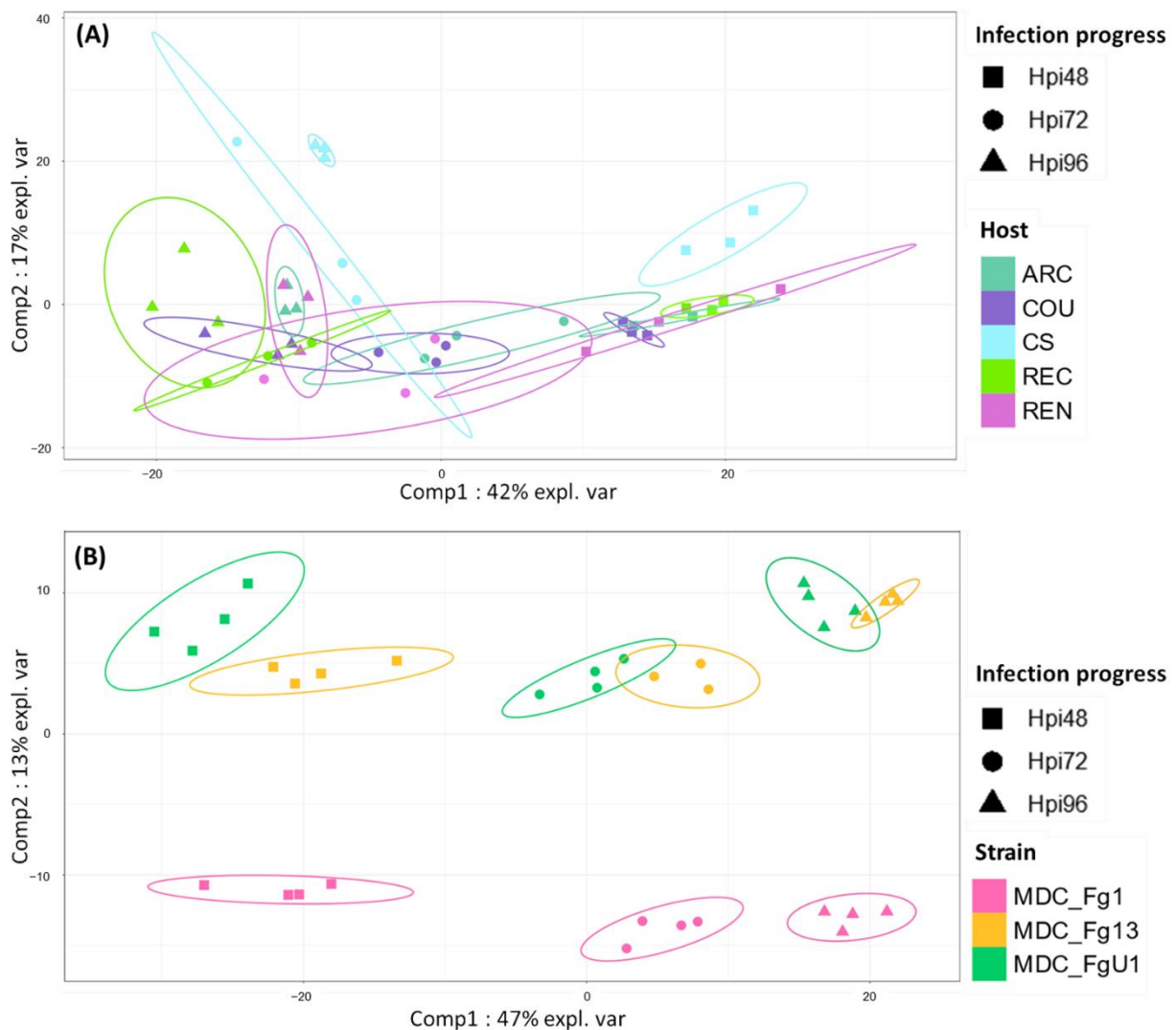


Figure 4. Discrimination of HostV (A) and PathoV (B) effectome gene sets expressed in all the hosts or by all the strains according to the experimental conditions. PLS-DA method was applied on the 433 genes expressed in all the hosts for HostV (A) to predict the host–infection progress combinations and on the 682 genes expressed by all the strains for PathoV (B) to predict the strain–infection progress combinations. The plots of the individuals extracted from the PLS-DA are represented on the two first components. For each condition, confidence ellipses are plotted to highlight discrimination strength (level set to 95%).

2.4. Different Wheat and *F. graminearum* Genetic Backgrounds Evidenced a Relevant Core Effectome Gene Set Expressed at Specific Infection Stages

As a whole, 357 effector genes were expressed in all the infected hosts by MDC_Fg1 and in Recital by the three strains; they represent an in planta core effectome of *F. graminearum* (Supplementary Table S4). Gene and sample Hierarchical Ascendant Clustering (HAC) applied to HostV and Pathov expression data, confirmed the strong impact of infection kinetics on quantitative differences in the core effectome gene set (Figure 5). Genes were expressed per waves and allowed to distinguish the early stages of infection (48 hpi) from the later ones. In comparison with HostV, the PathoV experiment clearly distinguished the intermediate time point (72 hpi) from 48 hpi. More than one-third of the core effectome gene set systematically displayed higher expression levels at 48 hpi as compared to 72 and 96 hpi in both experiments (clusters HostV-1, -2 and clusters PathoV-1, -2) while nearly 60% demonstrated higher expression levels at the latter stages of infection (clusters HostV-4, -5,

-6, -7 and clusters PathoV-3, -4, -6). Gathering up to 94% of identical gene accessions, these expression patterns were highly reproducible in both the HostV and PathoV experiments.

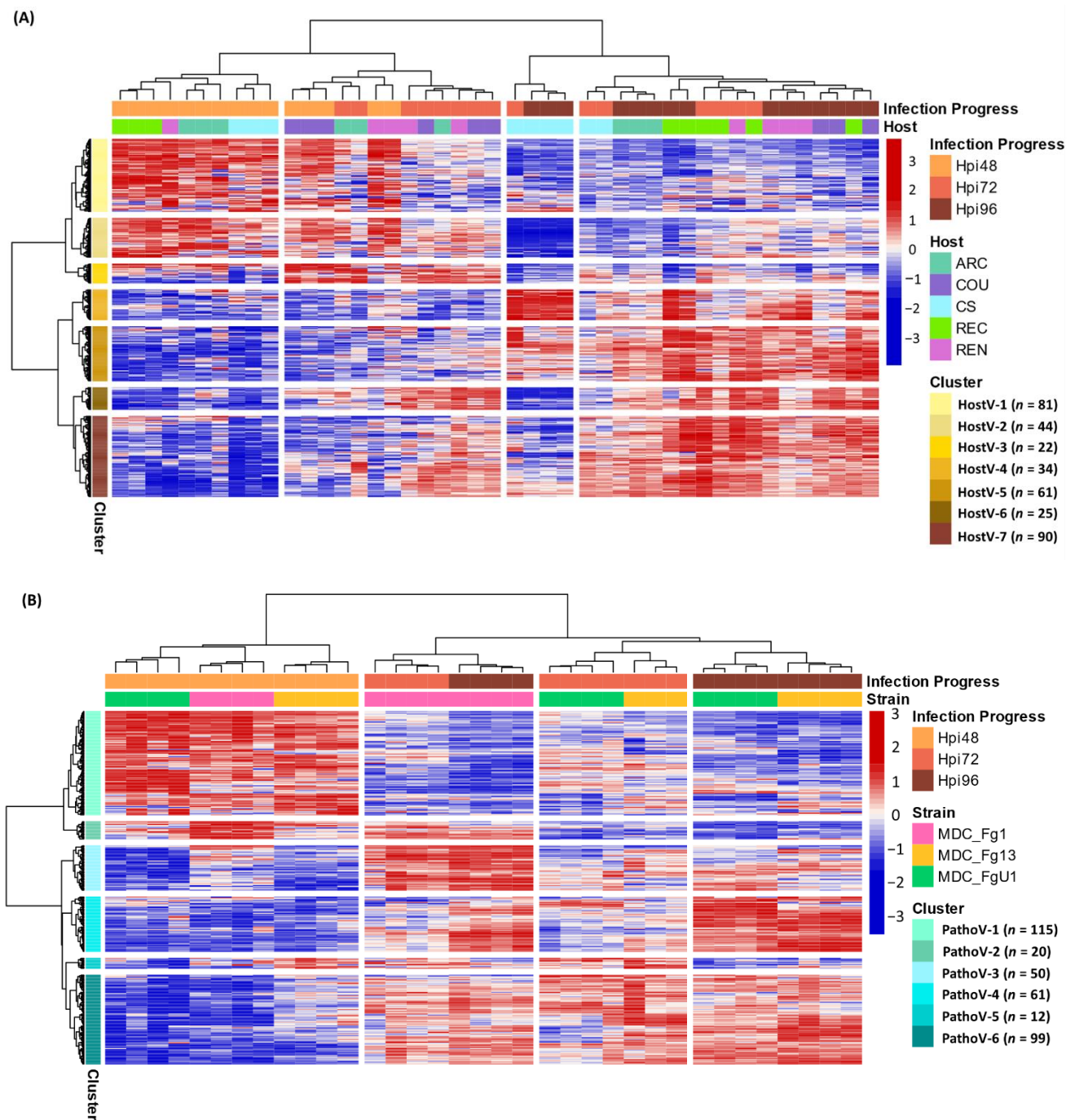


Figure 5. Expression regulation patterns of the core effectome genes along with the infection progress in HostV (A) and PathoV (B) data sets. The structure of gene and sample data sets were determined by HAC based on Ward's minimum variance method using the z-score transformed gene expression values. Heatmap color scales represent the z-score transformed expression values of the genes from the core effectome gene set for each sample. The clustering on top of the heatmap represents the experimental conditions which are labeled according to the factors Infection Progress and Host for the HostV experiment, and Infection Progress and Strain for the PathoV experiment. The clustering on the right side of the heatmap represents the genes, which are colored according to their cluster membership.

2.5. Host and Strain Driven Regulations of the Core Effectome Gene Set

2.5.1. Host Cultivar Effects

Basal gene regulations driven by the host genetic background were observed for 43% of the core effectome (154 H + I DEGs; Supplementary Table S3) while a difference in gene expression dynamics in different hosts was found for almost 17% of the core effectome (59 HxI DEGs). In addition, the HAC showed that all 72 hpi samples did not cluster together, thus demonstrating that expression patterns can depend on the host (Figure 5A). This exemplifies that according to the infected host, the magnitude of the gene expression increase or decrease could vary. Indeed, the 72 hpi samples of 'Arche' (ARC), 'Courtot' (COU) and 'Renan' (REN) hosts displayed more similarities with most of the 48 hpi samples while the 72 hpi samples of CS and REC hosts were clustered with the 96 hpi samples. These differences were mainly observed in the clusters HostV-1, -2, -4 and -5. For instance, in cluster HostV-4, the gene expression increase was stronger and continuous between 48 and 96 hpi in CS and REC while it mainly took place between 72 and 96 hpi in ARC, COU and REN hosts. Finally, MDC_Fg1 displayed specific gene expression patterns at 96 hpi in CS when compared to the other hosts. In the cluster HostV-2 and -4, MDC_Fg1 gene expression changes were larger when facing CS than the other hosts while in cluster HostV-6 and -7, changes proved to be smaller.

2.5.2. Fungal Strain Effects

Almost 65% of the core effectome gene set displayed strain effects, including 184 S+I DEGs and 46 SxI DEGs (Supplementary Table S3). This demonstrates that depending on the strain genetic background, basal gene expression level can be different. Although all 48 hpi samples were clustered together in the HAC, differences at the gene expression level discriminated the three strains (Figure 5B). For instance, clusters PathoV-2 and -3 clearly distinguished MDC_Fg13 and MDC_FgU1 from MDC_Fg1 displaying stronger expression levels while the cluster PathoV-6 clearly distinguished MDC_Fg13 with higher expression levels than the two other strains. The 72 and 96 hpi samples clearly separated MDC_Fg1 from MDC_Fg13 and MDC_FgU1 mainly through the gene clusters PathoV-2, -3 and -4. In cluster PathoV-5, the three strains were clearly discriminated from each other at each time point.

2.6. *Fusarium graminearum* Putative Effectome Displayed Several Targets in Wheat Spikes at Different Infection Stages

A predicted localization within the host was found for 268 proteins (75.07%) of the 357 proteins belonging to the core effectome (Figure 6, Supplementary Table S4). Nearly 90% of these proteins displayed a unique predicted localization. Apoplast localization was found for 85% (227 proteins) of the proteins while the others harbored at least one subcellular localization within the host. Both apoplast and cytoplasm localizations were predicted for 29 proteins. The host nucleus was the main subcellular target, gathering 65% (46) of the proteins with a predicted localization. The chloroplast was the second target with 22 proteins while the host mitochondria included only 11 proteins. Multiple predicted intracellular localizations were found for eight proteins. Genes supposed to be secreted in the apoplast or which failed to identify any known subcellular localization signal, displayed mainly increasing expression along with the infection progress. As for the genes coding for proteins supposed to enter host cells, the ratio between genes of increasing and decreasing expression was balanced.

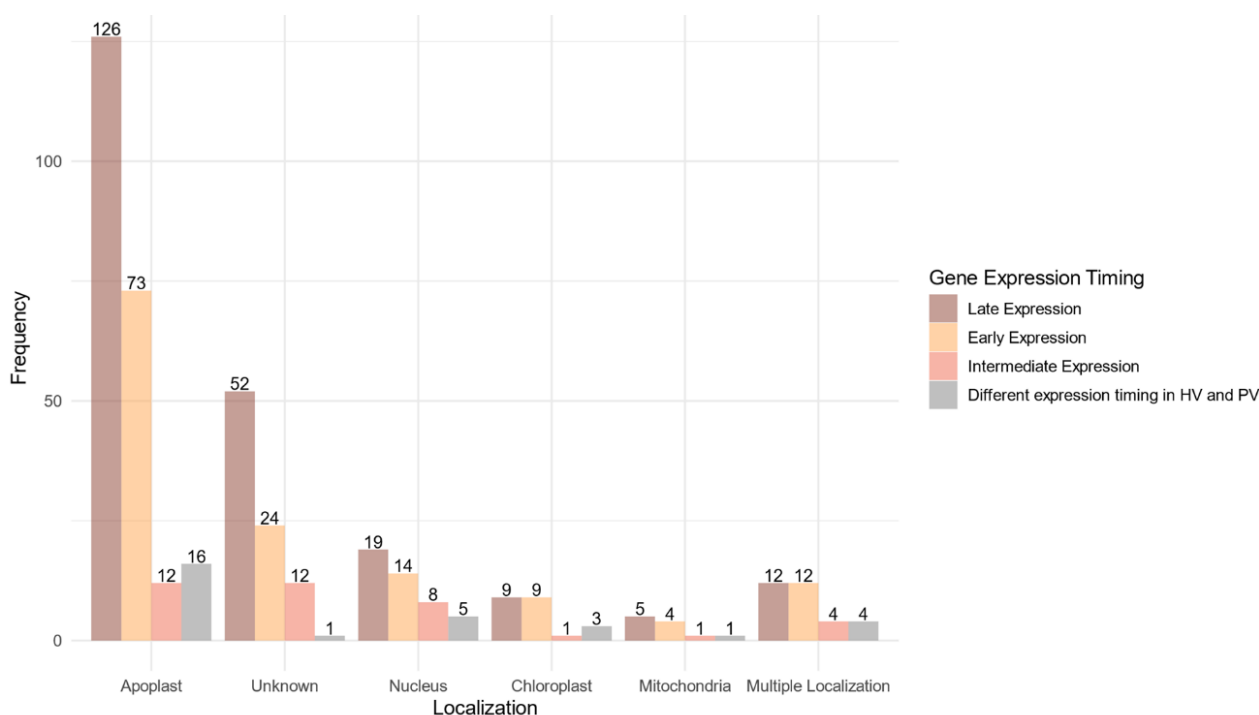


Figure 6. Localization within the host of the fungal proteins encoded by the core effectome gene set according to the expression timing during the infection progress. Barplots represent the frequencies of the predicted localizations within the host of the proteins coded by *F. graminearum* genes expressed at the early stages of the infection (Early Expression), at intermediate stages of infection (Intermediate Expression), at latter stages of infection (Late Expression) in both HostV and PathoV experiments or expressed with different dynamics between the HostV and PathoV experiments.

2.7. Identification of Additional Effector Features

2.7.1. In Silico Prediction

Based on protein size, net charge and amino acid content [53], EffectorP2.0 led to the identification of 66 putative effectors among the core effectome; 53 genes displayed apoplast localization (Supplementary Table S4). Nearly 55% of them showed an increasing expression pattern along with the infection progress while 32% showed a decreasing expression pattern. Three other genes harbored a chloroplast localization signal; all of them were of increasing expression along with the infection progress. Only 1 gene harbored a plant nuclear localization signal and 1 other with a mitochondria transit peptide; both displayed different expression dynamics between the HostV and PathoV experiments.

2.7.2. Localization in the Fast-Evolving Subgenome

Because previous comparative genomic studies demonstrated that *F. graminearum* putative pathogenicity-related genes were located in variable regions of the genome [49,54,55], the distribution of our core effectome on *F. graminearum* genome was studied (Supplementary Table S4). At both inter- and intrachromosomal level, spatial distribution of the core effector genes was uneven (Figure S3). Chromosome 1 accounted for 79 genes, chromosome 2 for 118 genes, chromosome 3 for 94 genes and chromosome 4 for 66 genes. With 13 and 12 genes per Mb, respectively, chromosomes 2 and 3 displayed higher effector gene densities than chromosomes 1 and 4 (6.7 and 7 genes per Mb, respectively). At the intrachromosomal level, genes were preferentially located in the telomeres and in the central regions of the chromosomes. Two-thirds of the core effectome genes were located in the fast-evolving subgenome [55]. While the distribution of intermediate and late expressed genes was relatively balanced on the genome, early expressed genes were mainly located on chromosome 2 (41.5%).

2.8. Predicted Functions of the Core Effectome Are Highly Diverse

As a whole, 140 Pfam and 66 GO terms characterizing 230 and 126 genes, respectively, were found in the core effectome (Supplementary Table S4). The gene set was significantly enriched in two GO Biological Process terms: ‘carbohydrate metabolic process’ and ‘proteolysis’ with 35 and 15 annotated genes, respectively. At the molecular function level, the core effectome was enriched in ‘metallocarboxypeptidase activity’, ‘hydrolase activity, hydrolyzing O-glycosyl compounds’, ‘polygalacturonase activity’, ‘hydrolase activity’, ‘alpha-L-arabinofuranosidase activity’ and ‘flavin adenine dinucleotide binding’ functions. Finally, the core effectome was enriched in the ‘extracellular region’ as GO Cellular Component term. The search for peptidases in MEROPS database and CAZymes in dbCAN database yielded 38 and 98 genes of the core effectome genes, including 4 and 16 EffectorP-predicted effectors, respectively. Moreover, 36 genes were found in PHI-base, including 22 matches with *F. graminearum*. Fifteen genes were assigned to the ‘unaffected pathogenicity’ category while 16 and 3 other genes were assigned to the ‘reduced virulence’ and ‘reduced virulence_unaffected pathogenicity’ categories, respectively. One gene matched with a *F. graminearum* accession and belonged to the ‘reduced virulence_increased virulence (hypervirulence)’ category. Finally, one gene corresponded to a validated effector that belonged to the category ‘effector (plant avirulence determinant)_increased virulence (hypervirulence)’. Effects on pathogenicity or virulence were shown for 8 EffectorP-predicted effectors.

3. Discussion

Taking advantage of different *F. graminearum* strains and wheat host cultivars, this study identified the in planta expression of 840 unique fungal genes harboring effector-associated features (i.e., genes encoding secreted proteins and differentially expressed during the FHB infection), including nine new accessions not included in the reference PH-1 genome. These represent almost 5% of *F. graminearum* pangenome and more than 35% of its putative pansecretome [49].

3.1. *Fusarium graminearum* Infection Involves a Highly Conserved Effectome

Transcriptome profiling of effector coding genes conducted on the three *F. graminearum* strains of contrasting aggressiveness and on the five hosts of contrasting susceptibility to FHB revealed highly conserved effector repertoires. We demonstrated that the three strains shared 90% of their effector-gene transcripts. While at the genomic scale, these three strains shared only 58% of their theoretical secretome [49], our results corroborate a previous in planta proteomics study demonstrating that nearly 100% of the whole identified secreted proteins were accumulated in the same three strains [20]. This emphasizes that the effective infection process of the three strains on wheat is based on a conserved effectome that controls critical plant processes to ensure the success of the infection. Similar results were already found in other pathosystems. For instance, the gene expression analysis of six *Puccinia triticina* strains highlighted a highly conserved infection strategy with 85.7% of the identified secretome genes expressed by all the strains during wheat infection [44]. Similar findings were also reported in the maize–*Exserohilum turcicum* interaction where 97% of the putative effector genes were shared by two strains [48]. Extending this analysis to the role of host genetic background on the expressed effectome, our data also demonstrated a highly conserved infection strategy in different wheat cultivars of contrasting susceptibility to FHB that engages a common effector repertoire shared at 91%. Few hosts’ specific gene expressions were already outlined at the whole-transcriptome scale [56]. Our results support similar conclusions with a special focus on the effectome gene set and are consistent with our previous proteomics study demonstrating the accumulation of the same fungal proteins in different wheat hosts [20]. A core effectome composed of 357 genes expressed by all the strains and in all wheat hosts (Figure 7) exemplified the highly conserved infection program established by *F. graminearum*. Because the interaction is systematically producing FHB disease regardless of the strain aggressiveness or the host susceptibility, these genes likely include key drivers of FHB in bread wheat. Their functions are thus supposed to be

crucial determinants of basal processes powering the FHB development in wheat, including 66 putative effector genes and 21 Phi-base matches known to be involved in pathogenicity.

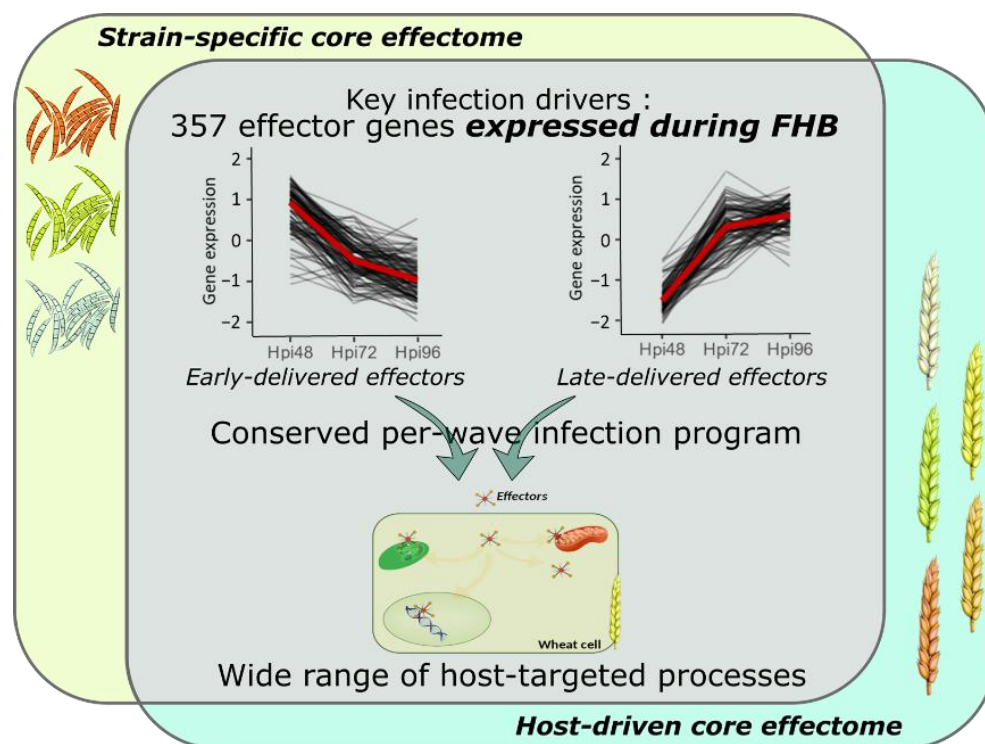


Figure 7. Model summarizing the conserved and complex *F. graminearum* infection strategy on wheat spikes. As a whole, 357 effector genes were identified as the key drivers of FHB infection expressed by all the strains and in all the infected hosts; they represent the *F. graminearum* core effectome. These genes were expressed at very specific infection stages in a per-wave manner, including genes highly expressed at the very beginning of the interaction with the wheat tissues and others highly expressed in the later stages of the infection. The timing of gene expression was mostly conserved independently of the strain or the host. Targeted processes within the host are highly diverse with a wide array of targeted compartments and predicted functions.

3.2. *F. graminearum* Core Effectors Are Delivered in a Conservative Per-Wave Expression

The core-effectome demonstrated to be deeply remodeled along with the infection progress, depicting the dynamic nature of its components. As previously shown, putative effector proteins were proved to be accumulated at specific stages of the infection process evidencing a specific transition that distinguishes early from late protein accumulations [19]. In line with this previous work, we also observed changing gene expression patterns at the same time (48 to 72 hpi transition), thus corroborating the major reorganization of the molecular arsenal that drives FHB infection. Furthermore, the fine-tuned timing of gene expression was mostly preserved in terms of dynamics for all the strains independently of their aggressiveness and in all the infected hosts independently of their susceptibility level, suggesting that *F. graminearum* set up a widely conserved genetic program with crucial functions required at very precise infection stages (Figure 7). This conserved infection program may be representative of *F. graminearum* generalist lifestyle, i.e., interacting with a wide range of hosts and spreading in different tissues [2,57], which results in a lower selection pressure and coevolution with a specific host species [43,58,59].

Besides these conserved infection patterns, some specific regulations in effector-genes were also found in the different fungal strains, but no clear link between gene expression magnitude and aggressiveness has been observed. Identified effector-genes were mainly located in the fast-evolving part of *F. graminearum* genome, characterized by genes of shorter size, larger variations in exon content and a higher proportion of synonymous

and nonsynonymous mutations, together with genes known to be highly transcribed during plant infection in comparison with fungal vegetative growth [49,54,55]. In our study, chromosome 2 for instance, displaying the highest density of polymorphism, also exhibited the highest effector gene density [54]. This polymorphism could explain a part of the observed strain-specific effects on effector-genes expression levels and further protein accumulations. Moreover, intrinsic characteristics of both MDC_Fg1, i.e., a French isolate [60], and ‘Chinese Spring’, i.e., an Asian spring cultivar, might be the main cause of *F. graminearum* specific expression patterns of the late-delivered effectors observed when facing ‘Chinese Spring’ in comparison to the European winter wheat cultivars, suggesting a remarkable ability to adapt to the different molecular contexts expressed in different wheat cultivars.

3.3. *F. graminearum* Infection Strategy Involves Integrative Host Cellular Processes

The search for localization signals within the *F. graminearum* secreted protein sequences revealed that putative effectors can target host apoplast, as well as different subcellular compartments including nucleus, chloroplast and mitochondria at several infection stages (Figure 7). Along with the relatively high diversity of predicted functions (66 GO terms and 140 Pfam), this supports that the infection success is based on a wide array of manipulated host pathways and echoes previous studies that evidenced the diverse nature of processes involved in FHB susceptibility [19,20,23].

Host apoplast appeared as the main target of the *F. graminearum* core effectome, including 53 putative genes with additional effector features, i.e., small cysteine-rich proteins. These genes gathered 65 CAZymes that depict the role of cell-wall degradation during FHB to promote host colonization and nutrient acquisition [61]. Eighteen others belonged to peptidases suggesting that *F. graminearum* is able to override host defense mechanisms especially by interacting with chitin and glucan-triggered immunity and inhibiting host enzymes and proteases as well as to acquire nutrients [62–65]. Besides these proteases, a guanine-specific ribonuclease was also predicted as a core apoplastic putative effector extending the control of plant stress responses to secreted nucleotidases [61,66]. In addition, three killer toxin KP4-like genes were also identified. Although a previous work has already shown their upregulation during wheat seedling rot disease and FHB, their role in virulence was proved only in seedling rot disease [67].

Intracellular core effectors of *F. graminearum* mainly targeted host nucleus, including two that match with validated virulence factors, a cysteine-rich secretory protein [68] and a PhoD-like phosphatase protein [69,70] along with one gene with additional effector features, i.e., a small cysteine rich protein. Through its eight predicted core nuclear proteases, *F. graminearum* might reprogram host gene expression by interfering with the plant’s transcription factors. This strategy was already found in the pathogenic bacteria *Xanthomonas euvesicatoria* and *Pseudomonas syringae* that target transcription factors involved in phytohormone pathways [71,72]. Nuclear effectors are also known to act on host transcription machinery by a direct binding on DNA, such as the *Melampsora larici-populina* Mlp124478 effector that represses genes involved in defense mechanisms [73]. A same strategy might be involved in the *F. graminearum* infection process though its own nuclear effectors.

Chloroplast and mitochondria were also important targets of *F. graminearum* core effectome, including three and one genes encoding small cysteine rich proteins, respectively, as well as a putative mitochondrial PhoD-like phosphatase virulence factor [69,70]. These organelles represent important biological hubs interconnecting primary metabolism, energy production, signaling pathways and plant responses to stress [74,75]. The inhibition of the defense mechanisms through the manipulation of chloroplast [76] and mitochondrial [77] processes was already evidenced in several plant–fungi interactions and proved here to be part of the *F. graminearum* infection strategy. In the case of the chloroplast, its central role has already been described in previous FHB studies [19,20,23]. Finally, effectors with multiple host targets were also detected, suggesting that one effector can achieve completely different functions during the infection progress. An effector targeting both the chloroplast

and the mitochondria was validated in poplar—*Melampsora larici-populina* [78,79] and, as it was outlined in *Blumeria graminis f. sp. hordei* with the BEC1054 RNase-like effector, those versatile effectors seem to disturb one specific process, such as a host's defense mechanisms, at several levels by interacting with multiple host proteins [80].

4. Materials and Methods

4.1. Experiments and Biological Material

The current study is based on two experiments, Host Variability (HostV) and Pathogen Variability (PathoV). In HostV, the aggressive strain MDC_Fg1 was inoculated in five bread wheat cultivars, including four French winter genotypes: 'Arche' (ARC), 'Courtot' (COU), 'Recital' (REC), 'Renan' (REN) and the Asian spring genotype 'Chinese Spring' (CS). In PathoV, three *F. graminearum* strains of contrasting aggressiveness, in decreasing order of aggressiveness MDC_Fg1, MDC_Fg13 and MDC_FgU1 [20] were inoculated in the susceptible wheat cultivar, 'Recital' (REC). For both experiments, samples were collected at 48, 72 and 96 h post-inoculation (48, 72 and 96 hpi) (Figure S4).

4.1.1. Preparation of the *Fusarium graminearum* Inoculum

Spores for all the strains were generated according to the same protocol. Mycelium was grown on Potato Dextrose Agar (PDA) medium (39 g of PDA for 1 L of reverse osmosis water) during eight days in the dark at 23 °C. To generate spores, mycelium plugs were suspended in a Mung Bean Broth medium (MBB, 40 g of organic mung bean per L of reverse osmosis water) during seven days in the dark at 23 °C and with an agitation of 150 rpm. Spores were isolated and stored in sterilized water at −20 °C.

4.1.2. Plant Growth Conditions

Wheat seeds were sown in buckets and kept at 20 °C during two weeks for germination. While Chinese spring plantlets were kept at 20 °C, an eight-week vernalization was performed at 4 °C and with a 8:16 (L:D) light cycle for the winter cultivars. Then, all plantlets were transplanted in 4 L pots and transferred into a controlled growth chamber with an automatic watering system. To allow tillering, plants were grown during three weeks at 17 °C/15 °C (day/night), a relative humidity of 70% and a 12:12 light cycle. Then, the experimental conditions were set as follow: 16:8 light cycle with 21 °C/17 °C (day/night) temperatures and a constant relative humidity of 80%.

4.1.3. Experimental Procedures

Complete factorial experiments were designed for HostV and PathoV. For the HostV experiment, the combinations of the five host cultivars and the three time points measured on three biological replicates represent a total of 45 samples. For the PathoV experiment, the combinations of the three strains and the three time points measured on four biological replicates (except for MDC_Fg13 × 72 hpi modality with 3 replicates) represent a total of 35 individuals. Experimental designs were surrounded by additional plants to limit any edge effects. Infection was performed at mid-anthesis by inoculating 10 µL of inoculum at a concentration of 10⁵ spores/mL in the floral cavity of the six central spikelets of three synchronous flowering spikes. For each individual, the inoculated spikelets were collected and immediately placed in liquid nitrogen. Samples were ground in fine powder and stored at −80 °C before RNA extraction.

4.1.4. RNA Extraction and Sequencing

Total RNA was extracted from 100 mg of the frozen powder described above, using a TRIzol protocol (TRI reagent[®], Sigma-Aldrich, St. Louis, MO, USA), followed by a FastDNase treatment (TURBO DNA-free[™] Kit, Thermo Fisher Scientific, Waltham, MA, USA). Electrophoresis with 1% agarose gel buffered in Tris-Acetate-EDTA was used to control sample quality. An amount of 10 µg of RNA per sample were used for sequencing. cDNA libraries were prepared with the TruSeq Stranded preparation kit reverse oriented

(Illumina, San Diego, CA, USA). For HostV samples, 2×150 base paired-end sequences were generated using Illumina HiSeq4000 and NovaSeq6000 at the Genoscope, the French National center of sequencing [81]. Sequencing of PathoV samples was performed using Illumina NovaSeq6000 at GeT platform of GenoTOUL [82].

4.2. *Fusarium graminearum* Pangenome Construction and Characterization

To construct the reference *F. graminearum* pangenome, we used all the genomes assembled and publicly accessible. A total of 19 genomes of different origins assembled from this species [49,54,55,83,84] and previously characterized [49] were used along with the reference strain PH-1 genome GCA_900044135.1 assembly [85,86]. The assemblies of three strains (CS3005, DAOM_233423, DAOM_241165) were publicly available. Fourteen strains (INRA-156, INRA-159, INRA-164, INRA-171, INRA-181, YL-1, HN9-1, HN-Z6, MDC_Fg13, MDC_Fg5, MDC_Fg8, MDC_202, MDC_Fg593 and MDC_Fg851) were available as Illumina raw data and were assembled with SPAdes v3.13.0 [87]. The two strains MDC_Fg1 and MDC_FgU1 were available as Pacbio long reads and were assembled with HGAP4 implemented in SMRT Link v5.0 [88]. Then, we performed a mapping of each assembled genome on the PH-1 reference genome using Minimap2 v2.12 [89]. Contigs unmapped and larger than 1 kb in size have been recovered. Additionally, redundant contigs have been removed with Cd-hit v4.6.7 [90]. In this way, we completed the PH-1 reference genome and represented a reference pangenome. On the other hand, to create the GFF/GTF annotation files of the reference assembled pangenome, we used the MAKER2 v2.31.10 annotation pipeline [91] with a combination of evidence-based methods (transcriptome data and homology with known proteomes) and ab initio gene prediction with SNAP v2013-02-16 [92] and Augustus v3.2.1 [93]. The repeating elements of the assembled pangenome were masked using the library of all the repeated elements from the Repbase database (1 February 2017 update) [94] using RepeatMasker v4.0.7 [95]. This *F. graminearum* pangenome is composed of 17,647 protein coding genes, adding 3502 genes to the GCA_900044135.1 assembly of the reference strain PH-1.

The putative pansecretome of *F. graminearum* was generated from the panproteome using SignalP v5.0b software [96] for conventionally secreted proteins and using ApoplastP v1.0.1 [97] for unconventionally apoplastic secreted proteins. This added 437 putative secreted proteins to the reference strain PH-1 and achieved a total of 2339 proteins, including 1656 conventionally secreted proteins. Localizer v1.0.4 [98] was used on protein mature sequences generated by SignalP v5.0b to predict the protein's subcellular localization within the host. Gene Ontology (GO) and Protein family (Pfam) annotations were generated with Interproscan v5.52-86.0 [99]. To identify putative genes related to pathogenicity, Blastp [100] searches were performed against the Pathogen–Host Interaction database v4-10 (PHI-base) [101,102] and against MEROPS v12.1 for peptidases and their inhibitors [103]. Carbohydrate-Active Enzymes (CAZymes) screening was performed with HMMER v3.1b2 [104] against the dbCAN HMM profile database v9.0 [105]. Secreted proteins with additional effector features, i.e., small cysteine rich proteins, were predicted with EffectorP2.0 software [53].

4.3. RNA-Seq Bioinformatic Analysis

The RNA-seq data obtained in both HostV and PathoV experiments were analyzed separately using a dual-genome mapping approach, thus generating two independent data sets (Figure S4). Calculations were performed on the supercomputer facilities of the Mésocentre Clermont Auvergne University [106] and on the TGCC infrastructure of the CEA [107].

4.3.1. Data Cleaning Step

Raw reads were trimmed for adapters and low quality bases (phred score < 20) with TrimGalore v0.6.5 [108]. Noncalled bases and polyA tails were trimmed from reads with homemade Perl scripts. Then, low complexity (compression size < 65%) and short (size

< 60 nucleotides) reads were discarded from the fastq files using homemade Perl scripts. Sample files were decontaminated by mapping with STAR v2.7.1.a [109] against a homemade database of potential contaminants composed of 1649 viral genomes, 9267 bacterial genomes and the human genome. The genomes were downloaded from the NCBI Reference Sequence Database [110]. Best whole genome assembly available for each organism or clade without gaps and unlocalized scaffolds were used while genomes with more than ten successive ambiguous bases were discarded. Ribosomal RNA reads were removed with SortMeRNA v4.2.0 [111] against a database of wheat and *F. graminearum* rRNAs built from noncoding RNA genes fasta file of *Triticum aestivum* GCA_900519105.1 assembly [112] and PH-1 GCA_900044135.1 genome assembly [85].

4.3.2. Mapping and Assignment

Genome and annotation files of *Triticum aestivum* v1.1 [113] and *F. graminearum* (pangenome) were combined into a host–pathogen genome. This combined genome contains 269,428 (high confidence and low confidence genes) wheat genes and 17,647 *F. graminearum* genes. The cleaned RNA-seq files were mapped against this combined genome with STAR v2.7.1a. For each species of the pathosystem, gene-level counts were generated with featureCounts software from the subread v2.0.1 package [114]. Only uniquely mapped read pairs were considered at the read count step.

4.4. Statistical Analysis of *F. graminearum* Expression Data

Only *F. graminearum* expression data were considered in this study. HostV and PathoV experiments were statistically analyzed independently (Figure S4). Statistical analysis was conducted on R v3.6.3 [115].

4.4.1. Gene Filtering and Normalization

For both experiments, genes were filtered according to their expression levels. HostV and PathoV genes were filtered per host and per strain, respectively, with a 4 Counts Per Million (CPM) threshold in at least 3 samples independently of the time point. Counts were normalized according to library size with the trimmed mean of M values (TMM) method implemented in edgeR package [116,117].

4.4.2. Differential Expression Analysis

The differential analysis is based on a negative binomial generalized linear model, where the logarithm of the proportion of normalized counts for a gene is modeled by all the factors describing the experiment. For the HostV experiment, the log₂ of the average normalized gene expression is an additive function of a time effect (3 modalities), a host effect (5 modalities) and an interaction between the time and the host (15 modalities). For the PathoV experiment, the log₂ of the average normalized gene expression is an additive function of a time effect (3 modalities), a strain effect (3 modalities) and an interaction between the two factors (9 modalities).

To make the processing of these two complex multifactorial designs easier, Differential Expression (DE) analysis was made using DiffAnalysis_edgeR function of DiCoExpress [118], which is a script-based tool implemented in R using pre-existing R packages, including edgeR for the differential analysis. This function generates automatically a large number of contrasts. In the HostV experiment, we considered the difference between two hosts at a given time point, the difference between two time points given a host and the interaction effect defined as the difference between two time points given a host minus the difference between the same two time points given a different host. It leads to a total of 77 contrasts. In the PathoV experiment, we considered the difference between two strains at a given time point, the difference between two time points for a given strain and the interaction defined as the difference between two time points given a strain minus the difference between the same two time points given a different strain. It leads to a total of 27 contrasts. For each contrast a likelihood ratio test was applied and raw *p*-values

were adjusted with the Benjamini–Hochberg procedure to control the false discovery rate. A gene was declared differentially expressed if its adjusted p -value is lower than 0.001. Enrichment in secretome genes between expressed and DE gene sets was performed using a hypergeometric test with the ‘phyper’ function of the R stats package [119] with a p -value threshold of 0.01.

4.4.3. Expression Pattern Characterization

For the following analyses, raw count values were normalized using the regularized logarithm transformation (rlog) implemented in the DESeq2 package [120] before being z -score transformed. To assess the capacity of the selected genes to discriminate the samples according to the host or strain \times time point combinations and their relevance to describe the infection stages, we performed Partial Least Squares Discriminant Analysis (PLS-DA) using the R package mixOmics [121] with 10 components and default parameters for the other options. Heatmaps representing gene expression patterns were made using the Pheatmap package [122]. The Hierarchical Ascendant Clustering (HAC) of genes and samples was performed with the ward.D2 agglomeration method [123] applied on the Euclidean distance matrices.

4.4.4. Functional Enrichment Analysis

Over-representation of GO terms and Pfam in gene sets was performed using a hypergeometric test with the ‘phyper’ function of the R stats package [119]. The p -values were adjusted for multiple testing with Benjamini–Hochberg method and a threshold of 0.01 was applied to select the enriched GO terms and Pfam.

4.5. Genomic Localization of Effectome Genes

Genomic localizations of the genes were extracted from the GTF annotation file of PH-1 and their distribution on the chromosomes according to their expression dynamics was generated with the karyoploteR package [124]. Exact positions of the fast and slow subgenome of *F. graminearum* were used to link gene positions and genome structural information [55,125].

5. Conclusions

We depicted a detailed map of *F. graminearum* effector genes expressed in planta during FHB infection. They demonstrated that *F. graminearum* displayed a remarkably conserved, fine-tuned and complex infection strategy. This work brings new information about the early stages of wheat–*F. graminearum* interaction and paves the way to further functional analysis of the key fungal molecular drivers of the infection. Taken together with the previous statements made at the proteomic scale on both plant and fungal sides [20,23], our results reinforce the assumption that wheat’s susceptibility to *F. graminearum* is determined by a conserved set of molecular players from both partners. Therefore, core-effectors may lead to the identification of the necessary host susceptibility factors targeted by *F. graminearum* and represent a valuable resource for wheat breeding.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms23031914/s1>.

Author Contributions: Designed research, T.L. and L.B.; performed transcriptomics experiments, F.R. and L.B.; sample preparation, F.R., L.B. and G.P.; conceived and performed bioinformatic pipeline, F.R., P.L. and T.A.; conceived and performed the modeling, F.R., M.-L.M. and L.B.; analyzed the data and prepared the figures, F.R. and L.B.; wrote the paper, F.R. and L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research and PhD grant (F.R.) were funded by the Region Auvergne-Rhône-Alpes and the FEDER supports. This work is supported by France Génomique (WheatOMICS project) and the Agence Nationale de la Recherche of the French government (NewMyco project, ANR-15-CE21-0010). The authors also acknowledge the support received from the Agence Nationale de la Recherche of

the French government through the program “Investissements d’Avenir” (16-IDEX-0001 CAP 20-25). IPS2 benefits from the support of the LabEx Saclay Plant Sciences-SPS (ANR-17-EUR-0007).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: PathoV transcriptomics data on *Fusarium graminearum* that support these findings were deposited in the BioProject repository database, reference number PRJNA795285. HostV transcriptomics data that support the findings were deposited in the BioProject repository database, reference number PRJNA795096. Other data are available in the Supplementary Material of this article.

Acknowledgments: The authors thank Richard Blanc for its valuable help in the plant cultivation, Florence Cambon and Olivier Soudière for their assistance in RNA extraction and fungal spore production, as well as Hélène Rimbert for helpful discussions on methodology and cluster use. The authors are also grateful to Jérôme Salse for coordinating WheatOmics project. Computer calculation and storage facilities were provided by the Mésocentre Clermont-Auvergne University and by the TGCC infrastructure of the CEA.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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Conclusion

L'objectif de ce premier chapitre était d'utiliser la variabilité génétique du blé et de *F. graminearum* pour décrire la diversité et la dynamique des gènes codant les effecteurs de l'agent pathogène exprimés au cours des premiers stades de l'interaction avec le blé. Nos travaux ont permis l'identification d'un pan-effectome putatif rassemblant 840 gènes codant des protéines sécrétées et dont l'expression est finement régulée au cours de l'infection. Avec 90% des gènes codant les effecteurs exprimés par les trois souches d'agressivité contrastée et 91% des gènes codant les effecteurs exprimés par la souche agressive exprimés chez l'ensemble des cultivars, nous avons mis en évidence l'existence d'une stratégie d'infection très conservée chez *F. graminearum*. L'intersection des résultats obtenus au cours de ces deux expérimentations a ensuite permis de proposer un core-effectome robuste de *F. graminearum* composé de 357 gènes exprimés et présentant des profils d'expression similaires quel que soit le fond génétique de la souche ou de l'hôte. Puisque quel que soit le niveau de sensibilité de l'hôte ou le niveau d'agressivité de la souche, l'issue de l'interaction est le succès de l'infection, ces effecteurs très conservés sont probablement des déterminants clefs de la sensibilité du blé à *F. graminearum*. Une telle conservation du programme génétique de *F. graminearum* avait notamment été démontré par les travaux de Francis Fabre à l'échelle protéomique (Fabre et al., 2019a). Ensuite, l'analyse des profils d'expression a démontré que cette stratégie d'infection très conservée repose également sur une fine régulation des gènes du core-effectome. En effet, ces derniers sont exprimés de manière séquentielle et à des stades très précis de l'infection avec une importante phase de transition entre 48hpi et 72hpi, indiquant ainsi que les effecteurs codés par ces gènes joue un rôle à un moment très précis du processus infectieux de *F. graminearum*. Nos résultats corroborent les résultats obtenus par Francis Fabre à l'échelle protéomique (Fabre et al., 2019b). Enfin, la recherche des localisations chez l'hôte a montré que les effecteurs ciblent en priorité l'apoplasme mais aussi les différents compartiments sous-cellulaires du blé dont le noyau est la cible la plus représentée avec 65% des signaux d'adressage intracellulaire. Ces résultats associés au grand nombre de fonctions prédites, suggèrent que le succès de l'infection repose sur la manipulation d'un grand nombre de processus biologiques du blé, illustrant ainsi la complexité de la stratégie d'infection de *F. graminearum*.

Les core-effecteurs putatifs identifiés au cours de cette étude nécessitent désormais une validation fonctionnelle. Sur la base de leur signal d'adressage prédit au noyau et de l'intensité de leurs changements d'expression au cours de l'infection, nous avons sélectionné, au sein du catalogue de core-effecteurs putatifs, 15 candidats devant faire l'objet d'une validation fonctionnelle : sécrétion et localisation subcellulaire chez la plante. De plus, ce catalogue de core-effecteurs représente une ressource importante pour identifier les facteurs de sensibilité ciblés chez le blé. La prochaine étape consiste donc à caractériser les déterminants de l'hôte du dialogue moléculaire et de connecter les deux composantes de ce pathosystème.

Chapitre II - Approche systémique et intégrative de l'interaction blé-*F. graminearum* pour identifier des facteurs de sensibilité candidats au FHB et définir leur rôle au sein de l'hôte

Contexte, enjeux et méthodologie

L'identification du catalogue de gènes de sensibilité requiert une compréhension fine du dialogue moléculaire établi entre le blé et *F. graminearum* au cours de l'infection (Fabre et al., 2020; Gorash et al., 2021). Les déterminants fongiques, i.e. les effecteurs, impliqués dans ce dialogue ont été décrits dans le premier chapitre de cette Thèse (Rocher et al., 2022). Il est désormais nécessaire d'identifier leurs cibles au sein de l'hôte, i.e. les facteurs de sensibilité, et de comprendre le rôle et l'impact de la manipulation de ces derniers sur les processus biologiques de la plante. Les réponses des plantes aux agents pathogènes sont des processus biologiques très complexes reposant sur de nombreuses voies de signalisation qui impliquent plusieurs étapes : la perception de signaux, la transduction de ces signaux et l'expression des gènes de réponses (Baillo et al., 2019). Les facteurs de transcriptions, acteurs à l'intersection de la transduction des signaux et de la mise en place des réponses, sont ainsi des hubs de régulation connectant de nombreux processus biologiques et permettant la mise en place de réponses moléculaires coordonnées (Tran et al., 2010; S.-A. Khan et al., 2018; Baillo et al., 2019). Ces caractéristiques en font des cibles importantes des effecteurs des agents pathogènes et ainsi des candidats particulièrement intéressants pour l'amélioration de caractères complexes tels que la sensibilité (M. Khan et al., 2018; Jaswal et al., 2020).

La caractérisation des réponses du blé au FHB a fait l'objet de nombreuses études moléculaires « large échelle » (Ding et al., 2011; Gottwald et al., 2012; Erayman et al., 2015; Nussbaumer et al., 2015; Ding et al., 2017; Pan et al., 2018; Wang et al., 2018; Brauer et al., 2019; Fabre et al., 2019b; Wang et al., 2019; Fabre et al., 2021; Yang et al., 2021). Cependant, à ce jour, que ce soit selon l'angle de la résistance ou de la sensibilité, les réseaux de régulation contrôlant les réponses du blé au FHB constitue une véritable boîte noire.

Au regard de la quantité d'information générée par les analyses « large échelle » et de la complexité des réseaux de régulation (Mochida et al., 2018), l'identification des gènes de sensibilité candidats au sein du corpus des réponses de l'hôte nécessite d'intégrer les données générées chez les deux protagonistes et ainsi de passer d'une approche réductionniste à une approche systémique de l'interaction (Peyraud et al., 2017; Nobori et al., 2018; Fabre et al., 2020). Enfin, l'identification de facteurs de sensibilité robustes et permettant le développement de nouvelles sources de résistance durables et large spectre nécessite de prendre en compte la diversité génétique tant du côté de l'agent pathogène que de l'hôte.

L'analyse des réponses du blé au FHB a été menée à partir des données RNA-seq générées au cours des deux expérimentations décrites dans le premier chapitre à savoir, l'infection d'un cultivar très sensible par trois souches d'agressivité contrastée et l'infection de cinq cultivars de sensibilité contrastée par une souche très agressive. Dans un premier temps, le réseau de régulation contrôlant les réponses du cultivar de blé sensible aux trois souches de *F. graminearum* a été caractérisé. Dans un second temps, l'intégration des réponses du blé avec les données d'expression des gènes codant les effecteurs conservés de *F. graminearum* et disposant d'un signal d'adressage nucléaire a permis d'identifier des facteurs de sensibilité putatifs. Enfin, l'expression de ces gènes de sensibilité putatifs identifiés a été étudié chez les cinq cultivars de sensibilité contrastée pour identifier un core-targetome.

Article 2 - System-level transcriptomics of wheat responses to FHB identifies gene regulatory networks and conserved master regulators as reliable candidate susceptibility genes

System-level transcriptomics of wheat responses to FHB identifies gene regulatory networks and conserved master regulators as reliable candidate susceptibility genes

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Abstract

Plant diseases are driven by an intricate set of defense mechanisms counterbalanced by the expression of host susceptibility factors promoted through the action of pathogen effectors. In spite of their central role in the establishment of the pathology, the primary components of plant susceptibility are still poorly understood and challenging to trace. Focusing on Fusarium head blight (FHB) in bread wheat and integrating plant transcriptomics responses from a susceptible cultivar facing *Fusarium graminearum* strains of different aggressiveness, we described unexpected differential expression of a conserved set of transcription factors and an original subset of master regulators were evidenced using regulation network computations. The dual-integration with the expression data of pathogen effector genes combined with database mining, demonstrated robust connections with plant molecular regulators and identified relevant candidate genes involved in plant immunity, mostly able to suppress plant defense mechanisms. Furthermore, taking advantage of wheat cultivars of contrasting susceptibility levels, a refined list of 123 conserved susceptibility gene candidates were proposed to be the necessary host's determinants for the establishment of a compatible interaction. In this respect, our findings provide new clues for improving FHB control in wheat and also could conceivably leverage further original researches dealing with a broader spectrum of plant pathogens.

Keywords: *Triticum aestivum*, *Fusarium graminearum*, system approach, regulation network, susceptibility factors

Introduction

The ascomycete fungus *Fusarium graminearum* (teleomorph *Gibberella zeae*) is the main causal agent of the Fusarium Head Blight (FHB) disease on bread wheat (Parry et al., 1995; Goswami and Kistler, 2004). Causing significant yield losses, grain quality alterations and the accumulation of carcinogenic mycotoxins (e.g., deoxynivalenol, DON) (Boyacıoğlu and Hettiarachchy, 1995; Argyris et al., 2003; Chen et al., 2019), FHB represents a major threat for wheat production (McMullen et al., 2012; Dahl and Wilson, 2018) and results in critical economic losses reaching up to USD 1.176 billion over 2015 and 2016 in the USA (Wilson et al., 2018). With rising temperatures and occasional increases in air humidity due to climate change, FHB outbreaks are expected to be more frequent and severe (Vaughan et al., 2016; Mylonas et al., 2020), making it necessary to develop efficient and sustainable management strategies (Xia et al., 2020). Wheat resistance to FHB is a complex and strictly quantitative trait with more than 625 reported Quantitative Trait Loci (QTLs) that displayed relatively minor effects especially when environmental conditions are conducive to the pathogen (Venske et al., 2019; Zheng et al., 2021). Hence, further researches are needed to identify alternative sources of resistance to improve breeding strategies and to efficiently control FHB.

For the last twenty years, the multiple evidences of plant genes required for pathogen infection (Vogel et al., 2002) have opened new opportunities to control natural immunity in plants. These genes, the so-called susceptibility genes, are involved in a wide range of plant fundamental processes that are hijacked by the pathogen through the delivery a large repertoire of effectors (O'Connell and Panstruga, 2006; van Schie and Takken, 2014; He et al., 2020; Jaswal et al., 2020). Resistance through the mutation of susceptibility genes (S genes) represents a promising alternative in breeding resistant cultivars, as exemplify by the broad spectrum resistance to powdery mildew conferred by the natural mutation of the Mlo gene in barley (Jørgensen, 1992). In the wheat-*F. graminearum* pathosystem, the role of susceptibility genes in the interaction outcome has already been demonstrated in several studies. For instance, the deletion of specific chromosome fragments led to an increase of the resistance level, indicating the existence of key FHB susceptibility genes in wheat genome (Ma et al., 2006; Garvin et al., 2015; Hales et al., 2020; Chhabra et al., 2021). Recently, direct functional evidences of the importance of FHB susceptibility factors in wheat were brought and revealed themselves to have a high application value in increasing wheat resistance to *F. graminearum*

(Nalam et al., 2015; Gordon et al., 2016; Hu et al., 2018; Li et al., 2019; Su et al., 2019; Brauer et al., 2020; Su et al., 2021), the most striking example being the natural mutation in the TaHRC gene that might underlie the resistance conferred by the Fhb1 QTL (Li et al., 2019; Su et al., 2019). Thus, identify the FHB susceptibility genes in wheat that drive the compatibility of the interaction with *F. graminearum* turns out to be a powerful way to expand the sources of sustainable resistance.

Mining a reliable catalogue of susceptibility genes requires to deepen our understanding of the molecular crosstalk between the two protagonists of the interaction (Fabre et al., 2020; Gorash et al., 2021). Biotic stress responses are based on complex signaling pathways involving signal perception, signal transduction and expression of stress-responsive genes (Baillo et al., 2019). Through their role in triggering gene expression, transcription factors (TFs) play a pivotal role in the signal transduction step and orchestrate coordinated plant responses. As a consequence, TFs represent hub nodes of the stress molecular responses enabling the activation or the repression of a large set of downstream target genes (Tran et al., 2010; S.-A. Khan et al., 2018; Baillo et al., 2019). Although their characteristics make them important pathogen's targets to control host plant responses (M. Khan et al., 2018; Jaswal et al., 2020) and thus interesting gene candidates to take advantage of complex trait improvement, the complexity of their regulation network requires to identify the downstream genes involved in the stress responses as well as all the impacted biological processes. Many studies have already reported the nature and diversity of wheat responses to *F. graminearum* using successful large-scale -omics studies (Ding et al., 2011; Gottwald et al., 2012; Erayman et al., 2015; Nussbaumer et al., 2015; Ding et al., 2017; Pan et al., 2018; L. Wang et al., 2018; Brauer et al., 2019; Fabre et al., 2019b; Wang et al., 2019; Fabre et al., 2021; Yang et al., 2021). However, no attempt has been made to decipher the complexity of the underlying regulation networks and its robustness when fungal strain of different aggressiveness and cultivars of contrasted susceptibilities are considered.

In this study, the molecular components underlying FHB susceptibility in bread wheat as well as their regulation processes are addressed using a mRNAseq-based profiling of responsive genes during a time course FHB infection. This comprehensive analysis has been conducted by merging information from (i) the characterization of the regulation network orchestrating the responses of a highly susceptible wheat cultivar facing three *F. graminearum*

strains of contrasting aggressiveness, (ii) the identification of susceptibility gene candidates through the integration of wheat responses to FHB with the expression data of fungal effector genes (Rocher et al., 2022) and (iii) the characterization of the expression of these susceptibility gene candidates in different wheat cultivars of contrasting susceptibility (Figure 1).

Materials and Methods

Experimental design and plant sample production

To perform a comprehensive characterization of wheat responses to FHB, a first experiment was conducted on the susceptible wheat cultivar 'Recital' which was inoculated with three *F. graminearum* strains of contrasting aggressiveness (MDC_Fg1, MDC_Fg13 and MDC_FgU1; in decreasing order of aggressiveness (Fabre et al., 2019a)) or with water as controls. To study the FHB responses of different wheat genetic backgrounds, five cultivars of contrasting susceptibility, 'Arche' (ARC), 'Courtot' (COU), 'Chinese Spring' (CS), 'Recital' (REC) and 'Renan' (REN) were inoculated with the single aggressive strain MDC_Fg1 or with water as controls. The two experiments were conducted to collect samples at 48, 72 and 96 hours post inoculation (hpi), which cover a pre-symptomatic period and the symptom onset stage (Fabre et al., 2019). Spore production as well as detailed plant growth conditions are described in Rocher et al. (Rocher et al., 2022). Complete factorial experiments were designed for both experiments. For the first experiment, the combinations of the four treatment conditions (three strains + one control) and the three time points measured on four biological replicates (except for MDC_Fg13 x 72hpi modality with 3 replicates) represented a total of 47 samples. For the second experiment, the combinations of the five cultivars, the two treatment conditions and the three time points measured on three biological replicates represented a total of 90 samples. The experiments were surrounded by additional plants to limit any edge effect. *F. graminearum* strains were inoculated at mid-anthesis by inoculating 10 μ L of a solution at a concentration of 10^5 spores/mL in the floral cavity of the six central spikelets of three synchronous flowering spikes (18 spikelets per biological replicate). For the mock samples, water was inoculated according to the same procedure. For each individual, only the inoculated spikelets were collected and immediately placed in liquid nitrogen. Samples were ground in fine powder and stored at -80 °C before RNA and DNA extractions.

Score symptom monitoring and analysis

The symptoms were scored using the 5-level rating scale given to each inoculated spikelet and described in (Fabre et al., 2019a). The symptoms produced by MDC_Fg1 on the five wheat cultivars were evaluated every 24h from 48hpi to 168hpi. A complete factorial experiment was designed from three additional biological replicates for each cultivar grown especially for the phenotyping procedure. The statistical analysis was conducted on the score symptoms averaged per biological replicate. The progress of symptom development was modeled using logistic regression curves using the following equation:

$$f(x) = \frac{Asym}{1 + e^{\left(\frac{-x - x_{mid}}{scal}\right)}} \text{ as described in (Gilligan, 1990),}$$

where x is the duration in hpi nested in the biological replicates, $Asym$ is the asymptote that modeled the maximum score symptom reached at the end of the experiment, x_{mid} is the time required to reach 50% of the $Asym$ score and, $scal$ is the time required to switch from 50% to 75% of the $Asym$ score. Modelization was performed using the `nlsList` function with the `SSlogis` setting from the `nlme` R package (Pineiro et al., 2022). $Asym$, x_{mid} and $scal$ parameters were retrieved for each individual and were analyzed with a one-way anova to test the cultivar effect.

RNA extraction and Sequencing

The detailed procedures have already been described for the same samples in Rocher et al. (Rocher et al., 2022). From 100mg of frozen powder, total RNA extraction was performed using a using a TRIzol protocol (TRI reagent[®], Sigma-Aldrich, St. Louis, MO, USA), followed by a FastDNase treatment (TURBO DNA-free[™] Kit, Thermo Fisher Scientific, Waltham, MA, USA). Sample quality was controlled by electrophoresis with 1% agarose gel buffered in Tris-Acetate-EDTA. A total of 10µg per sample was used for sequencing. The cDNA libraries were produced using the TruSeq Stranded preparation kit reverse oriented (Illumina, San Diego, CA, USA). For both experiments, 2x150 base paired-end sequences were generated. Samples of the first experiment dealing with the single cultivar 'Recital' were sequenced using Illumina NovaSeq6000 at GeT platform of GenoTOUL ("GeT – Génomique & transcriptomique," n.d.) and the samples of the second experiment dealing with the five wheat cultivars were

sequenced using Illumina HiSeq4000 and NovaSeq6000 at the Genoscope, the French National center of sequencing (CEA, 2021).

RNA-Seq Bioinformatic Analysis

Sequencing data from both experiments were analyzed separately, using a host-pathogen genome mapping approach. Calculations were performed on the supercomputer facilities of the Mésocentre Clermont Auvergne University (“UCA - Mésocentre,” n.d.) and on the TGCC infrastructure of the CEA (“CEA - HPC - TGCC,” n.d.). The construction of *F. graminearum* pangenome used in the mapping step is fully described in Rocher et al. (Rocher et al., 2022).

From raw reads, TrimGalore v0.6.5 (“Babraham Bioinformatics - Trim Galore!,” n.d.) was used to remove the adapters and low quality bases (phred score < 20), while homemade Perl scripts were developed to trim the non-called bases and the polyA tails. Reads exhibiting a low complexity level (compression size < 65%) and a short size (size < 60 nucleotides) were discarded using homemade Perl scripts. A decontamination step was performed by mapping with STAR v2.7.1a (Dobin et al., 2013) against a homemade database of potential contaminants composed of 1,649 viral genomes, 9,267 bacterial genomes and the human genome, downloaded from the NCBI Reference Sequence Database (NCBI, n.d.). The trimming of ribosomal RNA reads was performed with SortMeRNA v4.2.0 (Kopylova et al., 2012) against a database of wheat and *F. graminearum* rRNAs built from noncoding RNA genes fasta files of the *Triticum aestivum* v1.1 assembly (“Triticum_aestivum (IWGSC) - Ensembl Genomes,” n.d.) and PH-1 GCA_900044135.1 genome assembly (“Fusarium_graminearum - PH1 GCA_900044135.1 assembly,” n.d.). Genome and annotation files of *Triticum aestivum* v1.1 (“IWGSC RefSeq v1.1 Reference Genome and Annotation,” n.d.) and *F. graminearum* (pangenome) were merged into a host-pathogen genome containing 269,428 (high confidence and low confidence genes) wheat genes and 17,647 *F. graminearum* genes. The mapping against this combined genome was performed using STAR v2.7.1a. Considering only the uniquely mapped read pairs, gene-level counts were generated for each species using the featureCounts software of the subread v2.0.1 package (“The Subread package,” n.d.).

Wheat gene ontology and annotations

Gene Ontology (GO) terms of *Triticum aestivum* v1.1 genome were retrieved from ensemble ("Triticum_aestivum (IWGSC) - Ensembl Genomes," n.d.) using the BiomaRt R interface (Durinck et al., 2022). GO terms were then used to build a wheat annotation orgdb R package using the makeOrgPackage function of the AnnotationForge R package (Carlson and Pagès, 2022). To predict Transcription Factors, iTAK software (Zheng et al., 2016) was applied on the High (HC) and Low Confidence (LC) wheat proteomes v1.1. To use only the most robust TF genes in the subsequent analysis, we kept predicted genes that were identified as high confidence TF genes in Ramirez et al., 2018 (Ramírez-González et al., 2018).

Protein sequences of the susceptibility genes listed in (van Schie and Takken, 2014) were retrieved from the UniProt Knowledgebase (Boutet et al., 2007) leading to a protein database of 185 susceptibility factors. To identify putative genes related with susceptibility in wheat, Blastp (Altschul et al., 1990) searches were conducted between wheat HC and LC proteomes and our susceptibility gene database. An identity threshold of 60% was fixed.

Statistical analysis of wheat expression data

Statistical analysis was conducted on R v4.1.1 ("R: The R Project for Statistical Computing," n.d.). In the first experiment dealing with the single 'Recital' cultivar, genes were filtered per treatment modality using a 4 Counts Per Million (CPM) threshold in at least 3 samples independently of the time point. This resulted in an expression matrix of 49,505 genes. In the second experiment dealing with the five wheat cultivars, genes were filtered per cultivar by treatment combination with a 4 CPM threshold in at least 3 samples independently of the time point. Expression matrices of 'Arche', 'Courtot', 'Chinese Spring', 'Recital' and 'Renan' were composed of 45,181; 44,416; 46,306; 46,120 and 45,252 genes respectively. For both experiments, expression matrices were normalized according to library size with the trimmed mean of M values (TMM) method implemented in edgeR package (Robinson and Oshlack, 2010; Robinson et al., 2010). Differential Expression (DE) analysis was performed using a negative binomial generalized linear model applied on the logarithm of the proportion of normalized counts for a gene modeled by all the factors composing the experiments. For the 'Recital' experiment, the log₂ of the mean normalized gene expression is an additive function of a treatment effect (4 modalities), a time effect (3 modalities) and an interaction

between the treatment and the time (12 modalities). In the 'five cultivars' experiment, a model was designed for each wheat cultivar where the log₂ of the mean normalized gene expression is an additive function of a treatment effect (2 modalities), a time effect (3 modalities) and an intersection between the treatment and the time (6 modalities). DE analysis was conducted using the DiffAnalysis_EdgeR function of the DicoExpress script-based tool (Lambert et al., 2020), that is based on EdgeR package. The contrasts are automatically generated, making easier the analysis of complex experimental designs. In the 'Recital' experiment, a total of 48 contrasts were considered, which were defined for 12 of them as the difference between two time points given a treatment modality, for 18 of them as the difference between two treatment modalities given a time point and for the last 18 as the interaction term meaning the difference between two time points given a treatment modality minus the difference between the same two time points given another treatment modality. In the 'five cultivars' experiment, a total of 12 contrasts were considered for each wheat cultivar. They were defined for six of them as the difference between two time points given a treatment modality, for three of them as the difference between two treatment modalities given a time point and for the last three as the difference between two time points given a treatment modality minus the difference between the same two time points given another treatment modality. A likelihood ratio test was performed on each contrast and the false discovery rate was controlled with the Benjamini-Hochberg adjustment procedure of the raw p-values (Benjamini and Hochberg, 1995). An adjusted p-value threshold of 0.001 was set to declare a gene differentially expressed. The logarithm of Fold Changes (logFC) were visualized by spiderplots generated with the ggradar R package (Bion, 2022).

Expression pattern characterization

In the 'five cultivars' experiment, filtered expression matrices from each wheat have been merged into a single expression matrix. Prior to the analyses and for both experiments, filtered raw count values were normalized using the regularized logarithm transformation (rlog) implemented in the DESeq2 package (Love et al., 2014). Then, a z-score transformation was applied to the normalized count values. Gene expression patterns were described by heatmaps using the Pheatmap package (Kolde, 2021). The genes and samples were clustered using the ward.D2 agglomeration method applied on the Euclidean distance matrices (Murtagh and Legendre, 2014). To assess the capacity of the selected genes to classify the

infected samples of the first experiment according to the strain \times time point combinations, we performed Partial Least Squares Discriminant Analysis (PLS-DA) using the R package *mixOmics* (Rohart et al., 2017) with 10 components and default parameters for the other options. To characterize the cultivars responses to FHB, we performed PCA analyses at each time point using the *FactoMineR* R package (Lê et al., 2008). We retrieved the coordinates of the individuals on the two first PCA's components on which we performed a complete linear model where the position on the component is an additive function of a cultivar effect (5 modalities), a treatment effect (2 modalities) and an interaction between the two factors (10 modalities). A p-value threshold of 0.05 was applied.

Enrichment analysis

Enrichment in TF genes and TF families were performed using an hypergeometric test with the *phyper* function of the R *stats* package ("Hypergeometric function - RDocumentation," n.d.) with a p-value threshold of 0.05. Functional enrichment analysis were conducted using an hypergeometric test with the *enrichGO* function of the *clusterProfiler* v4.0 R package (Wu et al., 2021) on wheat's Biological Process (BP) GO terms. Benjamin-Hochberg procedure was used to adjust the p-values. An adjusted p-value threshold of 0.05 was set to declare over-represented a BP category.

Regulation Networks computation

Two directional networks were computed, i.e the TF regulation network and the Effector regulation network, using the *network_inference* function of the *DIANE* R package (Cassan et al., 2021) which is based on the *GENIE3* R package (Huynh-Thu et al., 2010). The *rlog* normalized counts of the selected genes were used as input expression values. In order to infer connections between regulator and target genes, Random Forests machine learning method is applied on the gene expression profiles to determine the influence of each regulator gene on each target gene. Because regression methods are very sensitive to correlation between regulator genes, highly correlated regulators were grouped using the *group_regressors* function of *DIANE* package before generating the TF regulation network, with a spearman correlation threshold of 95% in absolute value. For each group of highly correlated regulators, a new expression value is computed using only the average expression value of the positively correlated genes. No grouping based on correlations was applied for

the effector regulation network. For both networks, we applied the following procedure. To select the most robust gene pairs without using a hard-threshold on edge weights, the edge's importance matrix was computed using the 'MSEincrease_oob' option as importance metric in the network_inference function. Then, to statistically test the significance of each edge importance value the test_edges function was applied on the inferred network with the following settings: density = 0.02, nTrees = 1000, nShuffle = 1000. Edge importance p-values were adjusted according to Benjamin-Hochberg procedure. The final networks were built from edges with an adjusted p-value below 0.02 using the network_from_tests function. Network resume tables were created using the network_data function, which also performed a the Louvain algorithm (Blondel et al., 2008) to define communities of nodes within the networks. Network visualizations were performed using the visNetwork R package (visNetwork, 2022).

Genomic localization of putative susceptibility genes

Genomic localizations of the genes were retrieved from the GTF annotation file of wheat and were plotted on wheat genome using the karyoplotR R package (Gel and Serra, 2017). The localizations of the FHB meta-QTLs was downloaded from the supplementary materials in (Zheng et al., 2021).

Results

FHB development in bread wheat cultivars

The dynamics of symptom development was evaluated in the five wheat cultivars using a logistic model applied on the measured score symptoms and computing three parameters of the disease progress: *Asym*, *xmid* and *scal*. Although no significant difference was observed for *Asym*, 'Recital' *xmid* was shorter than the one of 'Arche', 'Courtot' and 'Chinese Spring' (Figure 2). 'Renan' also displayed a significantly lower *xmid* than both 'Arche' and 'Chinese Spring'. The *scal* of 'Recital' was significantly lower than the one measured in other cultivars except in 'Renan'. To refine the susceptibility ranking of the five cultivars, we performed a HAC on the three logistic regression parameters (Figure 2 inset). It clearly discriminated 'Recital' from the four other cultivars which were divided into two distinct groups with 'Renan' that was separated from 'Arche', 'Chinese Spring' and 'Courtot'. Based on the disease progress modeling, a susceptibility ranking of the cultivars was established as follow: 'Recital' (high

susceptibility), 'Renan' (intermediate susceptibility), 'Arche', 'Courtot', and 'Chinese Spring' (moderate susceptibility).

Transcriptional landscape of early FHB responses in the most susceptible wheat genotype 'Recital' facing different fungal strains revealed conserved changes

A total of 49,505 genes were found to be expressed in Recital and were used for the Differential Expression (DE) analysis. DE results were classified into 16 categories (Supplementary Table S1). Among the 41,891 differentially expressed genes (DEGs), we selected genes differentially expressed in response to at least one strain in comparison to the control (M) conditions. This resulted in a FHB-responsive gene set composed of 38,818 genes (92.6% of the whole DE gene set, Figure 3A) including 26,779 genes that were systematically regulated whatever the inoculated fungal strain ("All strains"-responsive gene set; 69% of the FHB-responsive gene set). Only 6,509 and 5,530 genes were differentially expressed in response to two of the three strains ("Strain accessory"-responsive gene set) and to one strain ("Strain specific"-responsive gene set), respectively. Among the "All strains"-responsive genes, 93% did not display any strain effect demonstrating that the different strains produced similar effects on the wheat transcriptome. Overall, the magnitude of 'Recital' responses to the three strains increased along with the infection progress (Figure 3B). At 48hpi, around 66% of the differences in gene expression between MDC_Fg1-, MDC_Fg13-, MDC_FgU1-inoculated and mock plants (fold changes, FC) did not exceed a ± 2 -fold change ($-1 \leq \log_2(\text{FC}) \leq 1$). At 72hpi and 96hpi in contrast, FC greater than ± 2 ($\log_2(\text{FC}) < -1$ or $\log_2(\text{FC}) > 1$) accounted for an average of 56% and 76% of the FHB-responsive genes whatever the inoculated strain, respectively.

To gain a better understanding of the strain impacts on wheat gene expression along with the infection progress, we computed a PLS-DA on the "All strains"-responsive gene set trying to discriminate strain \times time-point combinations (Figure 3C). The first component, explaining 70% of the total variance, clearly discriminated the three infection stages, with 72hpi acting as a transition phase between 48hpi and 96hpi. The second component, explaining 8% of the variance, slightly discriminated the wheat responses to MDC_Fg1 from the ones induced with the two other strains at 72hpi and 96hpi. These results were confirmed by the hierarchical ascendant clustering (HAC) of the infected samples on the "All strains"-

responsive gene set (Supplementary Figure S1) emphasizing that 'Recital' cultivar displayed similar quantitative responses to the three strains.

The FHB development induced major readjustments in the abundance of key wheat transcriptional regulators independently of strain aggressiveness

The "All strains"-responsive genes demonstrated the reprogramming and the temporal adjustments of the 'Recital' transcriptome along the infection progress with two balanced gene groups (Supplementary Figure S1). The first group, gathering 12,160 genes, displayed higher expression levels in control samples than in the infected ones, with a continuously decreasing gene expression along with the infection progress. On the opposite, the second group, gathering 14,619 genes, displayed lower expression levels in the control samples than in the infected samples depicting a continuously increasing gene expression along with the infection progress. The search of transcription factors (TF) among this "All strains"-responsive gene set identified 1,074 TF encoding genes from 53 TF families. In comparison with the whole expressed gene set, the "All strains"-responsive gene set was significantly enriched in TF genes ($p\text{-value} < 1.5 \times 10^{-12}$) with the over-representation of the AP2/ERF-ERF, NAC, Tify and WRKY TF families (corrected $p\text{-value} < 0.05$). Two expression groups were clearly identified including a set of 324 TF genes which were over-expressed in control samples in comparison with the infected ones and another set of 750 TF genes which were under-expressed in control samples in comparison with infected samples. TF genes belonging to the same family displayed similar expression patterns (Figure 4 and Supplementary Table S2). The B3, B3-ARF, C2C2-CO-like, C2C2-YABBY, MADS_MIKC, mTERF and Whirly families were mostly down-regulated in response to FHB while AP2/ERF-ERF, AP2/ERF-RAV, CAMTA, LOB, MYB, NAC, PLATZ, Tify and WRKY families were mostly up-regulated along with the infection progress.

Biological processes and functions of the core-responsive genes of the susceptible wheat genotype interacting with the three fungal strains

Down-regulated genes in response to the three strains were enriched in 312 biological process (BP) subcategories (Supplementary Table S3A). Those categories were mainly associated with fundamental biological processes. Primary metabolism was widely impacted through several processes: photosynthesis (mainly through light harvesting), polysaccharide

and monosaccharide synthesis, starch metabolism, as well as amino acid and nucleotide synthesis. Cell and tissue structure was also altered at several scales including cytoskeleton, organelle structure as well as plasma membrane and cell wall. Several other cell basal functions were disturbed though the down-regulation of genes involved in transduction, transport, protein sorting, transcription and DNA repair and modification. Likewise, reproductive and cell cycle related process were impacted and concerned 30% of the enriched BP terms including mitosis, meiosis, replication, chromosome separation, ovule and embryo development and, responses to auxin and cytokinin. Genes involved in terpenoid synthesis and in the negative regulation of the immune response were also down-regulated along with the infection progress.

Up-regulated genes in response to the three strains were enriched in 174 BP subcategories (Supplementary Table S3B). Genes involved in responses to several phytohormones, including gibberellin, salicylic acid, jasmonic acid, abscisic acid, cytokinin and ethylene, were induced in response to the different *F. graminearum* strains. Genes involved in the synthesis of signaling specialized metabolites (chorismate, indolalkylamine, olefin, amine compounds and oxylipin) were also over-represented. The biotic stress response associated categories (e.g., “systemic acquired resistance”, “receptor signaling”, “chitin catabolism”) as well as genes involved in the synthesis of several defense specialized metabolites (e.g., “sulfur containing compounds”, “phenylpropanoids”, “phenol compounds”, “trehalose” and “prephenate”) were sharply activated along with the infection. The antioxidant machinery was also activated during the infection progress with the over-representation of genes involved in the ROS catabolism, in the detoxification processes and in the synthesis of antioxidant specialized metabolites such as the glutathione and amine compounds. Genes associated with ion transport, calcium, ammonium and metal ions, as well as the trans-membrane transport of carbohydrates were up-regulated. Cell integrity seems to be altered with an enrichment in the catabolism of several cell wall components : xylan, lignin and amino-glycan. However, genes involved in the synthesis of cinnamic acid associated with the lignification process were also up-regulated along with the infection progress as were the genes involved in pollen pistil interaction, protein ubiquitination and lipid catabolism.

Establishing the transcription factor regulation network in response to FHB and searching for master regulators

From the “All strains”-responsive gene set described in the single ‘Recital’ susceptible cultivar facing the three different *F. graminearum* strains, we selected genes that were systematically expressed in the four other wheat cultivars of contrasting susceptibility (cv. ‘Arche’, ‘Courtot’, ‘Chinese Spring’ and ‘Renan’). As a whole, 20,629 genes (77%) were found in all wheat cultivars regardless of their susceptibility level and were used to build the regulation networks. Among these genes, 772 were predicted to encode TFs. To establish the directional network of FHB responses, a set of 269 regulators were defined among which 16 corresponded to groups of highly correlated TFs (> 95% in absolute value). Statistical tests performed on the network edges led to a directional network composed of 9,791 nodes and 28,581 edges (Supplementary File S1). The network was divided in 10 communities gathering 415 to 1792 nodes (Table 1) with 12 to 50 regulator nodes. Then, we identified 91 master regulator nodes, *i.e.* a regulator node that regulated other regulator nodes without being regulated itself. For example, TraesCS1B02G318800, encoding a MYB TF, had 34 direct targets including two TF genes, TraesCS6D02G362900 and TraesCS3D02G231200, which had 74 and 32 targets, respectively (Figure 3D). Among these master regulator nodes, seven were groups of highly correlated TFs leading to a total of 427 master TFs (Supplementary Table S4). These master regulators belonged to 44 families, including four main families that gathered nearly half of the set; *i.e.*, the WRKY (71 genes), the NAC (57 genes), the AP2/ERF-ERF (41 genes) and the MYB (40 genes) families. Most of the master regulators were induced in response to the FHB infection (Figure 5A); 368 master TF genes (Cluster 2) were over-expressed in infected samples in comparison with controls while only 59 (Cluster 1) were down-regulated.

Leveraging *F. graminearum* effector expression dynamics to identify new susceptibility gene candidates

To identify putative susceptibility genes (S genes) targeted by *F. graminearum*, we computed another directional network using 46 nuclear core-effectors of the three *F. graminearum* strains (Rocher et al. 2022) as putative regulators of the FHB responsive gene set (20,629 genes expressed in all wheat cultivars). Statistical tests performed on the edges led to a directional network composed of 17,393 edges connecting 41 nuclear effectors to 12,866 wheat targets (Supplementary File S2), of which 6,485 were shared with the TF

regulatory network of FHB responses. FHB responsive genes which were not TFs represented 12,362 genes, of which 5,981 were also found to be targeted by wheat TFs in the previous network. TF genes were significantly enriched (p -value <0.05) within the target network, with a total of 504 genes targeted by *F. graminearum* nuclear effectors. Among them, 338 genes were identified as master regulators in the TF regulatory network of FHB responses.

To narrow down the number of putative S genes and to identify the most robust ones, we blasted wheat genes targeted by *F. graminearum* effectors against a homemade ortholog database of known S genes established from the review of Van Schie et al. (2014). As a whole, 203 genes (Supplementary Table S5) matched with known S genes of which 163 were associated with defense suppression. Among the 203 putative S genes, 41 were master TFs, 24 were TFs and 138 were not TF genes. Among the master TF putative S genes, we found 33, 4 and 4 genes belonging to the WRKY, MYB and NAC families, respectively. Among TFs which were not considered as master regulators, only two unique families were found: the MYB and WRKY families gathering 17 and 7 genes, respectively. Among the non-TF putative S genes, almost half (67 genes) were also found to be regulated by TFs in the TF regulatory network of FHB responses.

Expression signatures of the putative S genes in the susceptible wheat cultivar

As a whole, the 203 identified putative S genes were mostly induced in response to FHB, including 150 genes which were up-regulated along with the infection progress and 4 genes over-expressed at 48hpi only in infected samples (Figure 5B; lower clusters) in comparison to control samples. Down-regulated patterns in response to FHB were observed for 49 genes (Figure 5B and Supplementary Table S5). Among the S genes involved in defense suppression, 130 genes were induced in response to FHB while 33 were down-regulated. Regarding the master regulators, all the WRKYs and NACs as well as three of the four MYBs were induced in response to FHB while only one MYB was down-regulated. Concerning the other TFs, all the WRKYs and the 13 MYBs were also induced in response to FHB while only 4 MYBs were down-regulated. For the non-TF S genes, 90 were induced along with the FHB progress with the maximum of expression reached at 96hpi, four were over-expressed in infected samples at 48hpi and 44 were over-expressed in controls. Noticeably, these expression patterns between infected and control samples were highly similar to the

responses evidenced in the other cultivars facing the aggressive MDC_Fg1 strain (Supplementary Figure S2).

Did putative FHB susceptibility genes discriminate different wheat genetic background?

To identify specific cultivar's FHB responses, we performed PCA on the S gene set for each time point (Figure 6A) as well as statistical tests on their components. For all the 3 time points, the first component, which explained 54.2%, 79.8% and 84.2% of the variance at 48hpi, 72hpi and 96hpi respectively, significantly discriminated the infected samples from the control ones. At 48hpi, the position of the infected 'Courtot' responses on the component 1 was significantly different from infected 'Chinese Spring' and 'Renan' responses, and the position of infected 'Recital' was significantly different from infected 'Renan' responses. At 48hpi, the second component (9.1%) was not significantly driven by the infected 'Arche' responses. At 72hpi, the position of infected 'Recital' responses on the component 1 was significantly different from infected 'Arche' and 'Courtot' responses, and the position of infected 'Chinese Spring' was significantly different from infected 'Arche' responses. At 72hpi, the second component (4.3%) not significantly distinguished the infected samples of 'Arche' and 'Courtot' from the infected samples of 'Chinese Spring', 'Recital' and 'Renan'. At 96hpi, the position of infected 'Recital' on component 1 was significantly different from all other infected cultivars, while the component 2 (5.1%) significantly discriminated infected condition from the control one for all cultivars as well as the infected 'Recital' from all other infected cultivars.

Among the 203 S genes, 123 genes displayed significant differences between control and infected samples for all 5 cultivars, of which 107 were involved in defense suppression and 53 encoded TFs. With a total 194 DEGs, 'Recital' displayed the highest number of FHB responsive DEGs. 'Chinese Spring', 'Arche', 'Renan' and 'Courtot' respectively displayed 187, 165, 150 and 137 FHB responsive DEGs. The magnitudes of FHB responses were different between the 5 cultivars (Figure 6B). This can be notably observed at the low FC ranges. At 48hpi, 'Renan', 'Chinese Spring' and 'Arche' displayed a relatively high proportion of FC responses < 2 ($-1 \leq \text{Log}_2(\text{FC}) \leq 1$), including 73.4%, 70.9% and 67.5% of the DEG respectively. Such low FC responses only represented 59.1% and 53.7% of the genes in 'Recital' and 'Courtot' respectively. At 72hpi, 'Arche' and 'Courtot' displayed 53.2% and 51.7% of their DEGs with $\text{FC} < 2$ while it accounted for only 26.6%, 18.2% and 17.2% in 'Renan', 'Chinese Spring'

and 'Recital'. At 96hpi, we distinguished two opposite patterns of responses with 'Courtot' displaying 43.3% of DEGs with $FC < 2$ while 'Recital' displayed 93.1% of its DEGS with $FC > 2$. 'Chinese Spring', 'Renan' and 'Arche' displayed 25.1%, 20.7% and 19.7% of their DEGs with $FC < 2$.

Genomic localization of the putative FHB susceptibility genes

The distribution of the S genes on the wheat genome was balanced at the sub-genome level with 67, 66 and 67 genes localized on sub-genomes A, B and D, respectively (Supplementary Figure S3). S genes were also localized over all the 21 wheat chromosomes (Table 2). Localization on the unknown chromosome accounted for three genes. A total of 24 S genes, were found within the 118 known FHB meta-QTLs (Zheng et al. 2021; Supplementary Table S6 and Supplementary Figure S3) on 14 different chromosomes. Among them, six genes including three WRKYs, two NACs and a single MYB, were identified as master regulators in the TF regulatory network of FHB responses. The 18 other genes were not TF genes. All master TF genes, except the MYB TF gene, were over-expressed in infected samples in comparison to the control samples. Regarding the non-TF genes, 11 genes were over-expressed in infected samples in comparison to the controls while 7 genes were under-expressed.

Discussion

Using *F. graminearum* strains of contrasting aggressiveness, we searched for consistent FHB induced transcriptional responses that are supposed to drive the infection process in 'Recital', a susceptible wheat cultivar. Gene regulatory network modeling as well as the responses from cultivars of different susceptibility to FHB were then leveraged to thoroughly characterized the regulation processes as well as to identify robust susceptibility genes that might be targeted by *F. graminearum*.

Transcriptome profiling of 'Recital' responses to the three *F. graminearum* strains of contrasting aggressiveness revealed an early and sharp alteration of wheat spike basal functioning through the down-regulation of genes involved in primary metabolism, in spike development and in cell structure, while genes involved in signaling pathways, biotic stress responses and detoxification processes were steeply induced from the infection outset. The control of 'Recital' transcriptome reprogramming in response to FHB involves a large number of TFs and TF families. Examining the relationship between these regulators showed that FHB-

responsive TFs displayed highly correlated expression patterns and evidenced major regulation hubs of the FHB responses. Four TF families, the WRKY, NAC, AP2/ERF and MYB families, that represented a total of 209 master regulator gene candidates induced along infection progress, were distinguished for their central role in FHB responses. Those four families are well known key players of biotic stress response that can positively or negatively affect the setup of defense mechanisms contributing to either resistance or susceptibility (Rushton et al., 2010; Ng et al., 2018; Baillo et al., 2019; Bian et al., 2021). In wheat resistant lines facing *F. graminearum*, two NAC TFs, the TaNAC032 (Soni et al., 2021) and the TaNACL-D1 (Perochon et al., 2019), as well as one WRKY TF, the TaWRKY70 (Kage et al., 2017), were already been associated with the setup of defense mechanisms, such as in lignin synthesis and in resistance related induced (RRI) metabolite production. On the opposite, the two NAC TFs, TaNAC2 (Zhang et al., 2018) and TaNAC30 (B. Wang et al., 2018) were shown to negatively regulate Reactive Oxygen Species (ROS) accumulation and to increase wheat susceptibility to *Puccinia striiformis*. In line with our results, those TF families were proved to be organized in complex regulation networks involving auto-regulatory processes, including intra-family cross-regulatory processes (Eulgem and Somssich, 2007; Nuruzzaman et al., 2013; Jiang et al., 2017; Ng et al., 2018; Paul et al., 2020) and inter-family cross-regulatory processes (Bemer et al., 2017; Cao et al., 2020). This concerted transcriptional modulation could represent an efficient way to target multiple biological processes and to set up fine-tuned responses to the fungal infection.

Integrating expression data from both protagonists of the interaction highlighted that transcription factors and especially master regulators could represent main direct or indirect targets of *F. graminearum* core nuclear effectors. As in the transcription network of FHB responses, we found that the four most represented families among the expected targeted TFs were the WRKY, NAC, MYB and AP2/ERF families, strengthening their prime role in regulating FHB responses. Those families were previously shown to be up-regulated in the 'Shaw' susceptible wheat cultivar in response to *F. graminearum* (Pan et al., 2018). TF manipulation by direct or indirect action of effectors were described in many plant-fungus interactions. Direct interactions between effectors and plant TFs were described in the symbiotic fungi *Glomus intraradices* that secretes the SP7 effector which interact with the AP2/ERF19 TF of *Medicago truncatula* in order to deactivate defense responses (Kloppholz et

al., 2011) but also in the hemibiotrophic fungi *Verticillium dahliae* with the VdSCP41 effector that targets the two transcription factors CBP60g and SARD1 in *A. thaliana* to alter their immunity functions (Qin et al., 2018). An example of indirect effect on a plant TF was described in the biotrophic pathogen *Ustilago maydis* that secretes the Tin2 effector which prevents the degradation of the ZmTTK1 protein kinase which in turn triggers the nuclear addressing of the ZmR1 maize TF (Tanaka et al., 2014).

Merging the S genes database (van Schie and Takken, 2014) and the *F. graminearum* *in planta* targets, we identified 203 S genes putatively targeted by *F. graminearum* core nuclear effectors, of which 64% were induced along with the infection progress and were proved to have a role in defense suppression with a wide range of mechanisms (van Schie and Takken, 2014). In line with this, the multi-gene model underlying the Fhb1 QTL suggest that the TaHRC gene product (Li et al., 2019; Su et al., 2019) might operate as a susceptibility factor targeted by *F. graminearum* to repress the WFhb-1 gene involved in antifungal activity (Paudel et al., 2020). Further evidences in the *F. graminearum* strategy also support the key role of defense suppressor genes in the compatibility of the interaction, such as the secretion of the arabinase Arb93B, able to repress the ROS-associated defense mechanisms (Hao et al., 2019). In this study, we identified nine wheat genes displaying a high degree of similarity with the Mildew resistance locus O (Mlo) gene and all of them were up-regulated upon FHB infection. Encoding a membrane protein involved in the negative regulation of immunity, Mlo is a well characterized susceptibility gene in barley and wheat to the biotroph pathogen *Blumeria graminis* and its KO mutation confers a broad spectrum resistance (Büschges et al., 1997; Engelhardt et al., 2018; Garcia-Ruiz et al., 2021). Resistance through the silencing of Mlo genes was also reported in pathosystems involving necrotrophs such as in the *Cucumis sativus* - *Corynespora cassicola* interaction (Yu et al., 2019). Additionally, four genes induced in response to *F. graminearum* matched the *A. thaliana* ENHANCED DISEASE RESISTANCE1 gene (EDR1), which alters defense responses by interacting with the immune regulators EDS1 and PAD4 (Neubauer et al., 2020). Six more up-regulated genes matching the DOWNY MILDEW RESISTANT 6 (DMR6) gene of *A. thaliana*, suggest another mechanism of defense suppression through the degradation of Salicylic Acid (SA), known to trigger the defense responses against biotrophic and hemibiotrophic pathogens (Robert-Seilaniantz et al., 2011; Zhang et al., 2017). Because the *A. thaliana* DMR6 gene and its barley orthologue were proved to promote FHB

susceptibility (Low et al., 2020) those six wheat genes represent reliable candidates S genes for further functional validation. Furthermore, TFs were also largely represented among the putative S genes involved in defense suppression with 58 TFs genes (90% of the effector targeted TF genes with a match in the S genes database) induced along infection progress, belonging to the WRKY (38 genes), NAC (4 genes) and MYB (16 genes) families. For instance, they included three WRKY genes with high similarity to the barley HvWRKY1 gene and eight WRKY genes matching the barley HvWRKY2 gene which are both negative regulators of plant defenses against the biotrophic pathogen *Blumeria graminis* by repressing the basal defense-related gene HvGER4c (Shen et al., 2007; Liu et al., 2014). By interacting with TF genes, *F. graminearum* appears to repress pivotal components of cell immunity and in this respect, it could be able to manipulate at a systemic scale a wide array of defense-related processes. This large number of putative targeted wheat genes appears consistent with the highly complex and fragmented nature of the FHB resistance controlled by 625 QTLs distributed throughout the genome (Venske et al., 2019; Zheng et al., 2021).

All the meta-QTLs, within which were localized our putative S genes, were associated with spreading within the spike and 13 were also associated with mycotoxin accumulation (Zheng et al., 2021). Taken together, these evidences indicate that *F. graminearum* targets several defense pathways and partly suppresses plant defense mechanisms to promote its successful establishment and development in the spike. This infection strategy deduced from the plant responses was further demonstrated to be highly conserved in the three *F. graminearum* strains. This finding fully corroborates previous results reporting that the same three strains shared 90% of their *in planta*-established effectome (Rocher et al., 2022).

While *F. graminearum* was able to infect the 5 wheat cultivars, only 61% of the S genes were significantly regulated in all the cultivars. Thus, pathogen's establishment in cultivar of contrasting susceptibility appeared to rely on a conserved corpus of S genes successfully manipulated in all wheat genetic backgrounds. The most susceptible wheat cultivar 'Recital' was clearly discriminated from the 4 others in terms of DEG number and response amplitude. However, there were no clear link between the susceptibility level, of the 4 other cultivars and their differences observed at the transcriptomic level. This exemplifies the complex nature of wheat - *F. graminearum* interaction and might highlight some redundancy in the processes targeted by *F. graminearum* effectors. Taking advantage of different wheat cultivars with

contrasting susceptibility level, this provides a robust list of 123 susceptibility gene candidates, including 107 defense suppressors and 53 TF genes, that responded to several *F. graminearum* strains and systematically regulated in different wheat genetic backgrounds.

To conclude, using a system-level transcriptomics data integration and mining genetic variability on both host and pathogen sides, we discovered unexpected conserved responses of wheat facing FHB. This portrays a distinctive signature of the intricate mechanisms that drive the outcome of the wheat-*F. graminearum* interaction and further enabled to delineate a set of core-transcription factors putatively involved in the orchestration of susceptibility factors. Such robust conservation emphasizes that wheat susceptibility to FHB could be one of the main drivers of disease establishment and as such, reshapes the way we consider plant responses to pathogens beyond the role and expression of the defense genes alone. In addition to their involvement in FHB, the identified candidate S genes could conceivably be of genetic value in controlling a broader spectrum of pathogens (Carella et al., 2018; He et al., 2020; Ceulemans et al., 2021), providing original clues for further researches.

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Figures and Tables

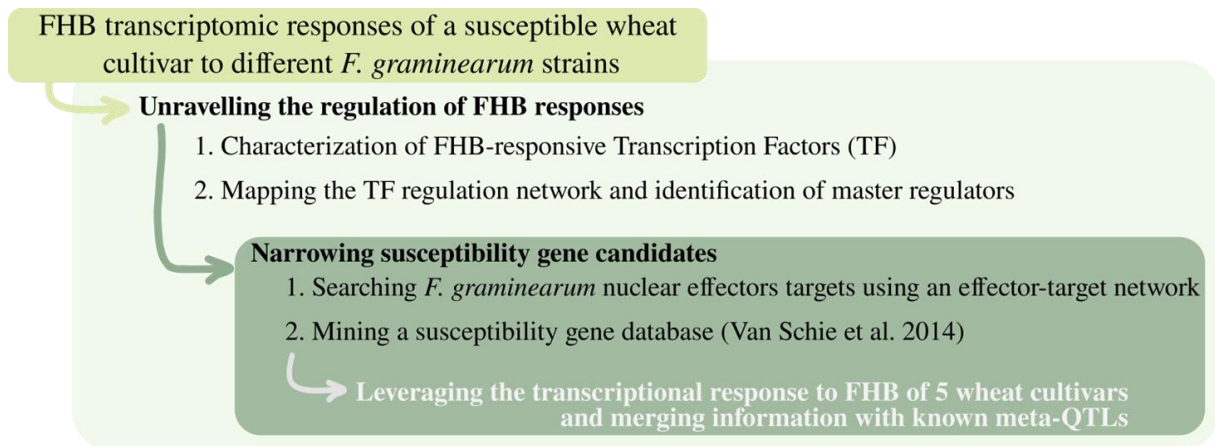


Figure 1. Reading grid of the approach used to decipher the mechanisms underlying wheat susceptibility to FHB and to identify relevant susceptibility genes.

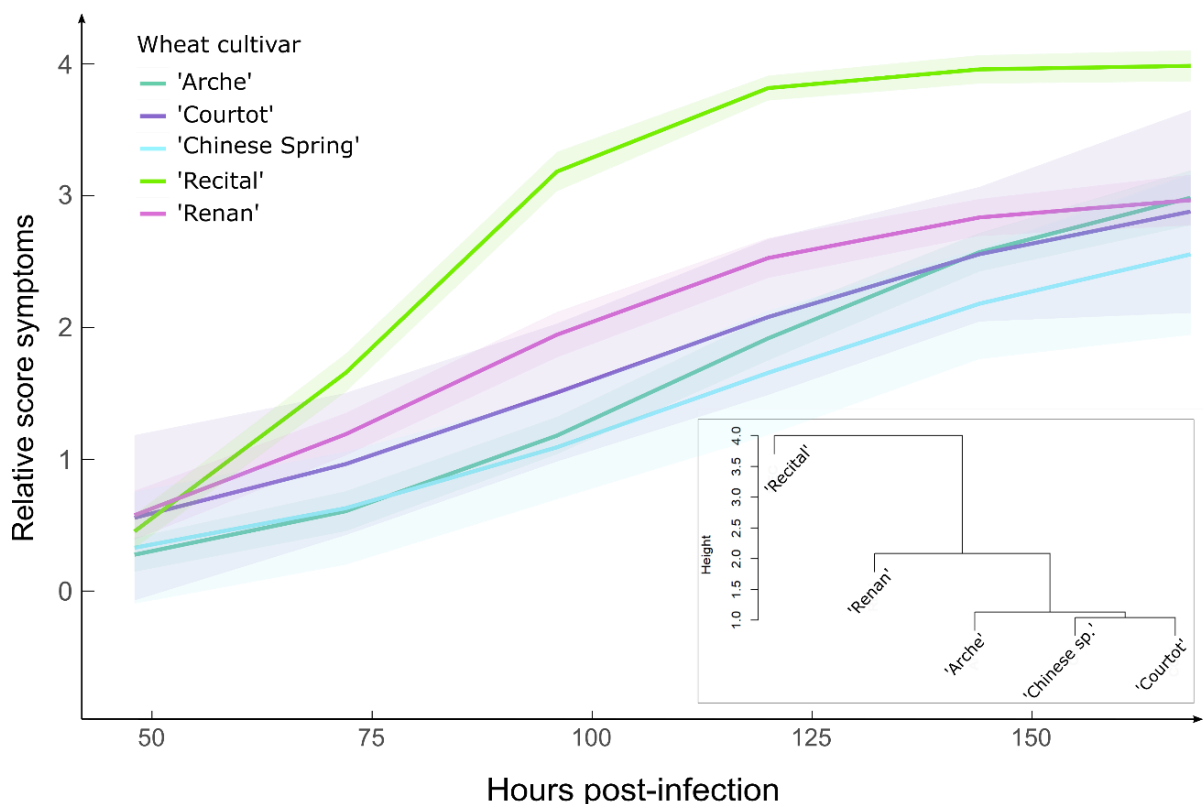


Figure 2. Dynamics of symptom development in the cultivars 'Arche', 'Courtot', 'Chinese Spring', 'Recital', 'Renan'. For each cultivar, symptom development was evaluated according to a logistic model (colored curves). The inset clustering tree represents the HAC performed on the logistic parameters.

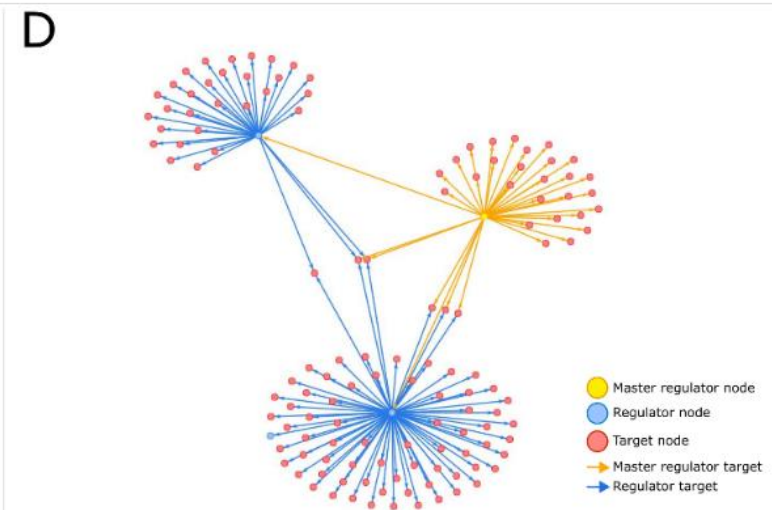
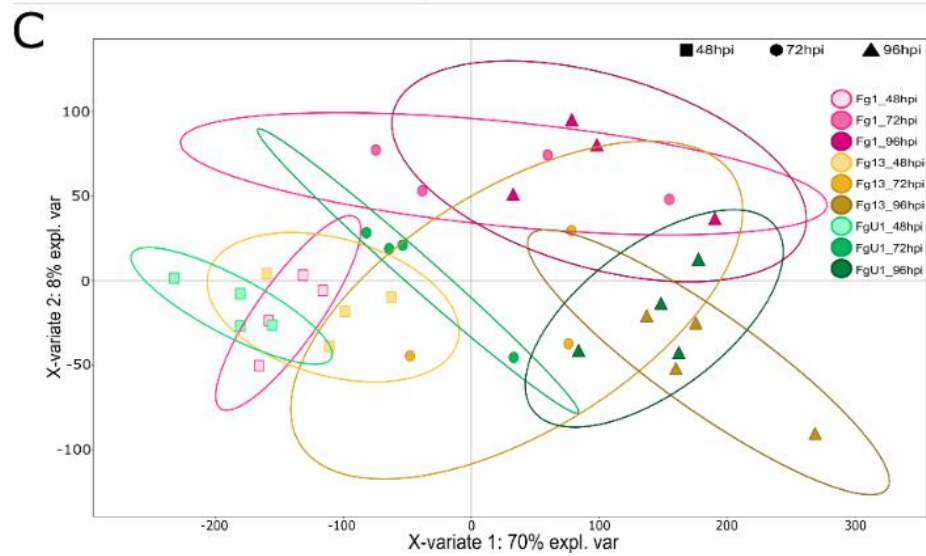
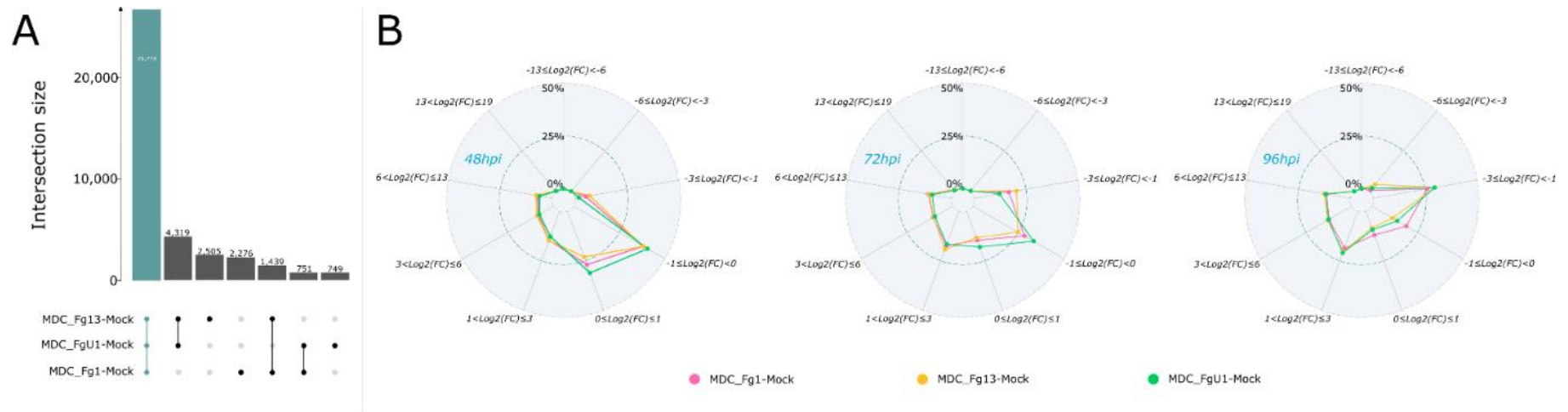


Figure 3. Characterization of 'Recital' responses to the three strains of contrasting aggressiveness. **(A)** The structure of the FHB-responsive gene set is represented by an upsetplot describing the number of genes significant for one ("Strain specific" –responsive gene set) , two ("Strain accessory" –responsive gene set) or three ("All Strain –responsive gene set, blue bar) strain-control comparisons. **(B)** The Variation amplitudes of gene expression for each Strain-Control comparison at 48hpi, 72hpi and 96hpi were represented with spider charts. Spider chart describes the $\log_2(\text{FC})$ between each strain (MDC_Fg1 in pink, MDC_Fg13 in yellow and MDC_FgU1 in green) and control. Colored points indicate the proportion of genes belonging to each of the nine $\log_2(\text{FC})$ ranges. Extreme values were the maximum and minimum $\log_2(\text{FC})$ of all time points and strain control comparisons. A negative $\log_2(\text{FC})$ indicates a lower expression level in the infected condition than in the control one and a positive $\log_2(\text{FC})$ indicates a higher expression level in infected condition than in the control one. Spider plots are composed of two grid circles, the middle grid circle indicating 25% and the maximum grid circle indicating 50%. **(C)** 'Recital' experimental conditions were classified according to the All strain - responsive gene set. PLS-DA method was applied on the 26,779 genes responsive to the three *F. graminearum* strains to predict the strain-infection progress combinations. The plots of the individuals extracted from the PLS-DA are represented on the two first components. For each condition, confidence ellipses are plotted to highlight discrimination strength (level set to 95%). **(D)** The complexity of the regulation network controlling 'Recital' FHB responses is illustrated by a directional network plot. Network was built using TF genes as regulators of all FHB responsive genes. The figure shows the targets of one master regulator (yellow node), TraesCS1B02G318800. Direct edges connecting the master regulator to its targets are indicated by yellow arrow. Master regulator's target genes are indicated in blue for the TF genes and in red for the not TF genes. Blue arrows represents the direct edges between the regulated TF genes and their own targets.

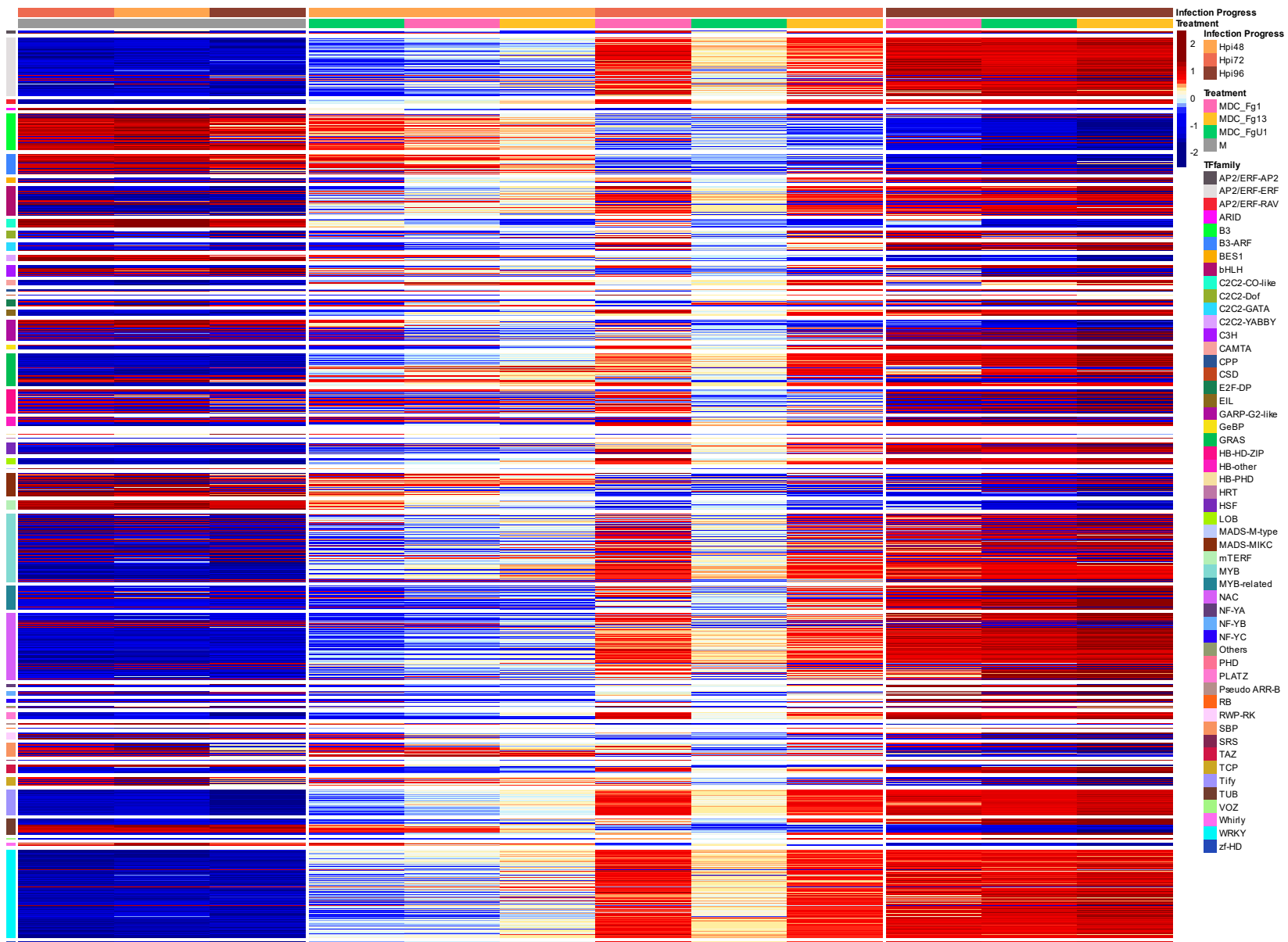


Figure 4. Expression regulation patterns of the All strain responsive TF encoding genes along with the infection progress 'Recital'. The structure of gene and sample data sets were determined by HAC based on Ward's minimum variance method using the z-score transformed gene expression values. Heatmap color scales represent the z-score transformed expression values of the TF genes from the All strain - responsive gene set for each condition. The clustering on top of the heatmap represents the experimental conditions which are labeled according to the factors Infection Progress and Treatment. On the right side of the heatmap TF genes were grouped and colored according to their TF family.

Table 1. Table TF regulation network description.

Community	Number of nodes	Number of regulator nodes	Number of master regulator nodes
C1	1,579	50	13
C2	1,428	24	14
C3	556	30	10
C4	933	50	21
C5	892	33	6
C6	1,792	14	5
C7	715	12	6
C8	415	18	8
C9	628	20	5
C10	853	18	3

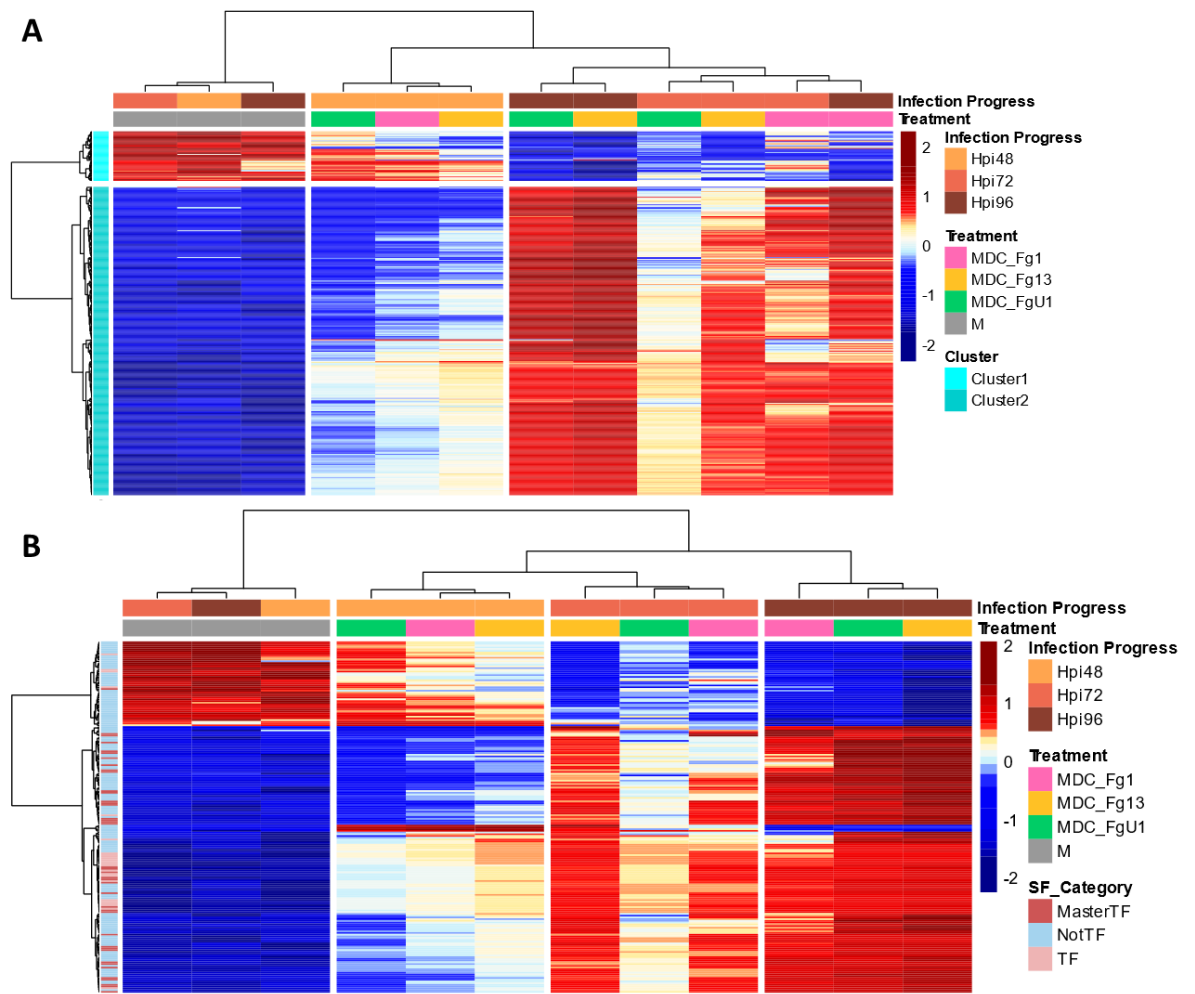
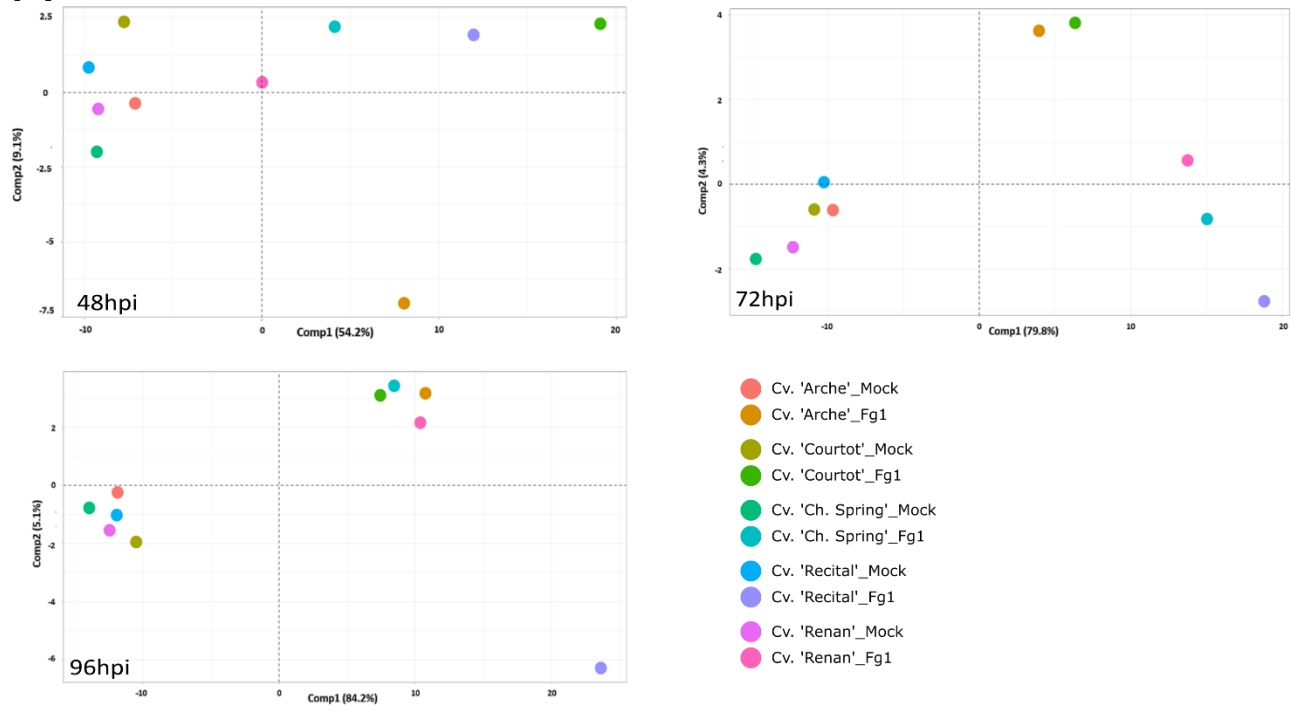


Figure 5. Expression regulation patterns of **(A)** the master TF regulating FHB response and **(B)** the putative susceptibility genes targeted by *F. graminearum* nuclear effectors in 'Recital' facing 3 strains of contrasting aggressiveness. The structure of gene and sample data sets were determined by HAC based on Ward's minimum variance method using the z-score transformed gene expression values. Heatmap color scales represent the z-score transformed expression values of the genes for each condition. The clustering on top of the heatmap represents the experimental conditions which are labeled according to the factors Infection Progress and Treatment. On the right side of the heatmap, genes were colored according to **(A)** their cluster membership and **(B)** the following *S* gene categories: Master TF, TF, not TF.

A



B

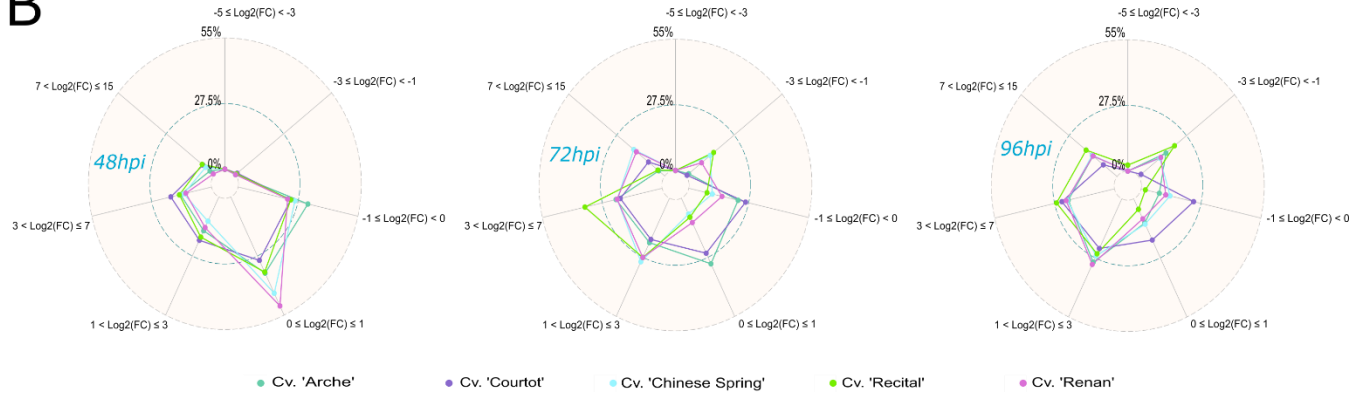


Figure 6. FHB responses of the five cultivars on the 203 putative S genes at 48hpi, 72hpi and 96hpi. **(A)** PCAs were computed on the z-score values of the S gene set at each time point. The plots of the individuals extracted from the PCAs are represented on the two first components, on which statistical tests were performed. **(B)** For each time point, a spider chart describes the $\log_2(\text{FC})$ between infected and control conditions in 'Arche', 'Courtot', 'Chinese Spring', 'Recital' and 'Renan'. Colored points indicate the proportion of genes belonging to each of the seven $\log_2(\text{FC})$ ranges in each cultivar. Extreme values were the maximum and minimum $\log_2(\text{FC})$ of all comparisons. A negative $\log_2(\text{FC})$ indicates a lower expression level in the infected condition than in the control one and a positive $\log_2(\text{FC})$ indicates a higher expression level in infected condition than in the control one. Spider charts are composed of two grid circles, the middle grid circle indicating 27.5% and the maximum grid circle indicating 55%.

Table 2. *S* genes distribution on wheat genome.

Chromosome	S gene number
chr1A	8
chr1B	9
chr1D	12
chr2A	11
chr2B	11
chr2D	10
chr3A	11
chr3B	8
chr3D	9
chr4A	11
chr4B	11
chr4D	9
chr5A	11
chr5B	10
chr5D	9
chr6A	8
chr6B	9
chr6D	8
chr7A	7
chr7B	8
chr7D	10
chrUn	3

Conclusion

L'objectif de ce deuxième chapitre était d'identifier des facteurs de sensibilité du blé au FHB mais aussi d'en comprendre leurs fonctions et leurs places au sein des processus biologiques de la plante hôte.

L'analyse des réponses du cultivar sensible 'Récital' aux trois souches d'agressivité contrastée a mis en évidence une vaste reprogrammation génétique, avec 26,779 gènes dérégulés quelle que soit la souche utilisée, et implique de nombreux facteurs de transcription. La caractérisation de la structure du réseau de régulation contrôlant cette reprogrammation a mis en évidence un réseau particulièrement complexe gouverné par des hubs majeurs de régulation, majoritairement induits au cours de l'infection. Près de la moitié de ces hubs appartiennent aux quatre familles de TFs suivantes : WRKY, NAC, AP2/ERF-ERF et MYB, qui sont des acteurs clés de la régulation des réponses des plantes aux stress biotiques et abiotiques (Rushton et al., 2010; Ng et al., 2018; Baillo et al., 2019; Bian et al., 2021) et qui semblent ici jouer un rôle central dans la mise en place des réponses du blé au FHB. Ces résultats illustrent ainsi que sensibilité du blé à *F. graminearum* est un caractère très complexe impliquant la régulation fine et coordonnée d'un grand nombre de processus biologiques par un ensemble de TFs très connectés qui représentent de véritables hubs biologiques au sein des mécanismes de transduction des signaux.

L'intégration des données d'expression du cultivar sensible 'Récital' et des effecteurs conservés chez les trois souches de *F. graminearum*, associée à l'utilisation d'une base de données de FS validés, a permis d'identifier 203 gènes de sensibilité putatifs dont 64% sont induits au cours de l'infection et joueraient un rôle clef dans la suppression des mécanismes de défense (van Schie and Takken, 2014), dont 58 gènes codant des TFs impliqués dans le réseau de régulation précédemment décrit et appartenant aux familles des WRKY, MYB et NAC. Ainsi, la stratégie d'infection de *F. graminearum* reposerait sur une manipulation à l'échelle systémique d'une large gamme de processus associés à la suppression des mécanismes de défense, lui permettant ainsi de pouvoir s'établir au sein des tissus.

Enfin, l'utilisation de cinq fonds génétiques hôtes a permis d'identifier un core-targetome putatifs composés de 123 gènes, dont 107 sont impliqués dans la suppression des mécanismes de défenses et 53 codent des TFs. *F. graminearum* étant capable de se développer dans l'ensemble de ces cultivars, ces gènes de sensibilité conservés représenteraient ainsi des déterminants hôtes nécessaires au succès de l'infection et constituent ainsi une ressource génétique de fort intérêt pour la recherche de résistances alternatives durables et large spectre chez le blé.

Nos résultats ont ainsi mis en évidence que la suppression des défenses est une étape clef du processus infectieux de *F. graminearum* et que les hubs de régulation représentent des cibles putatives privilégiées de ses effecteurs lui permettant ainsi de contrôler et de reprogrammer une large gamme de processus biologiques. Les interactions identifiées au cours de ces travaux entre les effecteurs nucléaires et les FS reposent sur des prédictions in silico et nécessitent évidemment une validation fonctionnelle en utilisant par exemple la méthode de Bimolecular fluorescence complementation (BiFC) (Kerppola, 2008).

Ce travail a mis en avant la nature complexe de la sensibilité du blé à *F. graminearum* qui se manifeste par la dérégulation d'un grand nombre de gènes impliqués dans le métabolisme primaire de la plante. Cependant, à ce jour, l'impact réel du FHB sur le statut physiologique de l'épi nécessite d'être caractérisé dans le but d'améliorer notre compréhension sur l'état de fonctionnement des tissus.

Chapitre III - Caractérisation de l'impact du dialogue moléculaire gouvernant l'interaction sur le statut physiologique de l'épi

Contexte, enjeux et méthodologie

Nos travaux ainsi que ceux menés par Francis Fabre (Fabre et al., 2019b, 2021) ont montré un impact fort du FHB sur les processus du métabolisme primaire du blé avec en particulier une reprogrammation importante des fonctions chloroplastiques. Le chloroplaste, organe siège des processus associés à la photosynthèse, est également un hub biologique important qui interconnecte un grand nombre de processus associés au métabolisme primaire, à la signalisation calcique et hormonale et aux mécanismes de défenses (Caplan et al., 2015; de Torres Zabala et al., 2015; Lu and Yao, 2018; Littlejohn et al., 2021). De plus, par le processus de signalisation rétrograde avec le noyau, par l'intermédiaire des stromules, le chloroplaste joue un rôle de senseur des stress et joue ainsi un rôle de médiateur dans la régulation transcriptomique de certains gènes du noyau dont beaucoup codent des protéines chloroplastiques (Caplan et al., 2015). Ainsi, le chloroplaste représente une cible privilégiée des effecteurs des agents pathogènes pour acquérir les nutriments nécessaires à leur développement ou supprimer les mécanismes de défense (Petre et al., 2016; Xu et al., 2019; Kretschmer et al., 2020).

Les méthodes actuelles de phénotypage de l'épi de blé au cours de l'infection par *F. graminearum* ne permettent pas d'apporter d'informations pertinentes concernant l'état de fonctionnement réel du chloroplaste et plus largement des autres organites. Afin de compléter les informations obtenues par l'approche transcriptomique, le statut physiologique des épis du cultivar sensible 'Récital' infectés par les deux souches de *F. graminearum* les plus contrastées en terme d'agressivité a été finement caractérisé par la mesure des échanges gazeux au cours des stades précoces de l'infection et avant l'apparitions des symptômes, de 24hpi à 72hpi. Cette technique d'écophysologie non-destructive a été appliquée avec succès à de nombreux pathosystèmes foliaires, permettant de décrire les effets des agents pathogènes étudiés sur les processus du métabolisme primaire de l'hôte (Horst et al., 2010; Carretero et al., 2011; Debona et al., 2014; Gortari et al., 2018).

L'épi étant un organe tri-dimensionnel très complexe, ces travaux ont été réalisés en collaboration avec Pierre Bancal, Chargé de Recherche au sein de l'UMR ECOSYS, qui a développé un prototype de chambre de mesure adapté à ce type d'organe (Fortineau and Bancal, 2018). L'association des résultats obtenus par l'approche transcriptomique et par l'approche d'écophysiole a permis de finement décrire les perturbations des processus de la photosynthèse et de la respiration induites par *F. graminearum* au cours de l'infection.

Article 3 - Gas exchange and transcriptome analysis revealed the effects of *F. graminearum* infection on wheat physiological status

Gas exchange and transcriptome analysis revealed the effects of *F. graminearum* infection on wheat physiological status

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Abstract

Wheat's susceptibility to *F. graminearum* results in a deep and sharp reprogramming of wide range of biological processes, including chloroplast associated functions. Chloroplast is well characterized biological hub at the interface of many processes such as photosynthesis, hormonal pathways, defense responses and thus might represent a major player of the molecular crosstalk established along FHB infection. Although the impacts of *F. graminearum* on chloroplast associated functions have been described at the molecular scale through proteomics and transcriptomics studies, phenotypic studies are needed to fill the gap between the observed molecular events and the actual impacts of the disease on the processes. Taking advantage of the gas exchange method, the effects of two *F. graminearum* strains of contrasting aggressiveness over spike's photosynthesis and respiration associated processes during an early infection time course were deeply characterized. A RNAseq-based expression profiling of the genes involved in the photosynthesis, respiration and stomatal movement processes was also performed using the same two strains. Combining information obtained from both complementary approaches, we revealed a strong increase of the respiration as well as an impairment of the photochemical and biochemical processes involved in photosynthesis, that was not related to the symptom development. In this respect, gas exchange approach has proven to be particularly robust to improve and deepen our understanding of the observed gene regulations.

Keywords: *Triticum aestivum*, Fusarium Head Blight, Ecophysiology, Gas Exchange variables, Transcriptomic

Introduction

Fusarium Head Blight (FHB), mainly caused by the Ascomycota fungus *Fusarium graminearum*, represents a major threat for wheat (Parry et al., 1995; Goswami and Kistler, 2004; McMullen et al., 2012; Dahl and Wilson, 2018). FHB has direct impacts on yield, grain quality and causes serious health concerns through the accumulation of carcinogenic mycotoxins (e.g., deoxynivalenol, DON) (Boyacioğlu and Hettiarachchy, 1995; Argyris et al., 2003; Chen et al., 2019). Existing control strategies, i.e. the combined use of fungicides, tolerant wheat genotypes and agricultural practices, are currently insufficient to fully control FHB epidemics (Dahl and Wilson, 2018; Xia et al., 2020). While breeding for resistance remains the most efficient tool to limit the damages associated with FHB outbreaks (Mesterhazy, 2020), FHB resistance trait is strictly quantitative and involves more than 625 Quantitative Trait Locus (Zheng et al., 2021) displaying relatively small effects (Bai et al., 2018; Steiner et al., 2017; Venske et al., 2019) and strong dependency to environmental conditions (Osman et al., 2015; Steiner et al., 2017). Over the past two decades, the multiple evidences of the involvement of host's genes, the so-called susceptibility genes (S genes), in the pathogen's establishment (Vogel, 2002) have opened new opportunities to identify new key determinants of plant diseases that can have direct application in breeding for resistance (Pavan et al., 2010; van Schie and Takken, 2014; Zaidi et al., 2018; Garcia-Ruiz et al., 2021). The importance of S genes in the outcome of wheat-*F. graminearum* interaction has already been demonstrated in several studies (Ma et al., 2006; Garvin et al., 2015; Hales et al., 2020; Chhabra et al., 2021; Nalam et al., 2015; Gordon et al., 2016; Hu et al., 2018; Li et al., 2019; Su et al., 2019; Brauer et al., 2020; Su et al., 2021), making the elucidation of the mechanisms underlying wheat susceptibility to FHB a promising approach to improve FHB resistance (Fabre et al., 2020; Gorash et al., 2021).

By definition, S genes are not only involved in the setup of a compatible interaction but also in a wide range of plant fundamental processes (Lapin and Van den Ackerveken, 2013; van Schie and Takken, 2014; Engelhardt et al., 2018; Garcia-Ruiz et al., 2021). Thus, beyond their identification, it is necessary to replace the S genes in their biological context. With the growing interest in the role of susceptibility in the wheat-*F. graminearum* pathosystem, it is necessary to extensively characterize the processes impacted along infection progress. While omics studies have resulted in large amounts of information on the processes impacted during

wheat's infection by *F. graminearum* (Fabre et al., 2020), the very complex nature of plant's susceptibility to pathogens makes it necessary to bridge the gap between the molecular information and the observed phenotype. In a recent proteomics study, chloroplast functions were found to be deeply reprogrammed in response to FHB and were thought to be a key component of wheat's susceptibility to FHB (Fabre et al., 2019b). Chloroplast is a central hub of the plant physiology that interconnects a wide array of biological processes, including photosynthesis, hormone synthesis (Jasmonate and Salicylic acid), programmed cell death signaling, calcium signaling and the synthesis of specialized metabolites, making it an important target of pathogen's effector to manipulate primary metabolism and the defense mechanisms (Caplan et al., 2015; de Torres Zabala et al., 2015; Lu and Yao, 2018; Kretschmer et al., 2020; Littlejohn et al., 2021).

Currently, the phenotypic characterization of wheat-*F. graminearum* interaction is mainly limited to the monitoring of symptoms, fungal mass and DON concentration (Mesterhazy, 2020) which does not bring any information on the impacted biological processes. Thus, breaking down the susceptibility trait into its physiological components is necessary. For this purpose, non-invasive technics such as gas-exchange measurements, that has been successfully applied to several pathosystems (Horst et al., 2010; Carretero et al., 2011; Debona et al., 2014; Gortari et al., 2018), can provide new insights of the physiological status of wheat's spikes during the interaction with *F. graminearum*.

The presented work addresses an in-depth characterization of the physiological status of wheat's spikes facing two *F. graminearum* strains of contrasting aggressiveness using gas exchange method. The main objectives were to investigate the pathogen-induced perturbations in photosynthesis and respiration and to connect the obtained data with the transcriptome profiling of wheat's FHB-responsive genes involved in those two biological processes.

Material and methods

Gene expression analysis

RNA-seq data

Gene expression data used in this study were generated from the susceptible wheat cultivar 'Recital' which was inoculated with three *F. graminearum* strains of contrasting aggressiveness (MDC_Fg1, MDC_Fg13 and MDC_FgU1; in decreasing order of aggressiveness (Fabre et al., 2019a)) or with water as controls (described in Rocher et al. In prep). The experiment, the bioinformatic pipeline and the differential expression analysis were detailed in chapter II.

Respiration and Photosynthesis- associated gene annotation

KEGG Orthology (KO) (Kanehisa and Goto, 2000) annotation of the High (HC) and Low Confidence (LC) wheat proteomes v1.1 ("IWGSC RefSeq v1.1 Reference Genome and Annotation," n.d.) was performed using GhostKOALA software with the "genus_prokaryotes + family_eukaryotes" option (Kanehisa et al., 2016). GhostKOALA results were then trimmed to keep only genes with an annotation generated from the plant kingdom. Respiration and Photosynthesis-associated KO lists were generated from the KEGG Modules part of the KEGG BRITE database (Kanehisa et al., 2006; "KEGG BRITE: KEGG Modules," n.d.).

Stomatal movement-associated gene annotation

Gene Ontology (GO) terms of *Triticum aestivum* v1.1 genome were retrieved from ensembl ("Triticum_aestivum (IWGSC) - Ensembl Genomes," n.d.) using the BiomaRt R interface (Durinck et al., 2022). GO terms were then used to build a wheat annotation orgdb R package using the makeOrgPackage function of the AnnotationForge R package (Carlson and Pagès, 2022). Following GO terms were used to retrieve wheat genes associated with stomatal movement: GO:1902456, GO:0090333. Gene functions were retrieved from UniProtKB database (Boutet et al., 2007).

Expression pattern characterization of the respiration and photosynthesis-associated genes

Statistical analyses were conducted on R v4.1.1 ("R: The R Project for Statistical Computing," n.d.). Only 'Recital' genes which were differentially expressed (DE) in response to the three strains in comparison to the control and with KO annotation terms belonging to

the respiration and photosynthesis-associated lists were used in this study. Prior to the analysis, raw count values were normalized using the regularized logarithm transformation (rlog) implemented in the DESeq2 package (Love et al., 2014). Then, a z-score transformation was applied to the normalized count values. Gene expression patterns were described by heatmaps using the Pheatmap package (Kolde, 2021). The genes and samples were clustered using the ward.D2 agglomeration method applied on the Euclidean distance matrices (Murtagh and Legendre, 2014).

Gas exchange experiments

Experimental designs and measurement protocol

To finely characterize FHB impacts on the physiological status of wheat's spikes we measured the gas exchanges of spikes under two light intensities, the first one with a Photosynthetic Photon Flux Density (PPFD) of $616 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ close to that of ambient light in the growth chamber (ambient light experiment) and the second one with a PPFD of $968 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (saturation light experiment, Fortineau and Bancal, 2018) (*Figure 1*). Both experiments were conducted in growth chambers with tightly controlled experimental conditions: 16:8 light cycle with a PPFD of $520 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at spike height, temperatures of $21^{\circ}\text{C}/17^{\circ}\text{C}$ (day/night) and a constant relative humidity of 80%. In both experiments, the susceptible cultivar 'Recital' was inoculated with the two most contrasting strains in terms of aggressiveness (MDC_Fg1 and MDC_FgU1; in decreasing order of aggressiveness) or with water as controls (*Figure 1*). Spore production as well as detailed plant growth conditions are described in Rocher et al. (Rocher et al., 2022). *F. graminearum* strains were inoculated at mid-anthesis by inoculating $10 \mu\text{L}$ of a solution at a concentration of 10^5 spores/mL in the floral cavity of all the spikelets of one spike. For the control samples, water was inoculated according to the same procedure. Complete factorial experiments were designed for both experiments. Gas exchange measurements were performed on six biological replicates for each of the three treatment modality in each experiment, representing a total of 18 plants per experiment. Gas exchange measurements were performed using a portable gas-exchange system (*Figure 1-(c)*) (LI-6400XT; LI-COR, Lincoln, USA) associated with a light chamber specifically designed for spikes and three-dimensional organs (*Figure 1-(b)*) (Fortineau and Bancal, 2018).

For both experiments, pre-inoculation measurements were performed one day before the inoculations. Then, measures were performed along a three point-kinetics on the same

plants, at 24 hours post inoculation (hpi), 48hpi and 72hpi, that cover the pre-symptomatic period (*Figure 1*). At each time point, measures were performed during light and dark periods, with a 3h time-interval between the two series of measures. In the saturation light experiment, plants were placed in a light adaptation chamber (*Figure 1-(a)*), composed of 4 vertical LED bars (BL120c, Valoya, Helsinki, Finland) and one top LED panel (GentleSpace gen², Philips Lighting France Signify, Créteil, France) for a total PPFD of 1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, during 10mn before the diurnal measures were performed. All parameters used in the experiments are detailed in Table 1. For each spike, after a stabilization period of 200s between the chamber closure and the first measure, a series of seven measures every 10s was performed. After the seventh measure, a match of the reference and sample infrared gas analyzers (IRGAs) was performed. Spike's temperature was monitored throughout the measurements using a thermocouple placed inside one of the central spikelets. Ambient CO₂ concentration was measured every 5s throughout the gas exchanges measures using a CO₂/H₂O gas analyzer (LI-840, LI-COR, Lincoln, USA). At the end of both experiments, inoculated spikes were scanned and their projected surface area were estimated using ImageJ software v.1.53e ("ImageJ," n.d.).

Score symptom monitoring and analysis

The symptoms were scored using the 5-level rating scale given to each inoculated spikelet and described in (Fabre et al., 2019a). The symptoms produced by MDC_Fg1 and MDC_FgU1 on the cultivar 'Recital' were assessed at 24hpi, 48hpi and 72hpi on the same spikes used for gas exchange measurements. Score symptoms from both experiments were simultaneously analyzed leading to a total of 12 biological replicates for each strain. The statistical analysis was conducted on the score symptoms averaged per biological replicate using a two-way repeated measures ANOVA, the kinetic factor was nested within each biological replicate, with the `anova_test` function of the `rstatix` package (KASSAMBARA, 2022). Pairwise comparisons were performed using pairwise t-tests with the `pairwise-t-test` function of the `rstatix` package (KASSAMBARA, 2022).

Ecophysiological variable computations and statistical analysis

The formulas used to compute the ecophysiological variables are detailed in the supplementary material. For all variables, we computed the mean of the measurement series (seven successive measures). All studied variables, except the absolute gross CO₂ assimilation

rate (P_{gross}) at 72hpi, were normalized according to the pre-inoculation point (X_0). This study focused on the following variables: Relative respiration rate (R/R_0), Relative net CO_2 assimilation rate (P_{net}/P_{net0}), Relative gross CO_2 assimilation rate (P_{gross}/P_{gross0}), Relative total conductance to CO_2 (g_{tc}/g_{tc0}) and Relative intercellular CO_2 concentration (C_i/C_{i0}). All those variables are in the supplementary table 1. For those variables, two-way repeated measures ANOVAs, with the kinetic factor nested within the biological replicate, were performed using the `anova_test` function of the `rstatix` package (KASSAMBARA, 2022). Pairwise comparisons were performed using pairwise t-tests with the `pairwise-t-test` function of the `rstatix` package (KASSAMBARA, 2022). Corrections for multiple testing were applied for all variables, except P_{gross}/P_{gross0} , using the Bonferroni adjustment method. The intensity of the P_{net}/P_{net0} and P_{gross}/P_{gross0} differences between 48hpi and 72hpi were compared for MDC_Fg1 and MDC_FgU1 using pairwise t-tests with the `pairwise-t-test` function of the `rstatix` package (KASSAMBARA, 2022). Light intensity and treatment effects were analyzed on the absolute P_{gross} at 72hpi using a two-way ANOVA with the `anova_test` function of the `rstatix` package (KASSAMBARA, 2022). Pairwise comparisons were performed using pairwise t-tests with the `pairwise-t-test` function of the `rstatix` package (KASSAMBARA, 2022).

Results

In situ FHB impacts on photosynthesis- and respiration-physiological parameters

Consequences of *F. graminearum* development in the spike's physiological functioning were assessed using two *F. graminearum* strains of contrasting aggressiveness, MDC_Fg1 (the most aggressive strain) and MDC_FgU1 (the less aggressive one) as previously reported in Fabre et al. (2019). In our study, symptom survey confirmed this previous work with clear differences as early as 48hpi between MDC_Fg1 and MDC_FgU1 (*Figure 2*). While a significant increase was observed in the induced symptoms for both strains between 48hpi and 72hpi, MDC_Fg1 induced symptoms earlier and with more intensity than MDC_FgU1, with an average score symptom at 72hpi that was twice as severe with MDC_Fg1 compared to MDC_FgU1.

FHB induced a strong increase of the spike's respiration rate

In response to MDC_Fg1 and MDC_FgU1 infection, a clear and significant increase of the relative respiration rate (R/R_0) was monitored at the whole spike level along with the

infection progress, for both ambient and saturation light experiments (Figure 3 and Table 2). In the control plants, a slight increase of the R/R0 was detected between 24hpi and 72hpi only in the ambient light experiment (Table 2). While, no differences were detected between the treatments at 24hpi, MDC_Fg1 and MDC_FgU1 induced a significantly higher R/R0 in the spikes than in the control plants at 48hpi and 72hpi, in both experiments (Figure 3). Significant strain-dependent responses were detected only in the ambient experiment, where MDC_Fg1 induced a higher R/R0 than MDC_FgU1 at both 48hpi and 72hpi (Figure 3).

FHB resulted in a decrease of the spike's CO₂ assimilation rate

While control samples did not displayed any changes of the relative net CO₂ assimilation rate (Pn/Pn0) during the experiment, MDC_Fg1 and MDC_FgU1 induced a significant drop of the Pn/Pn0 between 48hpi and 72hpi, independently of the incident lighting (Table 3). No differences were observed between conditions at both 24hpi and 48hpi but both strains induced a significantly lower Pn/Pn0 compared to control plants at 72hpi in both ambient and saturation light experiments (Figure 4). No strain-dependent changes allowed to distinguish 'Recital' responses to MDC_Fg1 and MDC_FgU1.

Similar trends were observed in the relative gross CO₂ assimilation rate (Pgross/Pgross0), with a significant drop of Pgross/Pgross0 between 48hpi and 72hpi for both strains while Pgross/Pgross0 remained stable in the control spikes (Table 4). Pgross/Pgross0 drop at 72hpi did not allow to distinguish the impact of the two strains in both experiments (Figure 5). The Pgross/Pgross0 drop between 48hpi and 72hpi was significantly lower than the Pn/Pn0 drop for both MDC_Fg1 and MDC_FgU1. While MDC_Fg1 induced a significant higher Pgross/Pgross0 than MDC_Fg13 at 24hpi in the ambient light experiment only, no differences of Pgross/Pgross0 were detected between the three conditions at 48hpi in both experiments. At 72hpi, MDC_Fg1 induced a significant lower Pgros/Pgross0 than in control plants in both experiments while the response to MDC_Fgu1 was significantly different to the control plants in the saturation light experiment only (Figure 5). In agreement with Pn/Pn0, no clear strain-dependent trends of Pgross/Pgross0 allowed to distinguish 'Recital' responses to MDC_Fg1 and MDC_FgU1.

Finally, we studied the effect of incident lighting intensity on the absolute gross CO₂ assimilation rate (Pgross) at 72hpi. No interaction effect was detected between the light intensity and the treatment factors, indicating a generic light response independent of the

treatments. Pgross intensity was significantly higher ($+1.2 \mu\text{mol/s/m}^2$) in the saturation light condition than in the ambient light (*Figure 6*).

FHB infection did not change the conductance to CO_2

The effects of FHB on the stomatal aperture was investigated using the relative total conductance to CO_2 (g_{tc}/g_{tc0}) and the relative CO_2 concentration in the sub-stomatal internal cavities (C_i/C_{i0}). The only significant factor impacting g_{tc}/g_{tc0} was the duration after spike inoculation. A significant reduction of the g_{tc}/g_{tc0} observed between 48hpi and 72hpi for both fungal and water inoculated plants in both experiments (*Figure 7*).

In the ambient light experiment, MDC_Fg1 and MDC_FgU1 induced a significant increase of the C_i/C_{i0} along with the infection duration while it remained stable in the control spikes (*Table 5A*). In saturation light experiment, only MDC_Fg1 induced a significant increase of the C_i/C_{i0} between 48hpi and 72hpi while a significant decrease was observed in the control spikes (*Table 5B*). In both experiments, no differences of C_i/C_{i0} were detected between conditions at 24hpi and 48hpi (*Figure 8*). At 72hpi, the C_i/C_{i0} was significantly higher in response to MDC_Fg1 and MDC_FgU1 than in the control spikes in the ambient light condition (*Figure 8A*) while only MDC_Fg1 induced a higher C_i/C_{i0} than the control spikes in the saturation light experiment (*Figure 8B*). However, in both experiments, no strain-specific changes in C_i/C_{i0} allowed to clearly distinguish 'Recital' responses to MDC_Fg1 and MDC_FgU1.

FHB impacts on photosynthesis- and respiration-related genes

F. graminearum effects on the genes involved in photosynthesis- and respiration-associated processes were studied using the FHB-responsive transcripts that were systematically regulated in the 'Recital' cultivar when facing each of the three *F. graminearum* strains of contrasting aggressiveness, MDC_Fg1, MDC_Fg13 and MDC_FgU1 (Rocher et al. In prep).

The photosynthesis-associated genes expression in response to FHB were widely repressed

A total of 256 genes involved in photosynthesis-associated processes were found to be significantly impacted by each of the fungal strains ("Photosynthesis responsive genes"). The Hierarchical Ascendant Clustering (HAC) applied on the conditions clearly discriminated the

infected modalities from the control as well as the infection stages for the infected modalities rather than the fungal strains (*Figure 9*).

Most of the “Photosynthesis responsive genes” were repressed in response to FHB. Around 81% (207 genes) of this gene set displayed higher expression in the control modality than in the infected ones (Cluster C1, Table 6A), with a strong expression drop between 48hpi and 72hpi in the infected spikes (*Figure 9*). On the opposite, only 49 genes displayed lower expression levels in the control modality than in the infected ones (Cluster C2, Table 6A), with a strong increase of their expression between 48hpi and 72hpi. Interestingly, all the genes involved in “Light harvesting” (30 genes), “Photosystem I” (19 genes), “F-type ATPase” (9 genes) and “Cytochrome b6/f complex” (1 gene) processes were under-expressed in response to FHB (Table 6A). Finally, 88% (22 genes), 74% (110 genes) and 70% (16 genes) of the genes involved in “Photosystem II”, “Carbon Fixation” and “Electron Transport” were also found to be under-expressed in response to FHB (Table 6A). Finally, only one gene, TraesCS5B02G516600, involved in the “Photosystem II” process and belonging to the first clustering group was differentially expressed between MDC_Fg1 and MDC_FgU1.

FHB-induced regulations in respiration-related genes showed a multifaceted response-

A total of 244 genes involved in the respiration-associated processes were found to be regulated upon FHB development (“Respiration responsive genes”). As for the photosynthesis-related genes, the HAC of the mRNA accumulation in each condition clearly discriminated the control plants from the infected ones as well as the infection stage for the infected modalities, while the strain aggressiveness only displayed minor effects on the variances (*Figure 10*).

FHB responses associated with the respiration process were divided in two balanced groups. The first group, gathering 125 genes, displayed decreasing mRNA expression in the infected plants (Cluster C1, Table 6B), with a strong expression drop between 48hpi and 72hpi (*Figure 10*). On the opposite, the second group, gathering 119 genes, displayed increasing mRNA expression in the infected plants (Cluster C2, Table 6B), with a strong expression burst between 48hpi and 72hpi. The “Oxidative Phosphorylation” process was the only process clearly under-expressed in the infected plants in comparison with the control ones, with 63% of the genes involved in this process being down regulated (Table 6B). On the opposite, the “Krebs cycle” and the “Pyruvate oxidation” processes were mostly over-expressed in the

infected plants in comparison with the control ones, with 67% and 86% of the genes involved in those processes being up-regulated, respectively (Table 6B). In the “Glycolysis” process, 50 and 48 genes displayed lower and higher expression levels in the infected plants than in the control ones, respectively (Table 6B). Only two genes (TraesCS1D02G215900 and TraesCS3B02G199500) belonging to the second group and involved in the “Glycolysis” and the “Krebs cycle” processes respectively, were differentially expressed between MDC_Fg1 and MDC_FgU1.

The stomatal movement-associated genes expression in response to FHB were widely repressed

A total of 7 genes involved in stomatal movement-associated processes were found to be significantly impacted by each of the fungal strains (“Stomata responsive genes”). The HAC applied on the conditions clearly discriminated the infected modalities from the control as well as the infection stages for the infected modalities rather than the fungal strains (*Figure 11*).

Except TraesCS5A02G337100, all the “Stomata responsive genes” displayed higher expression in the control modality than in the infected ones, with a strong expression drop between 48hpi and 72hpi in the infected spikes (*Figure 11*). Among down-regulated functions in response to FHB, 3 homeologs coding for “chloroplast calcium sensing receptors”, 2 homeologs coding for “mitogen-activated protein kinases” and 1 gene coding for a “key zinc finger transcription factor gene” were found (Table 7). The only up-regulated gene in response to FHB coded for a “phospholipase D” (Table 7).

Discussion

Taking advantage of two *F. graminearum* strains of contrasting aggressiveness, the impacts of FHB on spike’s basal functioning were assessed using complementary approaches at the ecophysiological and molecular level. This included the characterization of the gas exchanges of wheat spikes during the infection progress using a specific device for three dimensional organs along with the expression profiles of photosynthesis- and respiration-associated genes.

Increase in respiration rate upon FHB is not only explain by plant gene regulations

FHB infection clearly induced a strong increase of the respiration rate in ‘Recital’ spikes that could be associated with a strong energy demand due to an accelerated metabolic activity

or to the setup of plant defenses (Major et al., 2010; Debona et al., 2014; Gramig and Harris, 2015). However, contrary to the photosynthetic processes, no clear links between the expression of the genes involved in the respiration pathways and the measured respiration rate have been observed. This may be the direct consequence of the highly flexible nature of plant respiration that can employ alternative pathways that bypass the conventional ones (Plaxton and Podestá, 2006; van Dongen et al., 2011). For instance, plants are able to use other substrates than the pyruvate such as the malate, the formate but also amino and fatty acids (Plaxton and Podestá, 2006). The respiration case clearly exemplifies the importance of phenotypically characterize the disease impacts on the host's biological processes to complete the data obtained from omics studies and to improve our understanding of the interaction. One limit of this study is that FHB effects on photorespiration processes, known to be involved in plant defenses against pathogens through H_2O_2 accumulation (Sørhagen et al., 2013), were not assessed.

FHB induced an impairment of the photo- and bio-chemical processes associated with photosynthesis not only connected to the alteration of symptomatic tissues

Ecophysiological characterization of the CO_2 assimilation rate, especially with the increased light intensity, clearly revealed an impairment of the photosynthesis at both net and gross level between 48hpi and 72hpi regardless of the strain aggressiveness that could not be related to the symptom appearance. The intense drop of the net CO_2 assimilation rate at 72hpi was primarily due to the large increase of the respiration rate observed between 48hpi and 72hpi. Yet the gross assimilation rate also decreased despite in a smaller extent. Thus, the FHB impact on the net photosynthesis rate was a combination of a decrease in gross photosynthesis rate and an increase of the respiration rate. Interestingly, the total conductance to CO_2 largely increased from 0 to 24hpi in both control and inoculated spikes. This ontological change could be attributed to glume aperture around anthesis. What's more, FHB did not affect the total conductance to CO_2 , suggesting that neither the stomatal functioning nor the cuticular permeability to CO_2 were altered along the infection progress. Consequently, the observed photosynthesis drop by FHB could not be related to a reduced CO_2 influx in the tissue and the C_i increase within spike is a direct consequence of the drop in the net CO_2 assimilation rate. Because the effect of different CO_2 concentrations on the net CO_2 assimilation rate was not assessed, we cannot exclude an impairment of the conductance of the mesophyll. However, FHB infection was associated with a sharp down-regulation of the

genes associated with the photochemical and biochemical processes and this independently of the strain's aggressiveness. Transcriptomic results associated with photosynthesis-associated processes corroborates a previous in planta proteomic study that demonstrated a strong abundance decrease of the proteins involved in light harvesting, photosystems and electron transport chain between 48hpi and 72hpi (Fabre et al., 2019b). Those processes were also similarly impacted at the proteome scale in 'Recital' facing the same *F. graminearum* strains (Fabre et al., 2019a). What's more, the functions coded by the genes associated with stomatal movement that were down-regulated in response to FHB were associated with drought stress tolerance and stomatal closure regulation in the literature (Wang et al., 2014; Cui et al., 2015; de Zelicourt et al., 2016; Kulkarni et al., 2017; Wang et al., 2019; Li et al., 2022), indicating that stomatal closure was not induced upon FHB infection. For instance, the chloroplast calcium-sensing receptors (CAS), localized at the thylakoid membrane, were proved to induce stomatal closure in response to extracellular calcium-stimuli (Li et al., 2022). Besides their well characterized role played in drought stress tolerance, CAS are also key components of the PAMP triggered immunity, the salicylic acid synthesis, involved in immunity against biotrophic pathogens, but also in chloroplast-nucleus communication under biotic stress (Medina-Puche et al., 2020). Taken together our results suggests that the observed decrease of photosynthesis was neither a consequence of the symptom development nor an alteration of the stomatal functioning but the result of a direct or indirect alteration of the photochemical and biochemical processes. In line with our conclusions, Francesconi *et al.* (2020) demonstrated that the genes involved in stomatal closure were not induced in response to FHB in the susceptible cultivar 'Rebelde' and that the photosynthetic efficiency was altered at 72hpi (Francesconi and Balestra, 2020). Similar results were also obtained in other pathosystems (Robert et al., 2006; Carretero et al., 2011; Debona et al., 2014; Tatagiba et al., 2015; Nogueira Júnior et al., 2019). For instance, the infection of rice leaves by *Monographella albescens* resulted in a significant photosynthesis decrease prior symptom development which was mainly associated with a decrease of light capture and this photosynthesis impairment was also reported in asymptomatic tissues (Tatagiba et al., 2015). Finally, despite the impairment of the photosynthesis processes, spike's tissues still reacted to variation of light intensity suggesting that chloroplasts remained partly functional. While our results are promising, an extended phenotypic characterization of the physiological status of wheat's spikes facing FHB is needed. For instance, chlorophyll fluorescence imaging,

successfully applied in many pathosystems, might bring relevant information concerning the efficiency of the photosystems and the non-photochemical chlorophyll fluorescence quenching energy loss (Rolfe and Scholes, 2010; Backes et al., 2021).

To conclude, combining the molecular characterization of a pathosystem with ecophysiological methods allowed to refine our understanding of the host's processes impacted along the infection progress. Gas exchanges measurements has proven to be a powerful tool to finely phenotype a trait as complex as the susceptibility by going beyond the use of traditional variables such as the score symptom monitoring.

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Figures and Tables

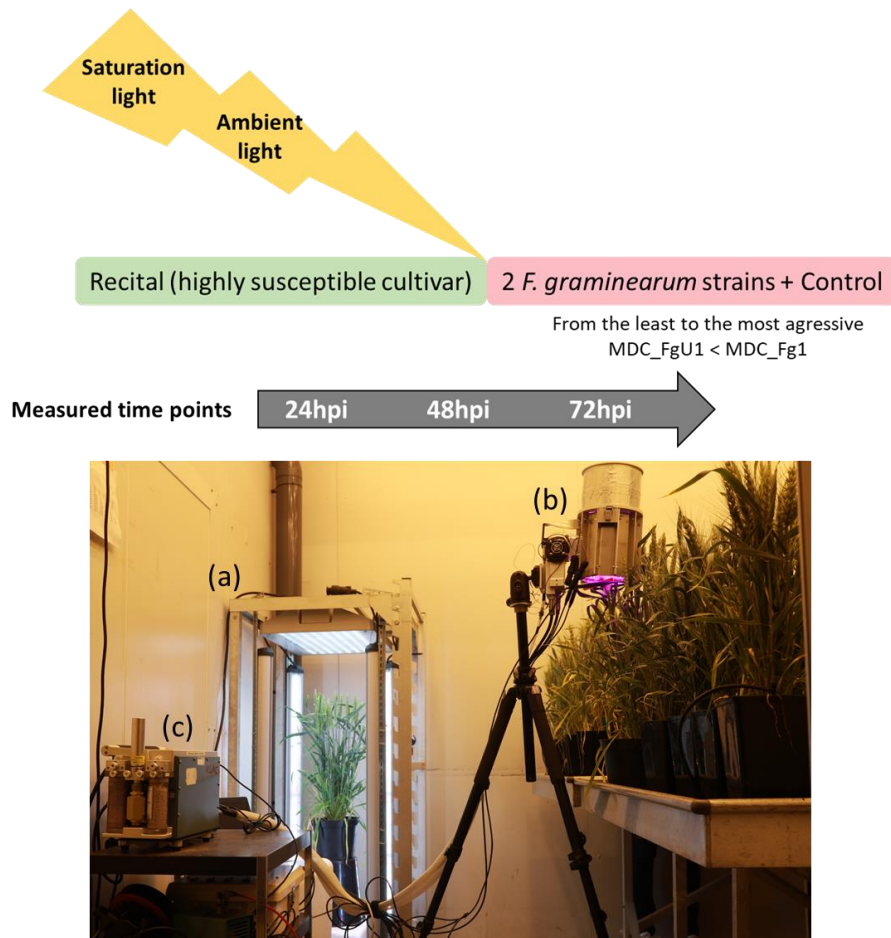


Figure 1. Overview of the experimental design of the study. Two ecophysiological experiments were performed with 2 light conditions, one with ambient light and the other one by adapting the plants at saturation light 15mn before the measures. For both experiments, the susceptible cultivar ‘Recital’ was inoculated with two *F. graminearum* strains, MDC_FgU1 and MDC_Fg1 in increasing order of aggressiveness, and with water for the controls. Gas exchange measures were performed at 24hpi, 48hpi and 72hpi (hpi: hours post inoculation). The photo shows (a) the chamber used for the light adaptation and (b) the measure chamber connected to (c) the LICOR 6400 device.

Table 1. Parameters set during the ambient and saturation light experiment during (A) diurnal and during (B) nocturnal measures.

(A)			(B)		
Parameters	Ambient light experiment	Saturation light experiment	Parameters	Ambient light experiment	Saturation light experiment
PPFD ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	616 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	968 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	PPFD ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	0 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
Reference CO ₂ (ppm)	400ppm	400ppm	Reference CO ₂ (ppm)	400ppm	400ppm
Flow ($\mu\text{mol}\cdot\text{s}^{-1}$)	600 $\mu\text{mol}\cdot\text{s}^{-1}$	600 $\mu\text{mol}\cdot\text{s}^{-1}$	Flow ($\mu\text{mol}\cdot\text{s}^{-1}$)	600 $\mu\text{mol}\cdot\text{s}^{-1}$	600 $\mu\text{mol}\cdot\text{s}^{-1}$
Block temperature	21°C	17°C	Block temperature	17°C	17°C

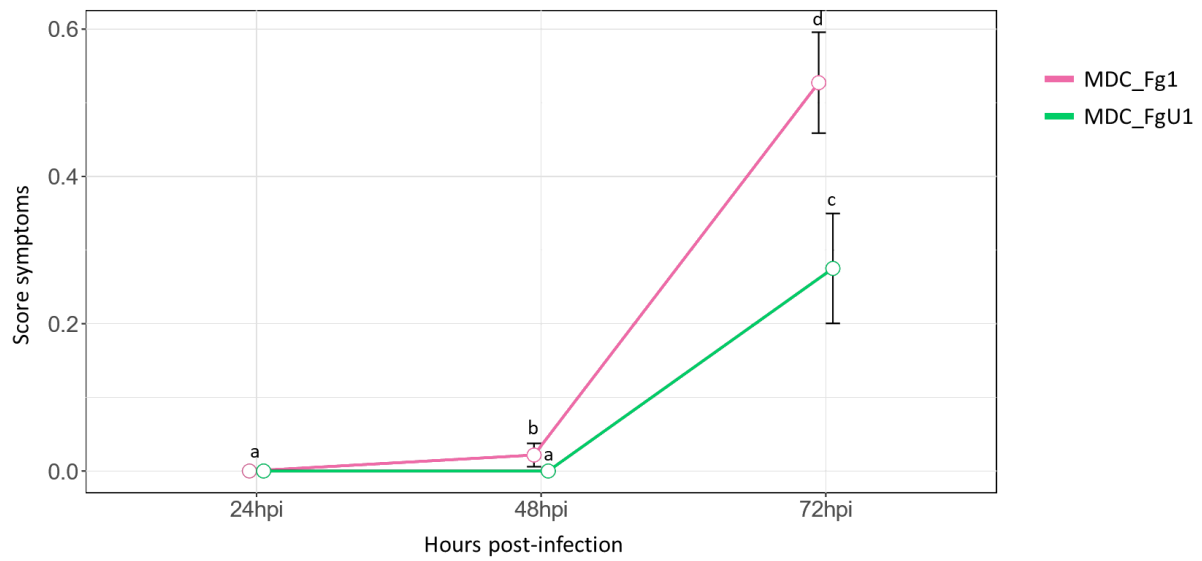


Figure 2. Symptom development induced by the two *F. graminearum* strains of contrasting aggressiveness. Line plots represent the score symptoms observed at 24hpi, 48hpi and 72hpi for MDC_Fg1 (pink) and MDC_Fg13 (green). Error bars represent the 95% confidence intervals. Significance threshold: p -value <0.05

Table 2. Statistical significance of the time point factor for a given treatment modality on Relative respiration rate of 'Recital' spikes measured in (A) ambient light and (B) saturation light conditions. Asterisks indicate a significant difference between two time points for one given treatment modality while ns indicate a nonsignificant difference. Significance threshold: Bonferroni adjusted p-value<0.05.

(A)				(B)			
Treatment modality	Tested time contrast	Adjusted p-value (Bonferroni)	Significance (Adjusted p-value<0,05)	Treatment modality	Tested time contrast	Adjusted p-value (Bonferroni)	Significance (Adjusted p-value<0,05)
MDC_Fg1	24hpi-48hpi	0.007	**	MDC_Fg1	24hpi-48hpi	0.025	*
MDC_Fg1	24hpi-72hpi	0.0000474	****	MDC_Fg1	24hpi-72hpi	0.000126	***
MDC_Fg1	48hpi-72hpi	0.002	**	MDC_Fg1	48hpi-72hpi	0.002	**
MDC_FgU1	24hpi-48hpi	0.001	**	MDC_FgU1	24hpi-48hpi	0.157	ns
MDC_FgU1	24hpi-72hpi	0.0000579	****	MDC_FgU1	24hpi-72hpi	0.00000849	****
MDC_FgU1	48hpi-72hpi	0.000429	***	MDC_FgU1	48hpi-72hpi	0.000759	***
Control	24hpi-48hpi	0.504	ns	Control	24hpi-48hpi	0.204	ns
Control	24hpi-72hpi	0.04	*	Control	24hpi-72hpi	0.234	ns
Control	48hpi-72hpi	0.064	ns	Control	48hpi-72hpi	1	ns

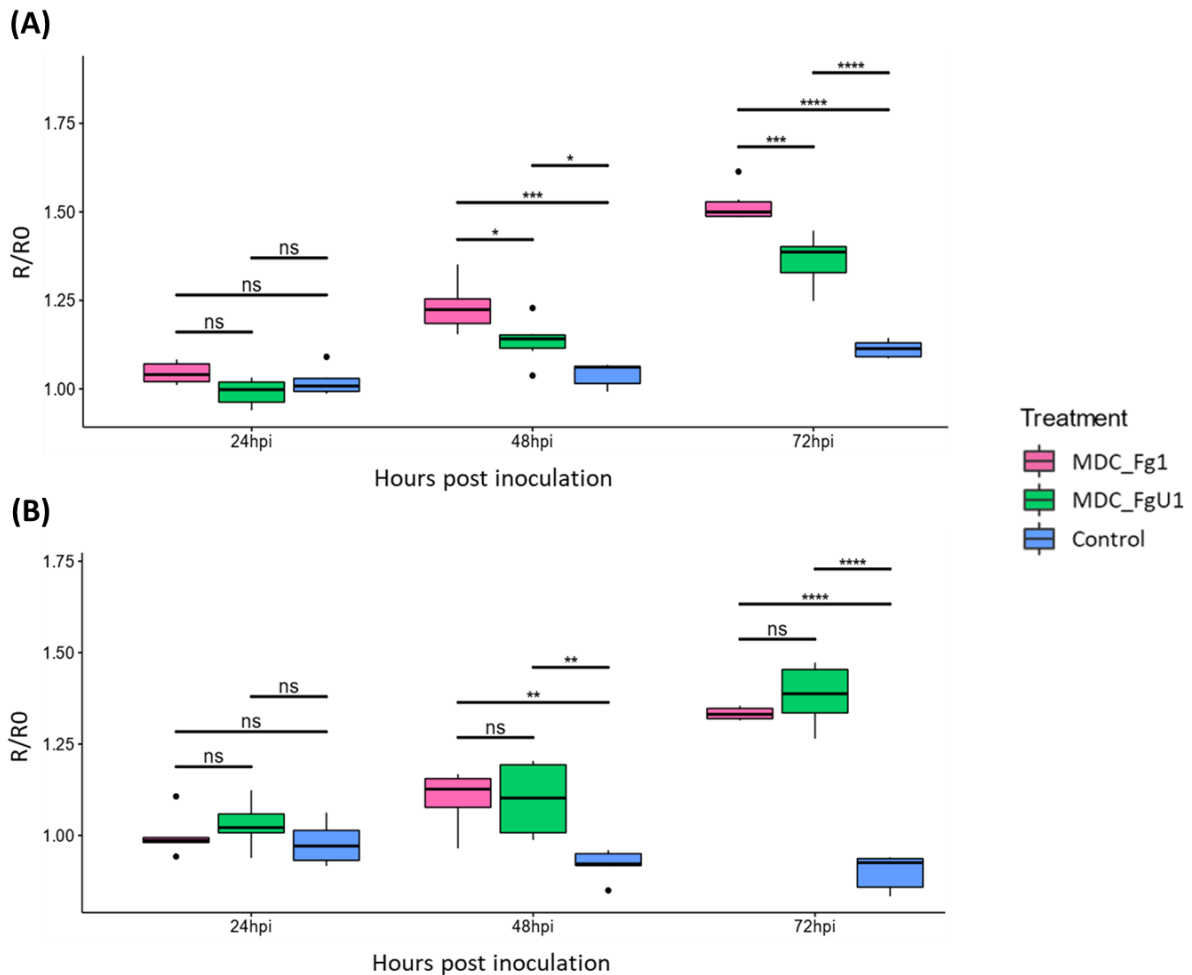


Figure 3. Relative respiration rate of 'Recital' spikes measured in response to treatment along infection progress. Boxplots represent the respiration rate of the spikes (R) measured at 24hpi, 48hpi and 72hpi after the inoculation of MDC_Fg1 (pink), MDC_FgU1 (green) and water (blue) for controls relatively to the pre-inoculation point (RO) in (A) ambient light and (B) saturation light conditions. Asterisks indicate a significant difference between two treatment modalities at one given time point while ns indicate a nonsignificant difference. Significance threshold: Bonferroni adjusted p-value<0.05.

Table 3. : Statistical significance of the time point factor for a given treatment modality on Relative net CO₂ assimilation rate of 'Recital' spikes measured in (A) ambient light and (B) saturation light conditions. Asterisks indicate a significant difference between two time points for one given treatment modality while ns indicate a nonsignificant difference. Significance threshold: Bonferroni adjusted p-value<0.05.

Treatment modality	Tested time contrast	Adjusted p-value (Bonferroni)	Significance (Adjusted p-value<0,05)
MDC_Fg1	24hpi-48hpi	0.171	ns
MDC_Fg1	24hpi-72hpi	0.002	**
MDC_Fg1	48hpi-72hpi	0.000756	***
MDC_FgU1	24hpi-48hpi	0.093	ns
MDC_FgU1	24hpi-72hpi	0.0000609	****
MDC_FgU1	48hpi-72hpi	0.000423	***
Control	24hpi-48hpi	1	ns
Control	24hpi-72hpi	0.068	ns
Control	48hpi-72hpi	0.077	ns

Treatment modality	Tested time contrast	Adjusted p-value (Bonferroni)	Significance (Adjusted p-value<0,05)
MDC_Fg1	24hpi-48hpi	0.02	*
MDC_Fg1	24hpi-72hpi	0.000289	***
MDC_Fg1	48hpi-72hpi	0.00036	***
MDC_FgU1	24hpi-48hpi	0.303	ns
MDC_FgU1	24hpi-72hpi	0.004	**
MDC_FgU1	48hpi-72hpi	0.006	**
Control	24hpi-48hpi	1	ns
Control	24hpi-72hpi	1	ns
Control	48hpi-72hpi	1	ns

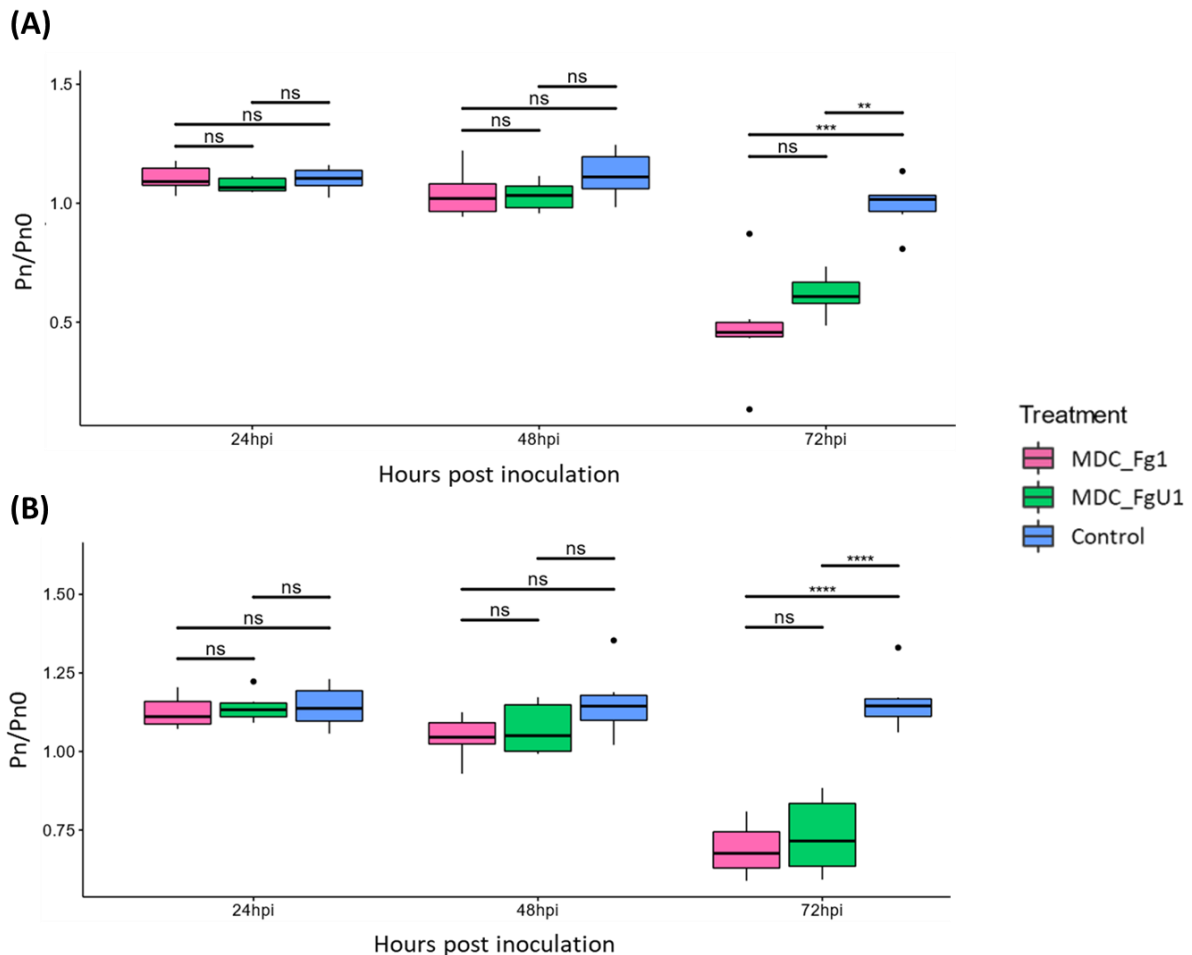


Figure 4. Relative net CO₂ assimilation rate of 'Recital' spikes measured in response to treatment along infection progress. Boxplots represent the net CO₂ assimilation rate of the spikes (P_n) measured at 24hpi, 48hpi and 72hpi after the inoculation of MDC_Fg1 (pink), MDC_FgU1 (green) and water (blue) for controls relatively to the pre-inoculation point (P_{n0}) in (A) ambient light and (B) saturation light conditions. Asterisks indicate a significant difference between two treatment modalities at one given time point while ns indicate a nonsignificant difference. Significance threshold: Bonferroni adjusted p-value<0.05.

Table 4. Statistical significance of the time point factor for a given treatment modality on Relative gross CO₂ assimilation rate of 'Recital' spikes measured in (A) ambient light and (B) saturation light conditions.

(A)				(B)			
Treatment modality	Tested time contrast	p-value	Significance (p-value<0,05)	Treatment modality	Tested time contrast	p-value	Significance (p-value<0,05)
MDC_Fg1	24hpi-48hpi	0.147	ns	MDC_Fg1	24hpi-48hpi	0.225	ns
MDC_Fg1	24hpi-72hpi	0.011	*	MDC_Fg1	24hpi-72hpi	0.002	**
MDC_Fg1	48hpi-72hpi	0.005	**	MDC_Fg1	48hpi-72hpi	0.000741	***
MDC_FgU1	24hpi-48hpi	0.042	*	MDC_FgU1	24hpi-48hpi	0.354	ns
MDC_FgU1	24hpi-72hpi	0.0000511	****	MDC_FgU1	24hpi-72hpi	0.018	*
MDC_FgU1	48hpi-72hpi	0.000292	***	MDC_FgU1	48hpi-72hpi	0.027	*
Control	24hpi-48hpi	0.454	ns	Control	24hpi-48hpi	0.695	ns
Control	24hpi-72hpi	0.276	ns	Control	24hpi-72hpi	0.533	ns
Control	48hpi-72hpi	0.163	ns	Control	48hpi-72hpi	0.701	ns

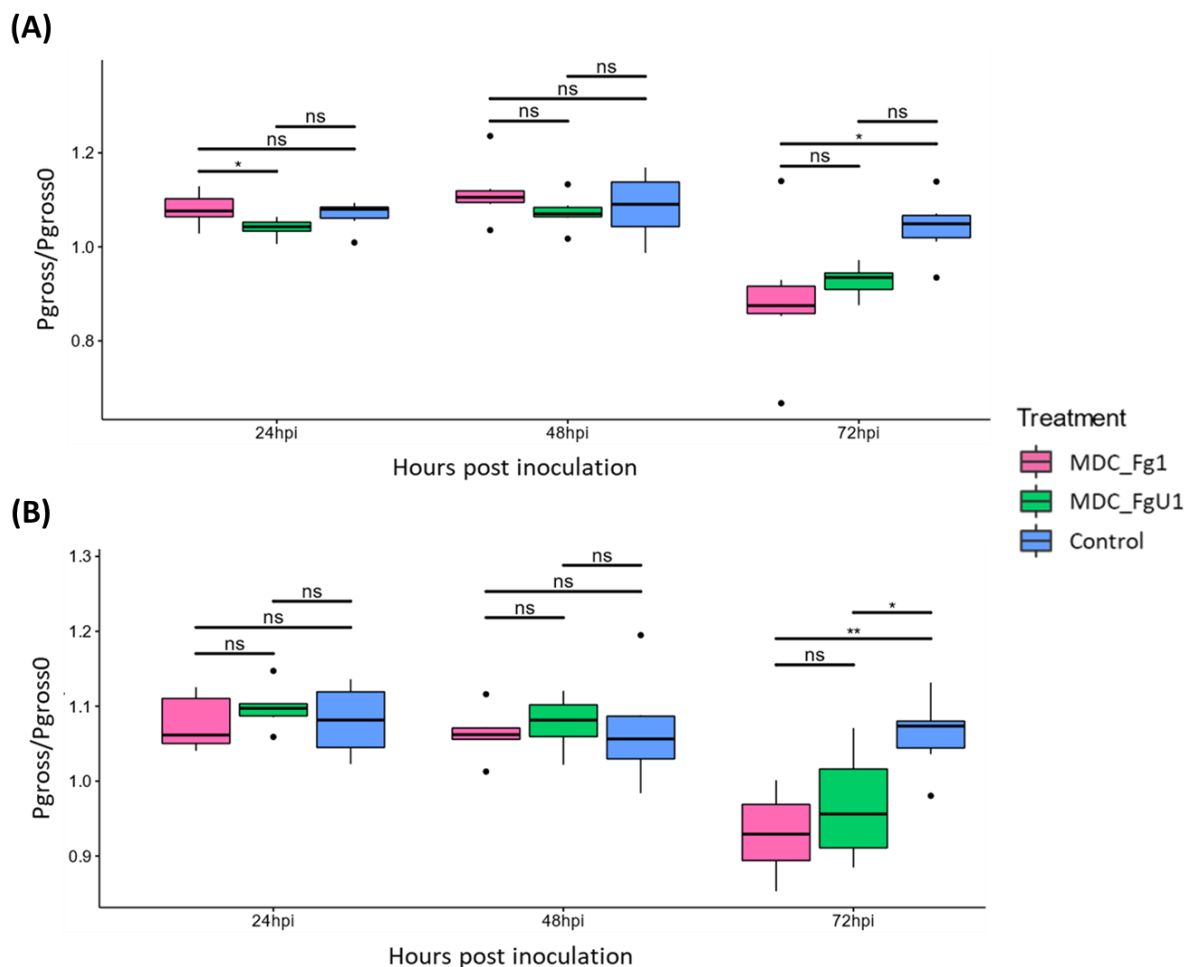


Figure 5. Relative gross CO₂ assimilation rate of 'Recital' spikes measured in response to treatment along infection progress. Boxplots represent the gross CO₂ assimilation rate of the spikes (P_{gross}) measured at 24hpi, 48hpi and 72hpi after the inoculation of MDC_Fg1 (pink), MDC_FgU1 (green) and water (blue) for controls relatively to the pre-inoculation point (P_{gross0}) in (A) ambient light and (B) saturation light conditions. Asterisks indicate a significant difference between two treatment modalities at one given time point while ns indicate a nonsignificant difference. Significance threshold: p-value<0.05.

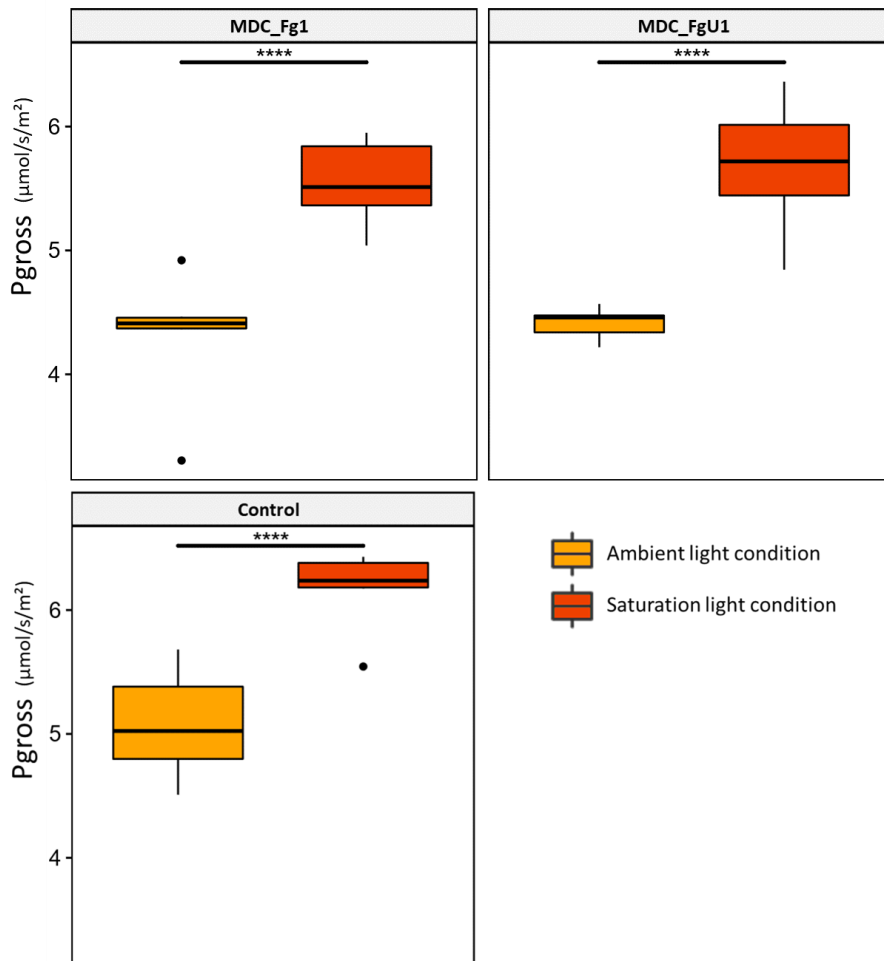


Figure 6. Absolute gross CO₂ assimilation rate of 'Recital' spikes measured at 72hpi in response to treatment and light condition. Boxplots represent the gross CO₂ assimilation rate of the spikes (Pgross) measured at 72hpi after the inoculation of MDC_Fg1 (left panel), MDC_FgU1 (central panel) and water (right panel) for controls in ambient light (orange) and saturation light (reddish orange) conditions. Asterisks indicate a significant difference between the two light modalities. Significance threshold: p -value < 0.05.

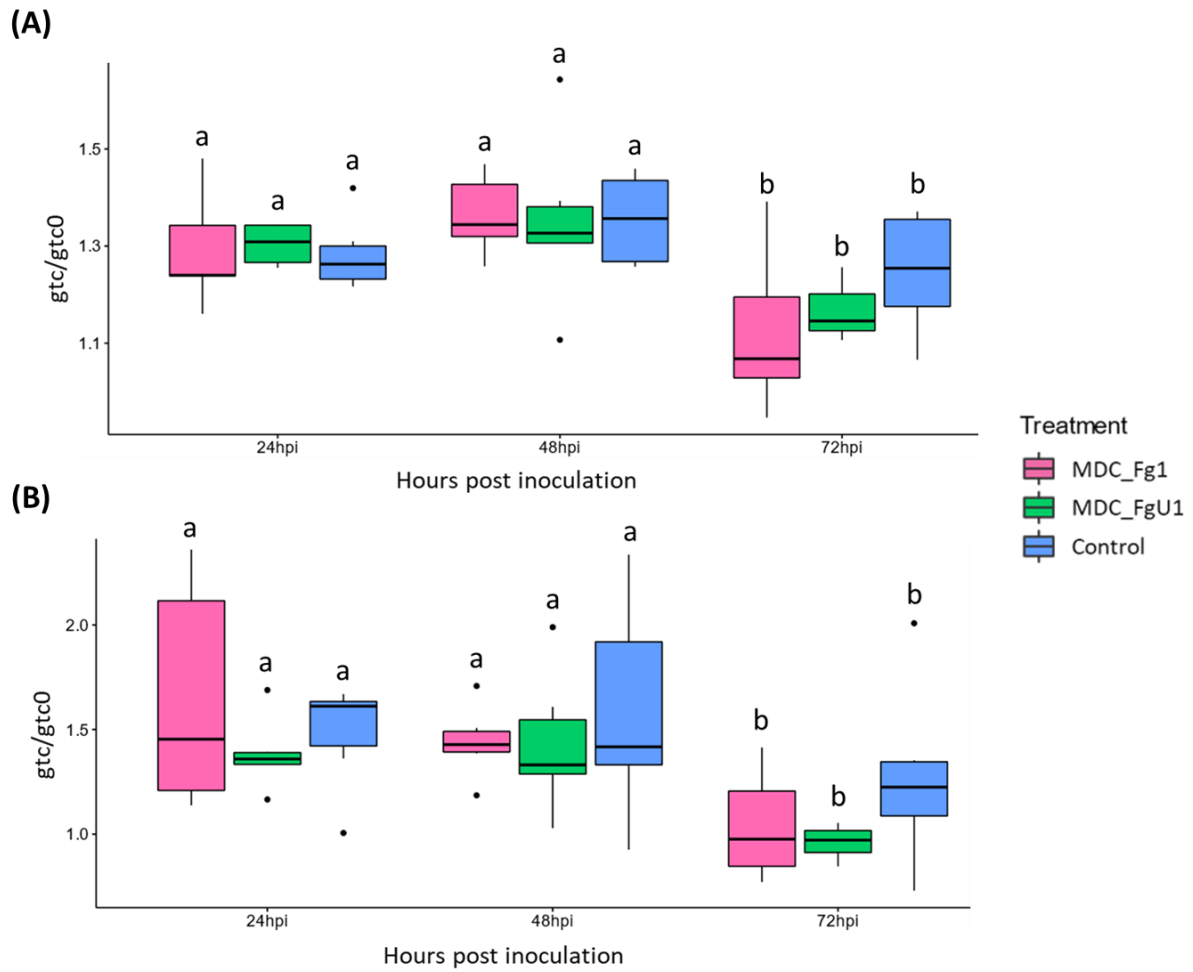


Figure 7. Relative total conductance to CO₂ of 'Recital' spikes measured in response to treatment along infection progress. Boxplots represent the total conductance to CO₂ of the spikes (gtc) measured at 24hpi, 48hpi and 72hpi after the inoculation of MDC_Fg1 (pink), MDC_FgU1 (green) and water (blue) for controls relatively to the pre-inoculation point (gtc0) in (A) ambient light and (B) saturation light conditions. Letters indicate a significant difference between time points. Significance threshold: p -value < 0.05.

Table 5. Statistical significance of the time point factor for a given treatment modality on Relative intercellular CO₂ concentration of ‘Recital’ spikes measured in (A) ambient light and (B) saturation light conditions. Asterisks indicate a significant difference between two time points for one given treatment modality while ns indicate a nonsignificant difference. Significance threshold: Bonferroni adjusted p-value<0.05.

(A)				(B)			
Treatment modality	Tested time contrast	p-value	Significance (p-value<0,05)	Treatment modality	Tested time contrast	p-value	Significance (p-value<0,05)
MDC_Fg1	24hpi-48hpi	0.125	ns	MDC_Fg1	24hpi-48hpi	1	ns
MDC_Fg1	24hpi-72hpi	0.003	**	MDC_Fg1	24hpi-72hpi	0.164	ns
MDC_Fg1	48hpi-72hpi	0.000756	***	MDC_Fg1	48hpi-72hpi	0.043	*
MDC_FgU1	24hpi-48hpi	0.106	ns	MDC_FgU1	24hpi-48hpi	0.885	ns
MDC_FgU1	24hpi-72hpi	0.000185	***	MDC_FgU1	24hpi-72hpi	0.119	ns
MDC_FgU1	48hpi-72hpi	0.000585	***	MDC_FgU1	48hpi-72hpi	0.468	ns
Control	24hpi-48hpi	1	ns	Control	24hpi-48hpi	1	ns
Control	24hpi-72hpi	0.268	ns	Control	24hpi-72hpi	0.139	ns
Control	48hpi-72hpi	0.792	ns	Control	48hpi-72hpi	0.035	*

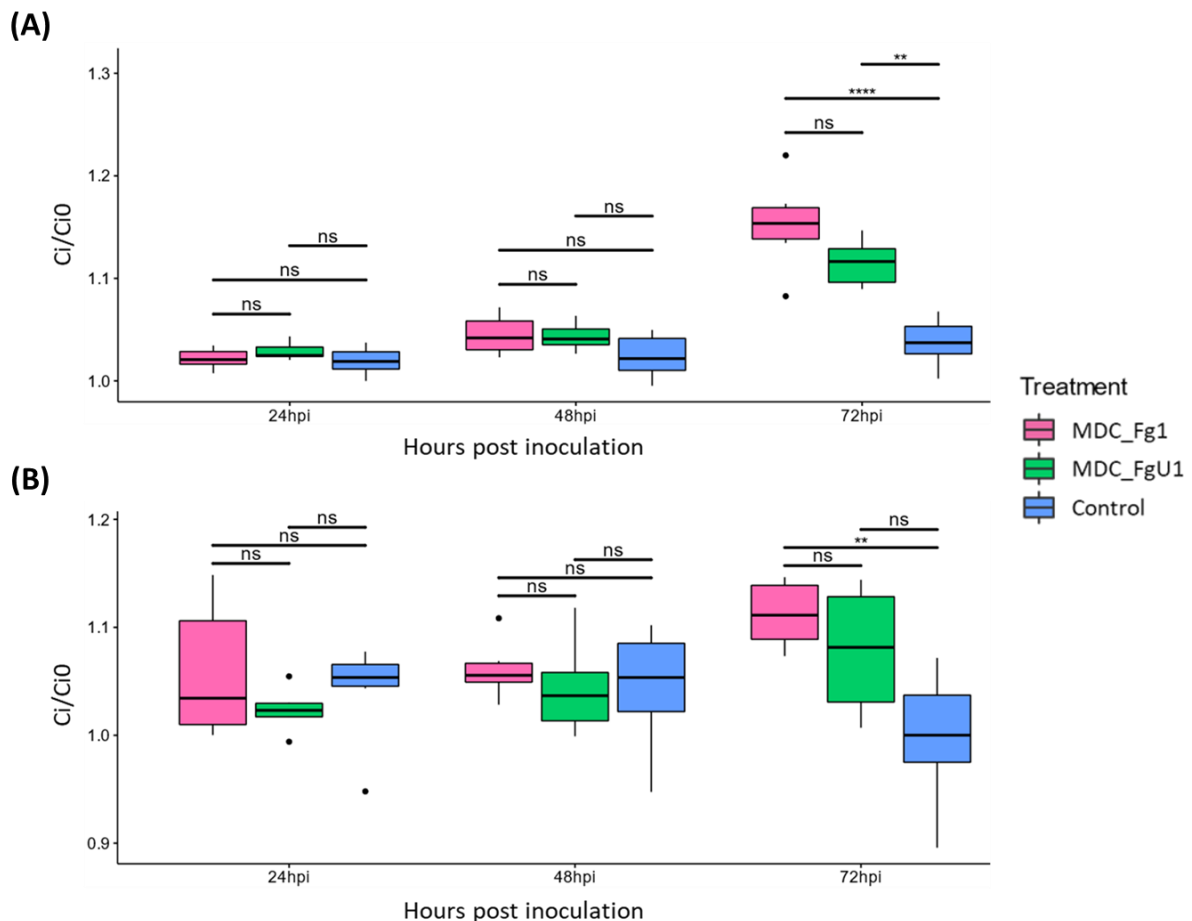


Figure 8. Relative intercellular CO₂ concentration of ‘Recital’ spikes measured in response to treatment along infection progress. Boxplots represent the intercellular CO₂ concentration of the spikes (Ci) measured at 24hpi, 48hpi and 72hpi after the inoculation of MDC_Fg1 (pink), MDC_FgU1 (green) and water (blue) for controls relatively to the pre-inoculation point (Ci0) in (A) ambient light and (B) saturation light conditions. Asterisks indicate a significant difference between two treatment modalities at one given time point while ns indicate a nonsignificant difference. Significance threshold: p-value<0.05.

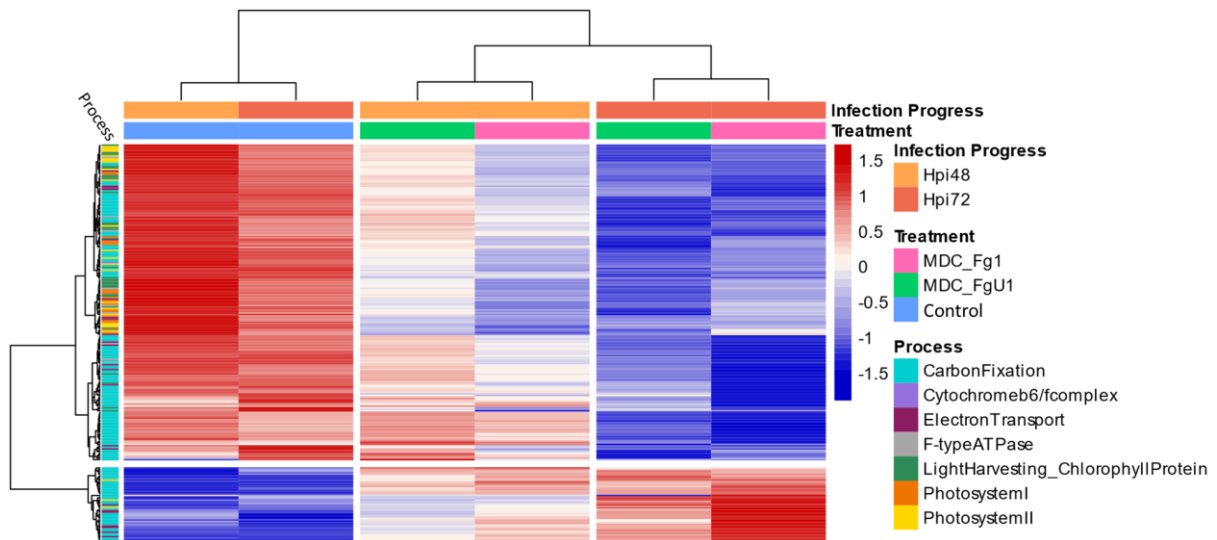


Figure 9. Expression regulation patterns of the genes involved in the photosynthesis associated processes in 'Recital' facing two strains of contrasting aggressiveness. The structure of gene and sample data sets were determined by HAC based on Ward's minimum variance method using the z-score transformed gene expression values. Heatmap color scales represent the z-score transformed expression values of the genes for each sample. The clustering on top of the heatmap represents the experimental conditions, labeled according to the factors Infection Progress and Treatment. On the right side of the heatmap, the genes were colored according to the process in which they are involved.

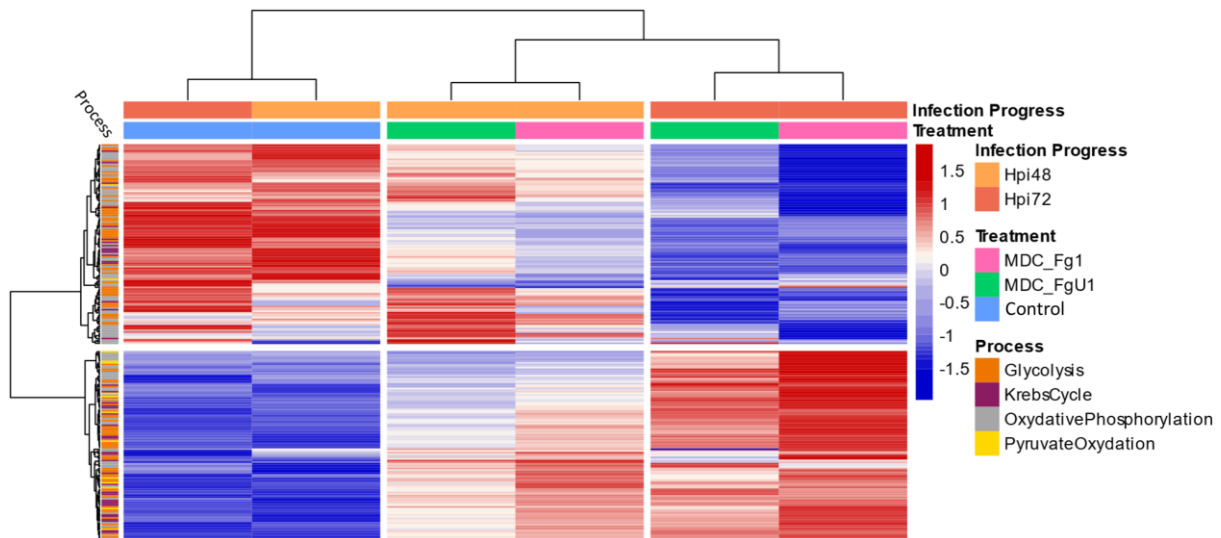


Figure 10. Expression regulation patterns of the genes involved in the respiration associated processes in 'Recital' facing two strains of contrasting aggressiveness. The structure of gene and sample data sets were determined by HAC based on Ward's minimum variance method using the z-score transformed gene expression values. Heatmap color scales represent the z-score transformed expression values of the genes for each sample. The clustering on top of the heatmap represents the experimental conditions, labeled according to the factors Infection Progress and Treatment. On the right side of the heatmap, the genes were colored according to the process in which they are involved.

Table 6. Resume of the photosynthesis- (A) and respiration-associated (B) processes affected by FHB at the molecular level. Concerning the photosynthesis-associated processes (A), the cluster C1 represents the genes repressed in response to FHB compared to control plants and the cluster C2 represents the genes induced in response to FHB compared to control plants. Concerning the respiration-associated processes (B), the cluster C1 represents the genes repressed in response to FHB compared to control plants and the cluster C2 represents the genes induced in response to FHB compared to control plants.

(A)

Biological process	Cluster	Gene number
Carbon fixation	C1	110
	C2	39
Cytochrome b6/f complex	C1	1
	C2	0
Electron transport	C1	16
	C2	7
F-type ATPase	C1	9
	C2	0
Light harvesting, Chlorophyll protein	C1	30
	C2	0
Photosystem I	C1	19
	C2	0
Photosystem II	C1	22
	C2	3

(B)

Biological process	Cluster	Gene number
Glycolysis	C1	50
	C2	48
Krebs cycle	C1	17
	C2	26
Pyruvate oxidation	C1	56
	C2	33
Oxidative phosphorylation	C1	2
	C2	12

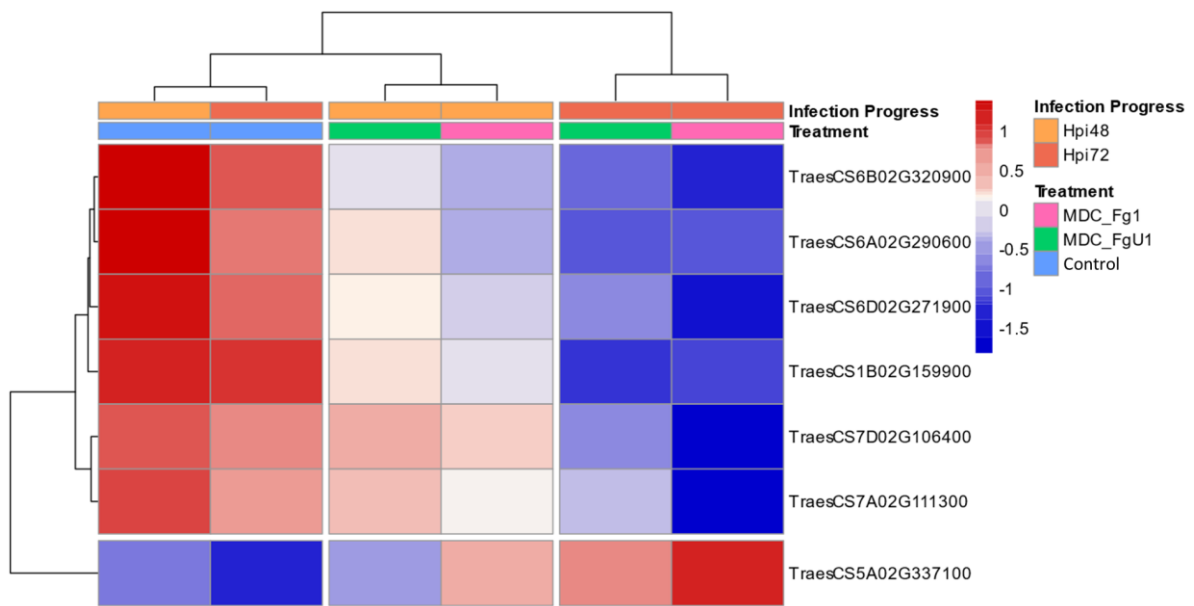


Figure 11. Expression regulation patterns of the genes involved in the stomatal movement in 'Recital' facing two strains of contrasting aggressiveness. The structure of gene and sample data sets were determined by HAC based on Ward's minimum variance method using the z-score transformed gene expression values. Heatmap color scales represent the z-score transformed expression values of the genes for each sample. The clustering on top of the heatmap represents the experimental conditions, labeled according to the factors Infection Progress and Treatment.

Table 7. Resume of the stomatal movement-associated genes affected by FHB at the molecular level.

Gene ID	Function	FHB response
TraesCS1B02G159900	Key zinc finger transcription factor gene	Under-expressed
TraesCS5A02G337100	Phospholipase D	Over-expressed
TraesCS6A02G290600	Chloroplast calcium sensing receptor	Under-expressed
TraesCS6B02G320900	Chloroplast calcium sensing receptor	Under-expressed
TraesCS6D02G271900	Chloroplast calcium sensing receptor	Under-expressed
TraesCS7A02G111300	Mitogen-activated protein kinase	Under-expressed
TraesCS7D02G106400	Mitogen-activated protein kinase	Under-expressed

Conclusion

Les résultats obtenus au cours de cette étude a mis en évidence une altération de l'assimilation nette de CO₂ en réponse au FHB entre 48hpi et 72hpi, résultant principalement d'une forte augmentation de la respiration mais aussi d'une altération de la photosynthèse brute entre 48hpi et 72hpi tandis qu'aucun impact sur la diffusion du CO₂ n'a été observé. De plus, aucun lien n'a pu être établi entre cette altération de l'assimilation du CO₂ et les différences d'intensité des symptômes induits par les deux souches de *F. graminearum*. Ensuite, malgré l'altération des processus photosynthétiques, les tissus de l'épi étaient toujours capables de réagir aux variations de l'intensité lumineuse, suggérant ainsi qu'à 72hpi, les chloroplastes restent en partie fonctionnels.

Enfin, bien que l'approche d'écophysiologie par échange de gaz gagnerait à être complétée par d'autres méthodes, telles que l'étude de la fluorescence de la chlorophylle et la mesure de la conductance du mésophylle, elle s'est révélée particulièrement robuste et complémentaire des données moléculaires. Les résultats obtenus au niveau de l'assimilation du CO₂ confirment la sous-régulation des gènes impliqués dans les processus photochimiques et biochimiques de la photosynthèse observée chez 'Récital' au cours de l'infection. De la même manière, l'absence d'effet sur la diffusivité du CO₂ est cohérente avec la sous-expression des gènes impliqués dans la fermeture stomatique. Enfin, la mesure des échanges de gaz a révélé tout son intérêt lors de la caractérisation de la respiration, processus pour lequel les seules données omiques se sont révélées insuffisantes pour conclure d'un effet du FHB.

Pour conclure, combiner différentes approches, telles que la transcriptomique et l'écophysiologie, est un levier puissant pour améliorer notre compréhension de l'impact d'une maladie aussi complexe que la FHB sur le fonctionnement des tissus infectés.

Discussion générale

Ce travail de Thèse, axé sur une approche systémique de l'interaction entre le blé tendre et *F. graminearum*, avait pour objectif d'identifier et de caractériser les déterminants du dialogue moléculaire établi au cours de l'infection. D'une part, ce travail a consisté à décrire la diversité du répertoire d'effecteurs sécrétés par l'agent pathogène au cours des stades précoces de l'interaction puis à identifier et définir la nature et le rôle des facteurs de sensibilité ciblés dans les processus biologiques de l'hôte. L'ensemble des résultats acquis au cours de ce travail de Thèse permet aujourd'hui de redéfinir plus précisément les acteurs clés gouvernant la sensibilité du blé à *F. graminearum*. Ils permettront dans les trois parties suivantes de discuter (i) de la conservation des effecteurs et des facteurs de sensibilité dans ces systèmes biologiques complexes, (ii) de la place des hubs de régulation dans les mécanismes de sensibilité et enfin (iii) de mettre en avant l'intérêt des approches de d'écophysiologie améliorer la compréhension des conséquences d'une pathologie sur le fonctionnement d'un organe complexe.

I. La sensibilité implique des effecteurs et des cibles conservés permettant la suppression de l'immunité

Quel que soit le niveau de sensibilité des cultivars de blé ou d'agressivité des souches de l'agent pathogène, l'interaction blé - *F. graminearum* produit systématiquement la maladie, suggérant que la sensibilité du blé au FHB est le produit de l'interaction entre des composantes conservées des deux protagonistes. Dans ce travail, l'analyse conjointe de l'expression, aux stades précoces de l'infection, des gènes codant des effecteurs putatifs chez trois souches de *F. graminearum* d'agressivité contrastée infectant un même cultivar sensible et chez une souche agressive infectant cinq cultivars de blé au cours des stades précoces de l'interaction a mis en évidence un programme d'infection fortement conservé, mettant en jeu un core-effectome composé de 357 gènes exprimés de manière séquentielle par l'ensemble des souches et dans tous les hôtes. Environ 30% de ces gènes avaient déjà été détectés *in planta* par des analyses de données protéomiques (Fabre et al., 2019b, 2019a).

Bien que le mode d'action des core-effecteurs identifiés au cours de nos travaux reste à caractériser, leur conservation suggèrent qu'ils jouent un rôle essentiel dans le succès de l'infection du blé par *F. graminearum* en ciblant des fonctions conservées chez les différents génotypes de blé, le core-targetome (Hemetsberger et al., 2015; Depotter and Doehlemann, 2020; Petre et al., 2020).

L'intégration des données d'expression des deux organismes, associée à l'exploration du catalogue de cibles putatives, grâce à une base donnée de facteurs de sensibilité (FS) connus et validés (van Schie and Takken, 2014), a permis l'identification, chez un cultivar très sensible, de 203 gènes de sensibilité candidats putativement ciblés par les core-effecteurs disposant d'un signal d'adressage au noyau. Parmi ce corpus de gènes, un core-targetome composé de 123 gènes, partagés par les cinq cultivars de blé utilisés au cours de ces travaux de Thèse, a été identifié. Ce core-targetome rassemble essentiellement des gènes induits au cours de l'infection (119 gènes) dont 87% jouent un rôle dans la suppression des mécanismes de défense. Plusieurs effecteurs de *F. graminearum* pourraient jouer un rôle majeur dans l'inhibition de la mort cellulaire (e.g., Bax inhibitor 1) et dans la régulation négative des mécanismes de défense (e.g., DMR6). Ces mécanismes apparaissent ainsi être des éléments clefs de la stratégie d'infection fongique (Hao et al., 2020; Jiang et al., 2020; Low et al., 2020) et confirment les travaux antérieurs de l'équipe (Chetouhi et al., 2016; Fabre et al., 2019b) qui ont également identifiés l'induction des protéines homologues de Bax inhibitor-1 et DMR6 chez le cultivar de blé sensible 'Récital' en réponse au FHB.

L'identification de nombreux gènes de sensibilité impliqués dans l'inhibition des mécanismes de défense et de la mort cellulaire démontre clairement l'importance de la phase biotrophe qui permet l'installation de *F. graminearum* dans les tissus et le succès de l'infection. Cette hypothèse est également soutenue par la mise en évidence que 55% des core-effecteurs disposant d'un adressage intracellulaire chez la plante (noyau, chloroplaste ou mitochondrie) présentaient leur pic d'expression à 96hpi, suggérant ainsi l'existence de processus de manipulation parasitaire à des stades avancés de l'infection (après l'apparition des symptômes). En accord avec cette observation, plus de la moitié des gènes de réponse du blé au FHB présentaient des niveaux d'expression élevés et supérieurs aux témoins même à des stades avancés de l'infection (96hpi). Ce dernier constat montre également clairement

que malgré le développement avancé des symptômes la machinerie transcriptionnelle reste active et que les tissus demeurent fonctionnels. Enfin, la caractérisation du statut physiologique de l'épi représente également un bon indicateur de son état de fonctionnement au cours de l'infection et montre clairement qu'à 72hpi les tissus restent capables d'ajuster les flux de carbone en réponse aux variations environnementales. Le corpus de données produit au cours de ce travail de Thèse complète parfaitement les travaux antérieurs de l'équipe qui suggéraient que l'ontogénie du grain était particulièrement préservée au cours de l'infection en dépit de symptômes de plus en plus marqués (Chetouhi et al., 2016). Le maintien de l'intégrité des tissus reproductifs est probablement un élément central du succès de l'infection qui pourrait en partie dépendre de la manipulation de la phase de remplissage du grain et exploiter les mécanismes qui préservent le grain d'une mort cellulaire qui condamnerait la multiplication de l'espèce.

Le maintien, au sein du règne végétal, des gènes de régulation négative des défenses qui facilitent l'installation de l'agent pathogène dans les tissus peut sembler de prime abord contre-intuitive (Watanabe and Lam, 2009; Falcone Ferreyra et al., 2015; Hasley et al., 2021; Thomazella et al., 2021; Koseoglou et al., 2022; Pirrello et al., 2022). Mais ces gènes, au-delà de leur rôle au sein des interactions avec les agents pathogènes, sont essentiels au fonctionnement de la plante et leur perte pourraient entraîner des effets délétères (Koseoglou et al., 2022). En effet, ces derniers permettent la régulation fine et coordonnée des processus antagonistes de croissance et de réponses de défenses des plantes garantissant ainsi l'adaptabilité de la plante à son environnement et donc l'optimisation de la croissance de la plante en l'absence de stress biotique (Tsuda and Somssich, 2015; Couto and Zipfel, 2016; Rodriguez et al., 2016; Ning et al., 2017). De plus, ces gènes représentent des cibles importantes des effecteurs des micro-organismes symbiotiques et sont nécessaires pour le succès de l'interaction (Kloppholz et al., 2011; Akum et al., 2015; Plett and Martin, 2015; Miwa and Okazaki, 2017). Ce rôle à l'intersection de plusieurs processus de la plante explique ainsi la fixation de ces gènes dans les génomes au cours de l'évolution.

II. La sensibilité du blé au FHB met en jeu de nombreux hubs de régulation

Parmi les gènes de sensibilité identifiés et conservés chez tous les cultivars de blé et en réponse aux trois souches de *F. graminearum*, 53 gènes, sur-exprimés au cours de l'infection, codent des TFs appartenant aux familles des WRKY (35 gènes), MYB (15 gènes) et NAC (3 gènes), connues pour être des acteurs majeurs de la régulation des réponses des plantes aux stress biotiques (Rushton et al., 2010; Ng et al., 2018; Baillo et al., 2019; Bian et al., 2021). La reconstruction du réseau de régulation contrôlant les réponses de 'Récital' aux trois souches de *F. graminearum* a permis de déterminer l'importance de ces TFs dans le devenir de l'interaction, avec 34 de ces gènes de sensibilité catégorisés « master regulators ». Ces derniers sont également répartis dans les deux groupes de TFs fortement corrélés qui présentent les nombres de connexions les plus élevés du réseau avec 2483 et 2227 gènes putativement régulés, respectivement. De plus, les gènes de sensibilité codant des TFs non catégorisés comme « master regulators » sont regroupés en un même groupe régulant un ensemble de 1432 gènes, lui-même putativement régulé par le groupe de « master regulators » le plus connecté du réseau. Ainsi, la réintégration des FS putatifs dans le réseau de régulation des réponses au FHB prédit *in silico* montre que les hubs de signalisation représentent des cibles privilégiées de *F. graminearum* chez le blé, leur manipulation permettant ainsi une reprogrammation coordonnée d'un grand nombre de gènes impliqués dans de nombreux processus biologiques.

De nombreuses études ont mis en évidence la sur-représentation des hubs de régulation des plantes parmi les cibles des effecteurs des agents pathogènes (Mukhtar et al., 2011; González-Fuente et al., 2020; Ceulemans et al., 2021). De plus, certains hubs représentent des points de convergence évolutive de la stratégie d'infection de nombreux agents pathogènes appartenant à des espèces, des genres et des règnes différents (Carella et al., 2018; González-Fuente et al., 2020; Ceulemans et al., 2021). A titre d'exemple, des expérimentations double hybride ont montré que le facteur de transcription TCP14 d'*A. thaliana*, impliqué dans la coordination des processus de développement, de synthèse d'acide salicylique et de mise en place de la résistance systémique acquise (Kieffer et al., 2011; Kim et al., 2014; Resentini et al., 2015; Yang et al., 2017), est la cible de 54 effecteurs sécrétés par la

bactérie *Pseudomonas syringae*, l'oomycète *Hyaloperonospora arabidopsidis* et le champignon *Golovinomyces orontii* (González-Fuente et al., 2020). Nos résultats illustrent ainsi la nécessité de réintégrer les facteurs de transcription dans leurs réseaux de régulation de la plante pour identifier les hubs mais aussi l'ensemble des processus impactés par la dérégulation de ces derniers.

Les réseaux de régulation des plantes imposant de fortes contraintes en terme de structure et de séquence (Carella et al., 2018), ces hubs de régulation représentent donc des cibles stables au cours du temps, peu sujettes aux mutations et qui par leurs rôle indispensables à la synchronisation des processus fondamentaux des plantes sont conservées chez un grand nombre d'espèces végétales. Ainsi, la capacité d'un agent pathogène à cibler les hubs de régulation des plantes lui conférerait un avantage évolutif et faciliterait l'expansion de sa gamme d'hôte (Carella et al., 2018).

III. L'écophysiologie, un levier pour améliorer la compréhension des réponses du blé au FHB

La caractérisation des réponses du cultivar sensible 'Récital' aux trois souches de *F. graminearum* montrent l'ampleur des processus cellulaires impactés par l'infection (54% des gènes exprimés), reflétant ainsi la complexité du phénotype de sensibilité qu'il est nécessaire de décomposer. Il est cependant difficile d'appréhender les conséquences phénotypiques du dialogue moléculaire entre le blé et *F. graminearum* sur le fonctionnement de l'épi sur la seule base des analyses transcriptomiques. Il est ainsi nécessaire d'utiliser d'autres approches complémentaires pour finement caractériser les processus impactés et valider les événements moléculaires observés par l'analyse des réponses transcriptomiques.

La mesure des échanges de gaz est une méthode non-destructive qui permet de suivre de manière résolutive l'évolution des processus du métabolisme primaire perturbés au cours de l'infection (Gortari et al., 2018; Haworth et al., 2018). Le phénotypage fin du statut physiologique du pathosystème à l'échelle de l'épi a permis d'étudier l'interaction sous un nouvel angle et s'est montré complémentaire et particulièrement pertinent pour améliorer l'interprétation des données générées à l'échelle du transcriptome.

La caractérisation de l'impact de souches d'agressivité contrastée sur les processus métaboliques de l'hôte a permis de confirmer la généricité des réponses du blé aux stades précoces de l'infection révélée par la conservation des effecteurs entre les souches et des réponses du cultivar 'Recital' à l'échelle moléculaire. Les mesures de l'assimilation du CO₂ et de la conductance stomatique associées aux données d'expression des gènes impliqués dans ces processus ont notamment permis de montrer une altération des processus photochimiques et biochimiques de l'appareil photosynthétique au cours de l'infection qui n'est pas directement liée au développement des symptômes. Enfin, l'approche écophysiological s'est révélée particulièrement robuste et efficace pour déterminer l'impact de *F. graminearum* sur la respiration de l'épi, processus pour lequel la caractérisation à l'échelle du transcriptome n'était pas suffisante et difficile du fait de la complexité du réseau d'acteurs mis en jeu. Ces travaux illustrent ainsi l'intérêt de combiner différentes approches et échelles phénotypiques pour caractériser un caractère aussi complexe que la sensibilité.

Toutefois, bien que prometteurs, ces premiers résultats nécessitent d'être complétés par l'utilisation de courbes de réponses de la photosynthèse à la concentration intercellulaire en CO₂, combinée à la mesure de la fluorescence de la chlorophylle qui permettrait notamment de déduire l'impact de l'infection sur l'état de fonctionnement des photosystèmes, la conductance du mésophylle au CO₂ et la capacité de carboxylation de la Rubisco (Moualeu-Ngangue et al., 2017; Burnett et al., 2019). Enfin, l'équilibre source-puit d'une plante est un processus dynamique, en particulier lors de la phase reproductive de la plante. Au-delà de l'impact local sur les paramètres photosynthétiques, le site de l'infection peut fortement modifier l'équilibre source-puit de la plante par la création d'un nouveau puit de nutriments associé aux besoins de l'agent pathogène qui entre en compétition avec les autres organes de la plante. Ainsi, l'infection peut entraîner d'importantes modifications du statut physiologique de l'hôte à l'échelle systémique pouvant se manifester par une augmentation de la photosynthèse au niveau des organes sains, une augmentation des flux de phloème à destination des tissus infectés (Horst et al., 2010; Bancal et al., 2012; Lapin and Van den Ackerveken, 2013). Il serait ainsi intéressant de caractériser l'effet de l'infection par *F. graminearum* sur le statut physiologique du blé à l'échelle systémique en mesurant les paramètres photosynthétiques au niveau de la feuille drapeau et en mesurant les

concentrations de certains minéraux dans l'épi (potassium, magnésium ou phosphore) qui sont des marqueurs des flux de phloème (Horst et al., 2010; Marschner, 2011).

Conclusion générale et perspectives

Notre compréhension actuelle de l'interaction entre le blé et *F. graminearum* résulte pour l'essentiel d'une approche dichotomique caractérisée par l'étude de manière indépendante des réponses de l'hôte d'une part et des déterminants fongiques de la pathogénicité d'autre part. A cette dichotomie s'ajoute le fait que les réponses de la plante ont été, jusqu'à aujourd'hui, principalement étudiées au travers des seuls mécanismes de défense et de résistance. Ces choix partiels ont conduit à ne considérer la sensibilité que comme un processus passif tout en excluant le plus souvent le rôle joué par l'agent pathogène au sein du dialogue moléculaire. Identifier les déterminants de la sensibilité d'une plante au développement d'une pathologie reste un enjeu majeur pour identifier de nouvelles résistances. Cet objectif exige de réconcilier toutes les composantes de ces systèmes biologiques complexes où s'expriment les stratégies d'infection de l'agent pathogène, i.e. l'effectome et l'ensemble des processus cibles de l'effectome chez la plante, i.e. le targetome (Lapin and Van den Ackerveken, 2013; Peyraud et al., 2017; Engelhardt et al., 2018; Arroyo-Velez et al., 2020; He et al., 2020; Garcia-Ruiz et al., 2021; Gorshkov and Tsers, 2022). En s'appuyant sur l'analyse de données transcriptomiques « large échelle », permettant l'analyse simultanée des gènes exprimés par la plante et le champignon (dual-transcriptome) aux différents stades de l'infection et en utilisant plusieurs fonds génétiques de l'hôte et de l'agent pathogène, ce travail a permis d'identifier des déterminants fongiques et hôtes clés gouvernant la sensibilité du blé tendre à *Fusarium graminearum*. Dans un premier temps, nos résultats ont démontré que la sensibilité du blé à *F. graminearum* repose sur une stratégie d'infection fortement conservée, complexe et finement régulée au cours des premiers stades de l'infection impliquant la manipulation d'un grand nombre de processus biologique chez le blé par un core-effectome. Nos travaux ont, ensuite, permis d'appréhender pour la première fois l'interaction blé - *F. graminearum* par une approche systémique intégrant les données obtenues chez les deux protagonistes. Cette approche s'est révélée particulièrement robuste et efficace pour identifier un corpus conservé de facteurs de sensibilité qui gouvernent le succès de l'infection et a, de plus, permis d'apporter de nouvelles preuves concernant

l'importance de la phase biotrophe dans le devenir de l'interaction. L'intégration des réponses du blé au FHB au sein de réseaux de régulation a clairement démontré que les facteurs de transcription jouant des rôles de hubs biologiques sont des cibles importantes et conservées de *F. graminearum*. Enfin, la combinaison de l'approche transcriptomique « large-échelle » avec la caractérisation fine du statut physiologique de l'épi a permis de grandement améliorer notre compréhension des impacts du FHB sur le fonctionnement de l'épi qui se manifestent notamment par un accroissement de la respiration et une altération des processus photochimiques et biochimiques de la photosynthèse.

L'ensemble des résultats obtenus ouvrent de nouvelles questions scientifiques détaillées au travers des perspectives suivantes.

I. Exploration de la diversité des interactions impliquant *F. graminearum* pour identifier les déterminants impliqués dans l'expansion de la gamme d'hôte

F. graminearum est un champignon généraliste capable de s'installer de manière endophyte dans de nombreuses espèces hôtes appartenant entre autres aux familles des graminées, des cucurbitacées, des légumineuses, des astéracées et des chénopodiacées (Goswami and Kistler, 2004; Mourellos et al., 2014; Suproniene et al., 2019) et de se développer dans de nombreux tissus d'une même plante hôte (Kazan et al., 2012). L'étude, chez plusieurs champignons symbiotiques ou pathogènes, des déterminants moléculaires permettant la colonisation de plusieurs espèces hôtes a notamment mis en évidence l'existence de core-effecteurs sécrétés quelle que soit l'espèce hôte (Hemetsberger et al., 2015; Plett et al., 2014; Lorrain et al., 2018; Redkar et al., 2022). Le succès de l'interaction entre *Fusarium oxysporum* et l'ensemble de ses plantes hôtes, éloignées sur le plan évolutif, est par exemple gouvernée par la sécrétion des effecteurs Early Root Compatibility effectors (ERCs) qui sont conservés chez l'ensemble des souches de l'espèce (Redkar et al., 2022). Les auteurs postulent également que ces ERCs cibleraient des processus évolutivement conservés au sein du règne végétal (Redkar et al., 2022). La conservation de tels facteurs de sensibilité chez les plantes peut notamment être illustré par le cas du gène MLO, apparu il y a environ

200 millions d'années, et qui malgré son rôle indispensable dans la pathogénicité de *B. graminis* avec l'ensemble de sa gamme d'hôte, a été maintenu au cours de l'évolution chez les angiospermes (Consonni et al., 2006; Kusch et al., 2016; Kusch and Panstruga, 2017; Jacott et al., 2021).

A ce jour, les déterminants fongiques et hôtes permettant la compatibilité de *F. graminearum* avec sa vaste gamme d'hôtes ne sont pas connus et nécessitent d'être explorés afin de déchiffrer les bases fondamentales de la pathogénicité de cet agent pathogène. Sur la base de nos travaux, les gènes de sensibilité codant des TFs et jouant des rôles de hubs biologiques, représenteraient des candidats pertinents dans l'établissement de cette compatibilité multi-hôtes. Les interactions entre effecteurs et FS putatifs du blé identifiés au cours de nos travaux doivent désormais faire l'objet de validations fonctionnelles. Ces dernières sont actuellement réalisées par Shimplal Ayilalath au cours des travaux de Thèse. L'identification de couples effecteurs-FS permettra dans un second temps de vérifier l'existence de ces interactions au sein des autres pathosystèmes impliquant *F. graminearum* et d'évaluer l'impact de la mutation de ces FS chez le blé et de leurs orthologues chez les autres plantes hôtes sur le devenir de l'interaction avec *F. graminearum*. Ces approches permettront d'apporter des preuves du rôle central joué par ces couples effecteurs-FS dans l'expansion de la gamme d'hôte de cet pathogène généraliste.

II. Rôle des petits ARNs au sein de l'interaction entre le blé et *F. graminearum*

Les travaux réalisés au cours de cette thèse se sont limités à identifier et caractériser les déterminants moléculaires de nature protéique gouvernant la sensibilité du blé à *F. graminearum*. Or, les petits ARNs jouent également un rôle de premier ordre tant du côté de l'agent pathogène que de l'hôte dans le devenir de l'interaction (Weiberg et al., 2014; Weiberg and Jin, 2015; Huang et al., 2019).

Tout d'abord, les petits RNAs endogènes sont des acteurs majeurs de la régulation de nombreux processus chez les plantes mais aussi chez les agents pathogènes. Chez les plantes, ces derniers garantissent notamment l'équilibre entre les processus de l'immunité et de la

croissance par la régulation fine de l'activation et de l'amplitude des réponses immunitaires (Qiao et al., 2021). C'est par exemple le cas de miR863-3p qui selon le contexte est capable d'inactiver à la fois des régulateurs négatifs et positifs des mécanismes de défense (Niu et al., 2016). Du côté de l'agent pathogène, la régulation des gènes codant les facteurs de virulence et en particulier les effecteurs sont sous le contrôle des petits ARNs, participant ainsi à la mise en place de manière séquentielle de la stratégie d'infection (M. Wang et al., 2015).

Outre leurs rôles endogènes, certains petits ARNs exercent leurs fonctions chez un autre organisme (« cross-kingdom small RNAs ») (Huang et al., 2019; Wang and Dean, 2020). Lors des interactions plantes-agents pathogènes, l'hôte est capable de transférer des petits ARNs dans l'agent pathogène lui permettant ainsi d'interférer avec la synthèse des facteurs de virulence de ce dernier (Cai et al., 2018). Réciproquement, les stratégies d'infection des agents pathogènes reposent également sur la sécrétion dans l'hôte de petits ARNs imitant ceux de l'hôte et permettant ainsi d'interférer avec la machinerie de silencing de l'hôte et d'inhiber la mise en place des réponses de défenses (Weiberg et al., 2013; M. Wang et al., 2015). A notre connaissance, seules deux études se sont intéressées à l'identification des petits ARNs effecteurs de *F. graminearum* (Jian and Liang, 2019; Werner et al., 2021). Jian et Liang (Jian and Liang, 2019) ont validés, en 2019, le rôle d'inhibiteur des mécanismes de défense du petit ARN Fg-sRNA1 qui cible et inactive le gène de défense TaCEBiP du blé. Enfin, la mise en place d'une approche de séquençage des petits ARNs par Werner et collaborateurs en 2021 (Werner et al., 2021) a permis l'identification de petits ARNs permettant la suppression des mécanismes de défense de deux espèces de graminées, l'orge et *Brachypodium distachyon*. Réciproquement, du côté du blé, le petit ARN microRNA1023 cible et inactive le gène FGSG_03101 de *F. graminearum* codant une alpha/beta hydrolase, inhibant le développement du champignon (Jiao and Peng, 2018).

Ainsi, les petits ARNs sont des déterminants importants du dialogue moléculaire entre le blé et *F. graminearum* et nécessite d'être explorés en détail pour améliorer notre compréhension de l'interaction (Kharbikar et al., 2019). L'utilisation de méthodes exploratoires *in planta* des petits ARNs exprimés au cours de l'infection tant du côté de l'hôte que du champignon associées à la recherche des cibles putatives dans les génomes représente un levier puissant pour réaliser un inventaire exhaustif de ces déterminants (Naidoo et al., 2018). Tout comme l'approche développée au cours de nos travaux, il sera nécessaire de

prendre en compte la variabilité génétique des deux protagonistes pour identifier les petits ARNs conservés qui sont nécessaires au succès de l'infection.

III. Valeur adaptative des déterminants gouvernant la sensibilité du blé à *F. graminearum* dans un contexte de changement climatique

L'utilisation des facteurs de sensibilité comme sources de résistance alternative nécessite de comprendre finement leur rôle au sein des différents processus de la plante et leurs effets sur la fitness de la plante dans différentes conditions environnementales (Engelhardt et al., 2018; Garcia-Ruiz et al., 2021; Koseoglou et al., 2022), en particulier lors de stress abiotiques qui dans un contexte de changement climatique s'additionnent aux stress biotiques (Velásquez et al., 2018; Zarattini et al., 2021). Cela concerne notamment les TFs qui sont impliqués dans la régulation des réponses des plantes à de nombreux stress qu'ils soient biotiques ou abiotiques, avec des effets synergiques ou antagonistes sur la tolérance des plantes à ces différents stress (Nuruzzaman et al., 2013; Y. Bai et al., 2018; Erpen et al., 2018; Wang et al., 2021). Au cours de nos travaux, nous avons par exemple identifié 3 gènes homéologues, ayant une similarité supérieure à 90% avec le TF HvWRKY1 de l'orge. Outre, son rôle dans la régulation négative des mécanismes de défense et la sensibilité de l'orge à *B. graminis* (Eckey et al., 2004; D. Liu et al., 2014), HvWRKY1 jouerait également un rôle important dans la tolérance de l'orge à de nombreux stress abiotiques tels que la sécheresse, la salinité et l'excès de cadmium (J. Zheng et al., 2021). Il est ainsi nécessaire d'évaluer l'effet de ces gènes de sensibilité du blé au FHB dans différentes conditions environnementales afin d'identifier des candidats n'impactant pas négativement la tolérance du blé à d'autres stress voire permettant une tolérance accrue à de multiples stress.

Dans un premier temps, l'implication de ces gènes dans la sensibilité du blé au FHB devra être vérifiée, puis l'effet de mutations de ces gènes sur la tolérance de la plante à différents stress abiotiques ainsi qu'à la combinaison de stress biotiques et abiotiques devra être évalué. Dans un second temps, la robustesse des facteurs de sensibilité identifiés, c'est-à-dire la capacité de maintien des fonctions exercées par ces derniers au sein de l'interaction blé - *F. graminearum* face à des perturbations internes ou externes (Félix and Barkoulas, 2015),

devra être évaluée dans des environnements fluctuants. Cet aspect fait l'objet du projet de Thèse de Larissa Adamik dont les travaux s'intéressent à la plasticité et la variabilité des réponses du blé à *F. graminearum* en conditions d'irrigation fluctuante.

IV. Assembler les pièces du puzzle : intégrer l'ensemble des informations générées à différentes échelles omiques

Au cours de nos travaux de Thèse, nous avons clairement démontré l'intérêt de l'intégration des données de *F. graminearum* et du blé pour identifier des FS. Cependant, nous ne nous sommes intéressés qu'à une des multiples facettes en jeu, les transcriptomes de la plante et de l'agent pathogène avec en ligne de mire les effecteurs et les facteurs de sensibilité. L'ensemble des travaux réalisés au sein de l'équipe (Fabre et al., 2019b, 2019a, 2021; Rocher et al., 2022), à l'échelle du protéome et du transcriptome, ont permis d'identifier et de finement caractériser des composantes clefs de l'interaction à la fois chez le blé et *F. graminearum*. Cependant, la sensibilité est un caractère très complexe gouverné par un super-réseau de régulation qui implique les sous-réseaux de la plante et de l'agent pathogène (Nobori et al., 2018). Ces deux sous-réseaux résultent eux même de l'interconnexion des différentes échelles omiques décrivant le phénotype interne des deux organismes (Peyraud et al., 2017; Großkinsky et al., 2018; Scossa et al., 2021). Il est maintenant nécessaire de connecter l'ensemble des connaissances acquises, c'est-à-dire assembler les pièces du puzzle, pour reconstruire l'histoire biologique du dialogue moléculaire de l'interaction entre le blé et *F. graminearum* et en comprendre la structure et la dynamique. A terme, l'intégration de l'ensemble des données générées dans le cadre du pathosystème permettra de finement décrire les mécanismes moléculaires et les voies biologiques gouvernant la sensibilité du blé au FHB. Ce travail d'intégration de l'ensemble des données générées dans le cadre de l'interaction blé – *F. graminearum* au sein de l'équipe est actuellement mis en œuvre par Samir Dou, post doctorant.

Références

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