Comparative transcriptome analysis of rutabaga (*Brassica napus*) cultivars in response to *Plasmodiophora brassicae*

by

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Abstract

Clubroot, a soil-borne disease caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is a threat to canola (*Brassica napus* L.) production in western Canada. Genetic resistance represents the most effective tool to manage this disease. To improve understanding of the mechanisms of resistance and pathogenesis in the clubroot pathosystem, the rutabaga (*B. napus* subsp. *rapifera* Metzg) cultivars 'Wilhelmsburger' (resistant) and 'Laurentian' (susceptible) were inoculated with *P. brassicae* pathotype 3A and their transcriptomes were analyzed at 7, 14 and 21 days after inoculation (dai) by RNA sequencing (RNA-seq). Thousands of transcripts with significant changes in expression were identified in each host at each time-point in inoculated vs. non-inoculated plants. Molecular responses at 7 and 14 dai supported clear differences in the clubroot resistance of the two genotypes. Both the resistant and the susceptible cultivars activated receptor-like protein (*RLP*) genes, resistance (*R*) genes and salicylic acid (SA) signaling as clubroot defense mechanisms. In addition, genes related to calcium signaling, and genes encoding leucine-rich repeat (LRR) receptor kinases, the respiratory burst oxidase homolog protein, and transcription factors such as WRKYs, ethylene responsive factors and bZIPs, appeared to be upregulated in 'Wilhelmsburger' to restrict *P. brassicae* development. Some of these genes are essential components of molecular defenses, including ethylene (ET) signaling and the oxidative burst. Our study highlights the importance of the activation of both SA- and ET-mediated responses in the resistant cultivar. A set of candidate genes showing contrasting patterns of expression between the resistant and susceptible cultivars was identified, representing potential targets for further study and validation through approaches such as gene editing.

Preface

This thesis is an original work by Ms. Qinqin Zhou, who conducted and analyzed all of the experiments, prepared the first draft and incorporated edits and suggestions for all chapters. Ms. Zhou designed the experiments in consultation with Dr. Leonardo Galindo-González, Research Associate in the Plant Pathology Lab, University of Alberta. Dr. Galindo-González also trained and assisted Ms. Zhou in conducting the data analysis, and edited several versions of Chapters 2 and 3. Ms. Zhou's supervisor, Dr. Stephen Strelkov, contributed to the development of the research concept, provided guidance on the project and edited several versions of all chapters. Ms. Zhou's co-supervisor, Dr. Sheau-Fang Hwang helped secure project funding and contributed to the original experimental context. Finally, Dr. Victor Manolii, Senior Technician in the Plant Pathology Lab, assisted with the experiments and provided expertise with respect to inoculations and handling of the pathogen and greenhouse material.

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List of Abbreviations

Abbreviation	Definition
ACS2	1-amino-cyclopropane-1-carboxylate synthase 2
AFLP	amplified fragment length polymorphism
AOC2	allene oxide cyclase 2
AOS	allene oxide synthase
ATL	Arabidopsis Tóxicos en Levadura
BAT5	bile acid transporter
BCn	offspring plants after several cycles of backcrossing
BSR-seq	bulked segregant RNA-seq
BTL10	BCA2Â zinc finger ATL 10
Bur-0	Arabidopsis ecotype Burren
bZIP	basic leucine zipper
CAC	clathrin adaptor complex
CaMBP	calmodulin-binding protein
CBP	calcium binding protein
CBP60g	CaMBP 60-like G
CCD	Canadian Clubroot Differential
cDNA	complementary DNA
Col-0	Arabidopsis ecotype Columbia
CR	clubroot resistant
CRISPR	clustered regularly interspaced palindromic repeat
CRISPR/Cas9	CRISPR-associated protein 9
CS	clubroot susceptible
Ct	cycle threshold
CYP79F1	cytochrome p450 79f1
CYP83A1	cytochrome P450, family 83, subfamily A, polypeptide 1
CYP94B1	cytochrome P450, family 94, subfamily B, polypeptide 1
CYP94C1	cytochrome P450, family 94, subfamily C, polypeptide 1
dai	days after inoculation
DAMP	damage-associated molecular pattern
DEG	differentially expressed gene
dH2O	distilled water
DI	disease index
DIR6	dirigent protein 6
DSB	double strand DNA break
E2	E2 ubiquitin-conjugating enzyme
E3	E3 ubiquitin ligase
ECD	European Clubroot Differential

EREBP	APETALA2/ethylene-responsive element binding protein
ERF	ethylene response factor
ET	ethylene
ETI	effector-triggered immunity
FAD7	fatty acid desaturase 7
GBS	genotyping by sequencing
GDI1	dissociation inhibitor 1
gDNA	genomic DNA
GWAS	genome-wide association studies
hai	hours after inoculation
HDR	homology-directed repair
ICS1	isochorismate synthase 1
ICS2	isochorismate synthase 2
JA	jasmonic acid
JA-Ile	jasmonoyl-L-isoleucine
JAZ	jasmonate-zim-domain proteins
log2FC	log2 fold-change
MAPK	mitogen-activated protein kinase
MAS	marker-assisted selection
MEKK	MAPK kinase kinase
MKK	MAPK kinase
MPK6	mitogen-activated protein kinase 6
MRC	major recognition complex
MYB15	MYB domain protein 15
NGS	next-generation sequencing
NHEJ	non-homologous end joining
NLP	necrosis and ethylene-inducing peptide 1-like protein
NPR1	nonexpresser of PR genes 1
NPR3	NPR1-like protein 3
OG	old brownish spindle galls
OPR1	12-oxophytodienoate reductase 1
PAM	protospacer adjacent motif
PAMP	pathogen-associated molecular pattern
PCA	principal component analysis
PEPR2	pep 1 receptor 2
PLSP2A	plastidic type I signal peptidase 2A
PME44	pectin methylesterase 44
PR	pathogenesis-related
PR1	pathogenesis-related gene 1
PRPS4	resistant to *Pseudomonas syringae* 4
PRR	pattern recognition receptor

PTI	PAMP-triggered immunity
qRT-PCR	quantitative Real-Time PCR
QTLs	quantitative trait loci
R gene	resistance gene
RAPD	PCR-based random amplified polymorphic DNA
RBOH	respiratory burst oxidase homolog
RFLP	restriction fragment length polymorphism
RIN	RNA integrity number
RLP	receptor-like protein
RNA-seq	RNA sequencing
ROS	reactive oxygen species
RPKM	reads per kb of transcript per million mapped reads
SA	salicylic acid
SAR	systemic acquired resistance
SARD1	SAR deficient 1
SCFE1	sclerotinia culture filtrate elicitor 1
sgRNA	single guide RNA
SL	symptomless roots
SNP	single nucleotide polymorphism
SOBIR1	protein suppressor of BIR1–1
SRA	Sequence Read Archive
SSR	simple sequence repeat
TF	transcription factor
TGA	TGACG motif-binding protein
TIR-NBS-LRR	nucleotide-binding site-leucine-rich repeat
TUA5	tubulin alpha-5
UBC11	ubiquitin conjugating enzyme
UBC9	ubiquitin conjugating enzyme 9
VHA-E1	vacuolar ATP synthase subunit E1
WAVH1	WAV3 homolog 1
YG	young white spindle galls with waxy appearance

Chapter 1 Introduction

1.1 General introduction to clubroot

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is an important soil-borne disease of crucifers characterized by the formation of club-shaped galls on the tap and lateral roots of infected hosts. The galls interfere with acquisition of nutrients and water from the soil, resulting in aboveground symptoms such as yellowing and wilting of the leaves, stunting, and premature ripening. Global crop losses caused by clubroot are estimated to be at least 10 to 15% (Dixon, 2009b). In Canada, clubroot is a significant threat to the production of canola (oilseed rape; *Brassica napus* L.), a valuable crop that brings $26.7 billion CAD annually to the national economy (LMC International, 2016).

Clubroot was likely introduced to Canada on infected fodder turnips by European settlers, and had become a major problem on cruciferous vegetables in Ontario, Quebec, British Columbia and the Atlantic Provinces by the early 20th century (Howard et al., 2010). The first report of clubroot on Canadian canola was in 1997 in Quebec (Strelkov et al., 2006). On the Prairies (Alberta, Saskatchewan and Manitoba), where most Canadian canola is grown (Canola Council of Canada, 2003), clubroot was first reported in 2003 in 12 fields in central Alberta (Tewari et al., 2005). Since then, the number of clubroot-infested fields in Alberta has increased every year, with more than 3,300 documented field infestations by 2019 (Strelkov et al., 2020b). The incidence of the disease is also increasing in Saskatchewan and Manitoba.

1.2 Management of clubroot

Various strategies can be helpful for the management of clubroot on canola, including the sanitization of field machinery to slow *P. brassicae* spread and long rotations out of susceptible hosts to reduce disease pressure. Unfortunately, these strategies have not been widely adopted by western Canadian farmers, for whom canola is one of the most profitable crops available (Strelkov et al., 2011; Hwang et al., 2014). Hence, farmers rely mostly on the planting of clubroot resistant (CR) canola cultivars for control of the disease (Peng et al., 2014a). The first CR canola was introduced in 2009, and by 2019 around 30 CR varieties were available from a variety of seed companies (Canola Council of Canada, 2019). While the basis of this resistance is not in the public domain, it appears that in most varieties resistance is under the control of a single gene (Rahman et al., 2014), likely derived from the European oilseed rape 'Mendel' (Fredua-Agyeman et al., 2018). The cropping of CR canola cultivars in short rotations over large regions has imposed significant selection pressure on *P. brassicae* populations, resulting in the emergence of new pathotypes able to overcome resistance (Strelkov et al., 2016, 2018). While efforts are underway by various public and private breeders to identify and introgress novel sources of resistance, it is important to apply novel approaches to accelerate this work.

1.3 Application of omics approaches to clubroot resistance studies

Advanced genomic and transcriptomic approaches, including genotyping by sequencing, genome-wide association studies, high-density single nucleotide polymorphism arrays, RNA sequencing (RNA-seq), and bulked segregant RNA-seq analysis, have been powerful tools for the study and understanding of clubroot resistance

(Li et al., 2016; Yu et al., 2017; Pang et al., 2018; Peng et al., 2018a; Fu et al., 2019b; Galindo-González et al., 2020). While genomic approaches have focused on the identification of quantitative resistance loci, the analysis of differentially expressed genes by RNA-seq can be important for understanding the mechanisms of resistance (and susceptibility) on a transcriptional level. An improved knowledge of host-pathogen interactions is critical for the long-term management of clubroot by helping to identify additional sources of resistance, breeding of varieties with more durable resistance, and rotating cultivars with differential resistance in *P. brassicae*-infested fields.

1.4 Hypothesis and objectives

The primary hypothesis underlying this thesis is that molecular variation in clubroot resistant and susceptible *B. napus* genotypes results in the differential expression of key genes in response to *P. brassicae*. These genes may be involved in various pathways, such as pathogen recognition, hormone-related pathways, and the metabolism of cell wall compounds, which mediate the interaction between host and pathogen. The differential regulation of these pathways in turn results in the observed compatible and incompatible interactions.

My work included three specific objectives: (1) to understand the effects of *P. brassicae* on the transcriptome of *B. napus* cultivars with differential resistance to the pathogen; (2) to describe the molecular response of the host in each specific interaction; and (3) to identify candidate genes that could be targeted for manipulation, such as via gene editing, to aid in future clubroot resistance breeding activities.

Chapter 2: Application of genomics and transcriptomics to accelerate development of clubroot resistant canola

2.1 Introduction

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is an important disease of the Brassicaceae. Both the yield and quality of *Brassica* crops are reduced by *P. brassicae* infection, with estimated losses ranging from 10 % to 15 % worldwide (Dixon, 2009b). In Canada, clubroot poses a significant threat to the production of canola (oilseed rape; *Brassica napus* L.) (Strelkov and Hwang, 2014), a crop that contributes 26.7 billion CAD annually to the national economy (LMC International, 2016). The clubroot pathogen produces long-lived resting spores (Hwang et al., 2013), making management of the disease difficult. Strategies focused on long rotations out of canola to prevent increases in soil-borne inoculum, and the sanitization of field machinery to slow its spread, have had limited acceptance by farmers (Strelkov et al., 2011). Other management approaches, such as the application of lime or soil amendments, give inconsistent results, are prohibitively expensive, or have not been thoroughly validated for canola cropping systems (Hwang et al., 2014).

The deployment of clubroot resistant (CR) canola cultivars represents the most important strategy for the management of this disease. A CR winter oilseed rape, 'Mendel', was released commercially in Europe in the 2000s (Diederichsen et al., 2009). In Canada, the first CR canola cultivar, '45H29', was released in 2009, followed quickly by many CR cultivars from multiple seed companies. By 2019, there were nearly 30 CR canola cultivars available on the Canadian market (Canola Council of Canada, 2019). The resistance in many of these cultivars appears to be derived from the European Clubroot

Differential (ECD) 04 (*B. rapa* line AABBCC; Buczacki et al., 1975) or 'Mendel' (Rahman et al., 2014; Fredua-Agyeman et al., 2018). Unfortunately, resistance can erode quickly as a result of selection pressure on pathogen populations, when CR cultivars are planted in short rotations over large regions. New pathotypes of *P. brassicae* capable of overcoming the resistance in most CR canola cultivars were identified in Canada within four years of the introduction of the resistance trait (Strelkov et al., 2016) and continue to become more common (Strelkov et al., 2018). Similarly, pathotypes of *P. brassicae* capable of overcoming the resistance in 'Mendel' have been reported from Germany (Zamani-Noor, 2017).

A good understanding of host-pathogen interactions is important for the identification and appropriate deployment of resistance genes in plant breeding (Boyd et al., 2013). However, the dissection of the molecular basis of host reactions to *P. brassicae* lags behind other systems. In the past several decades, efforts have been made to elucidate the lifecycle of *P. brassicae* and the importance of plant hormones in gall development. The lifecycle of *P. brassicae* consists of three phases, survival in soil, root hair infection, and cortical infection (Kageyama and Asano, 2009). Roots infected by *P. brassicae* exhibit hypertrophy and hyperplasia, resulting in the formation of large galls that interfere with the uptake of water and nutrients from the soil. These galls develop during the secondary phase of infection, and are associated with disturbances in plant hormone homeostasis. Auxin, cytokinin and brassinosteroids, have been suggested as having key roles in gall development (Ludwig-Müller, 2014). While the molecular basis for hormone regulation remained unknown in earlier reports, more recent transcriptional studies have aided in explaining the mechanisms of action of these hormones. For

example, cytokinin and auxin have been suggested to account for the hyperplasia observed in infected tissues, while auxin on its own accounts for the hypertrophy (Siemens et al., 2006; Jahn et al., 2013). Brassinosteroid synthesis was suggested as necessary for the development of galls only recently, based on transcriptomic analysis of *P. brassicae*-infected *Arabidopsis thaliana* (Schuller et al., 2014).

In addition to the central role of hormones, other molecular defense mechanisms are also important to target genes that might be bred into resistant cultivars. Plants have a two-layer innate immune system against pathogens. The first layer is responsible for general defenses and is activated by pattern recognition receptors (PRRs) that detect conserved pathogen-associated molecular patterns (PAMPs); this type of defense is known as PAMP-triggered immunity (PTI). PTI can be overcome by pathogen effectors, and therefore a second layer of immunity is activated by specific disease resistance (*R*) genes that recognize these effectors; this type of defense is known as effector-triggered immunity (ETI), which accounts for a stronger and narrower defense (Jones and Dangl, 2006). Plant hormones, including salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), are at the core of immune signaling components generated in both PTI and ETI (Mine et al., 2014). Effector-triggered immunity also activates complex defense responses through the action of defense-related transcription factors (Nejat et al., 2017). Therefore, a network of genes with different functions accounts for the defense mechanisms of plants against pathogens. Improved understanding of the complex networks of interactions between hosts and *P. brassicae* will help in the development of canola cultivars with broader and more durable resistance.

The application of omics approaches is becoming increasingly common in studies of the biology of Brassicas and provides insights into the molecular mechanisms that underlie disease resistance in these plants at the genomic, transcriptomic, proteomic, and metabolomic levels (Francisco et al., 2016). High-throughput and precise marker and candidate gene identification through omics studies are promising tools for future breeding activities (Collard and Mackill, 2008). Here we review how advanced genomic and transcriptomic approaches can lead to the cost-effective identification of useful markers and candidate genes associated with or conferring clubroot resistance. Furthermore, we propose that validation and utilization of candidate genes through the genome-editing technology of the CRISPR/Cas9 system will speed up the breeding of CR cultivars.

2.2 Impact of marker-assisted selection (MAS) on clubroot resistance breeding

The goal of traditional breeding is to use recurrent crossing to increase clubroot resistance in *B. napus* and other Brassicas, and to add this resistance to clubroot susceptible (CS) varieties with desirable agronomic traits, such as earlier flowering and good seed quality (Rahman et al., 2011). The fundamental requirement, as well as the major challenge to achieve this goal, is selecting plant genotypes with resistance to different *P. brassicae* pathotypes. Although phenotypic selection, which refers to host inoculation followed by disease rating, can be performed to assess clubroot resistance, this methodology tends to be time-consuming, laborious and can be affected by environmental factors. Marker-assisted selection, a breeding process in which DNA marker detection and selection are combined with conventional breeding (Jiang, 2013), has become an important strategy for clubroot resistance breeding (Matsumoto et al.,

2012; Hirani et al., 2016; Liu et al., 2018). Once markers have been related to a phenotype, recurrent disease rating is no longer necessary, which can speed up the breeding process.

Various DNA markers have been developed and applied for the identification and mapping of clubroot resistance loci; these include hybridization-based restriction fragment length polymorphisms (RFLPs) (Voorrips et al., 1997), PCR-based random amplified polymorphic DNA (RAPD) (Kuginuki et al., 1997), amplified fragment length polymorphisms (AFLPs) (Voorrips et al., 1997) and simple sequence repeats (SSRs) (Suwabe et al., 2003). However, many of these markers are low-output (e.g., do not cover the full genome), time-consuming and/or expensive. In recent years, SNP markers, representing single base variations between DNA sequences of individuals or lines, have become highly desirable for genomic selection because they are abundant in the genomes and flexible for high-throughput detection approaches based on next-generation sequencing (NGS) (Mammadov et al., 2012).

The use of MAS has contributed greatly to CR breeding programmes. Several *B. napus* CR varieties have been developed through the introgression of resistance loci from *B. rapa*, where identified markers linked to these loci were used in plant screening. For example, clubroot resistance linked to several SSR markers was transferred into the canola 'Topas' from the Chinese cabbage hybrid 'Qulihuang' (Hirani et al., 2016); the CR locus *Rcr1* flanked by several SSR markers was introgressed from the pak choy 'Flower Nabana' into canola breeding lines (Chu et al., 2014; Rahman et al., 2014); and several resistance genes linked to SSRs and intron polymorphic markers were introduced from

three CR Chinese cabbage varieties into *B. napus* (Liu et al., 2018). Compared with selection based solely on phenotype, combining MAS saves time, resources and effort, by simplifying screening and allowing earlier and individual-plant selection (Collard and Mackill, 2008; Liu et al., 2018). In addition, MAS facilitates the pyramiding or stacking of multiple resistance genes into a single background, which may improve the durability of clubroot resistance (Matsumoto et al., 2012). Such gene pyramiding cannot be achieved by phenotypic selection, due to its limitation in recognizing resistance genes/loci. Therefore, MAS is still the mainstay in CR breeding by the canola industry. In the presence of other resistance genes/loci, the effectiveness of applying MAS in clubroot resistance breeding will continue to increase as CR loci/genes are mapped more precisely using advanced omics techniques.

2.3 Use of the *Arabidopsis thaliana* genome to identify and map clubroot resistance loci

Early genome sequencing technologies contributed to the release of the genome sequence of *A. thaliana* (Arabidopsis Genome Initiative, 2000). *Brassica* species and *A. thaliana* are in the same family, the Brassicaceae, and are both hosts of *P. brassicae* (Dixon, 2009b; Al-Shehbaz, 2012). Therefore, the available *A. thaliana* genome information can serve as a bridge to understand clubroot resistance in *Brassica* species. For example, two major CR loci *Crr1* and *Crr2*, which reside on different genomic regions in *B. rapa* and showed differential resistance to *P. brassicae* isolates, correspond to a small region on chromosome 4 of *A. thaliana*. This region is known as one of the clusters of disease resistance genes, termed the major recognition complexes (MRCs), suggesting species-specific evolution of *Crr1* and *Crr2* due to either chromosomal

rearrangements of two genes clustered in the MRCs region or to functional diversification of duplicated genes that originated from a single gene (Suwabe et al., 2003, 2006). Sequence-Tagged Site markers developed based on genomic information from *A. thaliana* enabled the construction of a fine map of *Crr3* in *B. rapa* (Saito et al., 2006). The *A. thaliana* genome information also facilitated map-based cloning and characterization of two CR genes in *B. rapa*, *Crr1a* and *Cra*, which were Toll-interleukin receptor (TIR)-nucleotide-binding site (NBS)-leucine-rich repeat (LRR)-type *R* genes (Ueno et al., 2012; Hatakeyama et al., 2013). The evidence of TIR-NBS-LRR-type *R* genes in clubroot resistance also enabled identification of more candidate CR genes in other studies (Table 1). Thus, *A. thaliana* genome-based studies provided valuable information about clubroot resistance when *Brassica* genomes were not available.

2.4 Contributions of NGS technologies to resistance breeding

Next-generation sequencing technologies, which allow the rapid and cost-effective generation of genome-scale data (Wetterstrand, 2019), opened up new opportunities to develop crops with improved traits and increased productivity (Varshney et al., 2014). Access to NGS enabled development of genotyping by sequencing (GBS) and the availability of genomic and transcriptomic sequences has helped to determine genome-wide polymorphism that can be developed into high-density SNP arrays (Elshire et al., 2011; Clarke et al., 2016). High-density SNP arrays and GBS have been widely used to screen SNPs and construct high-density genetic maps, which have become important for increasing the efficiency and cost effectiveness of MAS, improving resolution of target loci (Mammadov et al., 2012), and helping to predict candidate genes (Afzal et al., 2018). High-throughput SNPs also can be used for genome-wide association

studies (GWAS), speeding up candidate gene identification (Varshney et al., 2014). Next-generation sequencing also enables cost-effective sequencing of all transcripts in a sample of a specific treatment, termed RNA sequencing (RNA-seq) (Wang et al., 2009). RNA-seq can be combined with bulked segregant analysis to map interesting loci precisely and predict candidate genes (Liu et al., 2012). Sequence data derived from RNA-seq can also be used to measure the levels of gene expression under different conditions, and thus to determine contrasting transcriptional states (Varshney et al., 2005). Therefore, RNA-seq can provide insights regarding the molecular defense network activated by host infection. More importantly, RNA-seq can be applied to the identification of candidate genes involved in functional networks related to a desired trait (Martin et al., 2013). These factors contribute to an increasing interest in the application of omics approaches to the development of CR *B. napus*. Details of the contributions of each approach are discussed below.

2.5 Identification of SNP markers linked with clubroot resistance through genomic and transcriptomic approaches

Next-generation sequencing has facilitated the construction of reference genome sequences of several *Brassica* species. So far, reference genomes have been published for *B. rapa* (Wang et al., 2011; Cai et al., 2017), *B. oleracea* (Liu et al., 2014) (Parkin et al. 2014), *B. nigra* (Yang et al., 2016), *B. napus* (Chalhoub et al., 2014; Bayer et al., 2017), and *B. juncea* (Yang et al., 2016). These genomic sequences provide valuable information to identify quantitative trait loci (QTLs), detect associated genes and evaluate sequence variation of these genes (Delourme et al., 2018). The utility of *Brassica* reference genome sequences also enhances the detection and genotyping of genome-wide SNPs of *Brassica*

species when combined with other approaches such as GBS, high-density SNP arrays, and BSR-seq (Clarke et al., 2016; Lee et al., 2016; Yu et al., 2016, 2017; Huang et al., 2017; Dakouri et al., 2018; Pang et al., 2018).

Genotyping by sequencing is a recently emerged genomics approach for exploring plant genetic diversity on a genome-wide scale. The GBS approach uses enzyme-based complexity reduction and DNA barcoded adaptors to generate multiplex libraries of samples that are ready for NGS sequencing. Reads obtained from NGS sequencing are aligned with reference genomes for SNP identification and genotyping. Compared with traditional marker development, which requires a two-step process for marker detection and genotyping, GBS provides simultaneous detection of polymorphisms and genotyping (Elshire et al., 2011). Although recently developed, GBS has been applied for genetic analysis and marker development in many crops, such as soybean (*Glycine max* L.), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) (Huang et al., 2014; Jarquín et al., 2014; Bélanger et al., 2016) (Bélanger et al. 2016). Similarly, GBS also can be applied for mapping CR loci and developing markers associated with clubroot resistance when combined with linkage analysis, since the polymorphisms generated can be linked to the CR phenotype after inoculation (Figure 2.1). In addition, candidate genes for clubroot resistance may be identified in mapped regions. Lee et al., (2016) genotyped 4,103 SNPs from F_2 populations of the two *B. olearacea* inbred lines, C1176 (CS) and C1220 (CR), and constructed a high-density genetic map spanning 879.9 cM with an average interval of 1.15 cM using these SNP markers. Combined with a clubroot resistance test, they identified two (CRQTL-GN_1 and CRQTL-GN_2) major QTLs for resistance to *P. brassicae* race 9 and one (CRQTL-YC) QTL for resistance to race 2. Furthermore, they

predicted that the paralogue of *CRc* in a block of chromosome C03 in *B. oleracea*, corresponding to a syntenic region in chromosome 5 in *A. thaliana* (Schranz et al., 2006), may be the candidate gene conferring resistance to *P. brassicae* (Table 1). Similarly, Yu et al., (2017) constructed a genetic map of CR loci based on 1,584 high-quality SNP markers in BC_1 plants derived from the two *B. rapa* lines, T19 (CR) and ACDC (CS). Three CR loci *Rcr4*, *Rcr8*, and *Rcr9* were identified on chromosomes A03, A02, and A08, respectively. Among these loci, *Rcr4* conferred resistance to *P. brassicae* pathotypes 2, 3, 5, 6 and 8, while *Rcr8* and *Rcr9* conferred resistance only to pathotype 5X. Additionally, several *R* genes encoding TIR-NBS-LRR proteins in each region were identified as CR candidate genes (Table 1).

High-density SNP arrays, another SNP genotyping technology, can also be applied to screen for CR loci and identify candidate genes within genomic regions (Figure 2.1). In contrast to GBS, this approach identifies and genotypes SNPs using commercial SNP arrays instead of whole genome re-sequencing, allowing easier sample preparation and straightforward comparisons (Mason et al., 2017). The 60K *Brassica* SNP array (Clarke et al., 2016) has been used to identify genome-wide loci for clubroot incidence and six resistance-associated traits in *B. oleracea* inbred lines tested with *P. brassicae* race 4 (Peng et al., 2018a). A high-resolution map was constructed using around 3,000 SNPs identified between the two parental lines, and 23 QTLs were found for three clubroot resistance associated traits, including disease incidence, number of fibrous roots and *P. brassicae* content in the roots. Among these QTLs, three novel CR loci were identified on chromosomes C6 and C8, where several pathogen-responsive genes were found, including those encoding R proteins, receptor binding proteins, a root

hair defective 3 gene, auxin-related proteins, pathogen-responsive factors and transcriptional factors.

The SNP genotyping technologies also facilitate GWAS, which are now increasingly used to identify candidate genes/loci underlying traits of interest in *B. napus* (Delourme et al., 2018). This approach identified SNPs through the exploration of recombination events in natural populations and analysis of the association between SNPs and the trait of interest (Korte and Farlow, 2013). Genome-wide association studies do not require the development of a mapping population, and provide higher resolution for QTL mapping than linkage analysis, since they take advantage of historical recombination in natural populations (Nordborg and Weigel, 2008). In addition, GWAS can be used in foundational experiments to provide information on the genetic architecture of a trait, selecting the parents for mapping analysis, and identifying candidate genes (Korte and Farlow, 2013). Therefore, GWAS can also be an important component of the resistance breeding toolbox (Figure 2.1). Li et al., (2016) carried out GWAS on 472 *B. napus* accessions from a natural global population to elucidate the genetic control and identify CR QTLs and candidate genes for resistance to *P. brassicae* pathotype 4. Within the nine identified CR QTLs, 28 *R* genes and 30 additional genes, whose homologues were expressed differentially in *B. rapa* in response to the same *P. brassicae* pathotype in a previous study (Chen et al., 2016a), were identified as CR candidate genes (Table 1).

Another advantage of GWAS approaches is that they enable the investigation of environmental influences on disease outcomes (Gage et al., 2016). The development of

clubroot is affected by environmental parameters such as soil pH and nitrogen content (Dixon, 2009a; Gossen et al., 2014). Understanding the interactions between environmental effects and disease resistance should help to increase the efficiency of use of CR sources. Laperche et al., (2017) carried out linkage analyses and GWAS to detect CR loci and evaluated their sensitivity to nitrogen supplements in a group of 92 diverse *B. napus* accessions and 108 lines generated from a cross of *B. napus* 'Darmor-bzh' (CR) and 'Yudal' (CS). They indicated that although soil nitrogen conditions and/or *P. brassicae* isolate can disturb the effects of some CR loci, the two major loci *C09* and *A03b* detected in their study could be useful in the development of genotypes with strong clubroot resistance, regardless of soil nitrogen conditions (Laperche et al., 2017).

2.6 Transcriptomic approaches for uncovering host response mechanisms following *P. brassicae* inoculation and predicting candidate genes for clubroot resistance

2.6.1 RNA-seq combined with bulked segregant analysis to map CR loci and identify candidate genes conferring clubroot resistance

The combination of bulked segregant analysis (Michelmore et al., 1991) and RNA-seq (Wang et al., 2009), known as BSR-Seq, has great potential to aid in the development of clubroot resistance (Figure 2.1). The application of BSR-Seq to the mapping of CR loci is based on polymorphic transcripts from resistant and susceptible bulk samples of offspring from highly susceptible and resistant parents. This approach has been used to precisely map CR loci and identify candidate genes conferring clubroot resistance in several studies (Yu et al., 2016; Huang et al., 2017; Dakouri et al., 2018; Pang et al., 2018; Chang et al., 2019; Table 1). For example, a BSR-seq study of *Rcr1* in *B. rapa* indicated that the most likely candidate genes for this locus were *Bra019409* and

Bra019410 (Yu et al., 2016). Other studies showed the associations of two *B. rapa* genes (*Bra019410* and *Bra019413*) with *Rcr2* (Huang et al., 2017), three *B. rapa* genes (*Bra001160*, *Bra001161*, and *Bra001175*) with *Crd* (Pang et al., 2018), two *B. oleracea* genes (*Bo7g108760* and *Bo7g109000*) with *Rcr7* (Dakouri et al., 2018), and five *B. rapa* genes (*Bra010552*, *Bra010588*, *Bra010589*, *Bra010590* and *Bra010663*) and one *B. nigra* gene (*BniB015819*) with *Rcr6* (Chang et al., 2019).

2.6.2 Differential expression analysis to explore defense mechanisms and find candidate genes for clubroot resistance

Although the previously mentioned approaches are very useful for identifying CR loci and helping to predict candidate genes conferring CR, they do not allow exploration of the underlying molecular mechanisms of action. Most candidate genes identified through mapping-based studies were *R* genes that encoded TIR-NBS-LRR proteins. There may be other genes, however, involved in the defense network acting against pathogens like *P. brassicae*. Those genes are often overlooked because they do not represent the most common mechanism of receptor (*R*-gene)-mediated resistance. Uncovering additional genes may allow for (i) an improved understanding of the biological basis of host defense, (ii) identification of key components of gene regulation, and (iii) a comparison of candidate gene functions in resistant and susceptible hosts. Differential expression analysis plays an important role in uncovering the other genes involved in the host response to *P. brassicae* by revealing mechanisms of clubroot resistance (Figure 2.1). Furthermore, the power of this strategy to explore host-response mechanisms is increasing as differential analyses can be performed to compare the transcriptional status in multiple hosts/pathotypes/stages. Several transcriptomic-level

differential expression analyses of host responses to *P. brassicae* have been conducted using either microarrays or RNA-seq (Tables 2, 3, 4).

Initially, the adoption of transcriptomic analysis was slow due to the high cost of sequencing, limiting studies to the interaction between *P. brassicae* and *A. thaliana*, a plant with an extensive genomics toolkit. To date, there have been at least seven transcriptomic studies of *A. thaliana* following *P. brassicae* infection, four of which were conducted using microarrays, two by RNA-seq and one using both approaches (Tables 2, 3). These studies assessed changes in gene expression levels in the host during pathogenesis, building a foundation to understand the molecular mechanisms involved in the response to *P. brassicae* (Siemens et al., 2006; Agarwal et al., 2011; Jubault et al., 2013; Schuller et al., 2014; Malinowski et al., 2016; Zhao et al., 2017; Irani et al., 2018). Some of these studies found significant upregulation of genes controlling plant hormones, such as auxins and cytokinins, during clubroot development (Siemens et al., 2006; Schuller et al., 2014; Malinowski et al., 2016; Irani et al., 2018). Schuller et al., (2014) compared individual root cells at different stages of the disease, confirming the activation of auxin and cytokinin genes, but also providing evidence of the activation of genes involved in brassinosteroid synthesis in enlarged cells and the central cylinder. Jubault et al., (2013) compared resistant and susceptible interactions between *A. thaliana* and *P. brassicae* and suggested that reduced or delayed metabolic diversion, faster and/or stronger upregulation of genes involved in classical defense responses, and downregulation of cell enlargement and division are major factors resulting in normal root growth in resistant hosts (Table 2). In a comparison of above and below-ground tissues following infection, Irani et al., (2018) found that genes associated with cell wall

modification, sucrose and starch biosynthesis, and several transcription factor classes were regulated differentially between the tissue types.

Currently, there is an increasing interest in the application of RNA-seq approaches to examine defense mechanisms in the Brassicas to *P. brassicae* infection. The greater genomic complexity of *Brassica* species relative to *A. thaliana* makes it difficult to extrapolate information gained from the latter to the former (Town et al., 2006; Delourme et al., 2018). RNA-seq provides many advantages over microarrays, such as the detection of new transcripts, detection of expression over a larger dynamic range, and lower costs (Wang et al., 2009; Zhao et al., 2014). At least nine RNA-seq studies have explored the defense mechanisms of *Brassica* spp. to *P. brassicae* (Table 4), most of which compared the transcriptomes of CR and CS hosts following inoculation (Chu et al., 2014; Chen et al., 2016a, 2016b; Zhang et al., 2016; Jia et al., 2017; Luo et al., 2018; Ciaghi et al., 2019; Mei et al., 2019; Wang et al., 2019). Some of these studies found a faster and/or stronger activation of defense-related genes, including *R* genes, receptor-kinase related genes, respiratory burst oxidase homolog (*RBOH*) and chitinase genes (Chen et al., 2016a; Zhang et al., 2016; Zhao et al., 2017; Mei et al., 2019; Wang et al., 2019), in CR vs. CS plants during primary or early secondary infection, consistent with the results obtained with *A. thaliana* (Jubault et al., 2013).

Differential expression analysis allows an understanding of the defense mechanisms triggered downstream of a CR gene/locus (*R* genes), and may help to identify key regulators involved in this response. Two studies have been conducted to understand the specific defense mechanisms mediated by the previously identified CR

loci *Crb* and *Rcr1*. Chen et al., (2016a) compared differentially expressed genes (DEGs) in near-isogenic lines of *B. rapa* that possessed or lacked the *Crb* locus, and found that a possible mechanism of *Crb*-mediated clubroot resistance included a stronger ETI response, with SA signaling occurring in the early stages of *P. brassicae* challenge. Chu et al., (2014) compared DEGs between segregating F_1 populations of *B. rapa* with or without the *Rcr1* locus, and suggested that resistance may be related to upregulation of genes involved in the JA and ET-related pathways, callose biosynthesis, indole-containing compound biosynthesis, and suppression of genes related to auxin synthesis and cell growth/development. Among several defense-related genes found in the *Rcr1* locus by Chu et al., (2014) were *Bra019412* and *Bra019413*, which matched TIR-NBS-LRR genes, and *Bra038776* matching a plasma membrane cysteine-rich receptor-like protein kinase. The latter has been reported to be involved in defense against various plant pathogens, including *P. brassicae* (Wang et al., 2019). Given their similarity to other genes involved in mediating host-pathogen interactions, the role(s) of these genes may warrant further study.

Transcriptomic analyses are important in elucidating the molecular mechanisms of the host response to *P. brassicae* (Tables 2 to 4) and may help to determine the function of key genes. Not all differentially expressed genes, however, can be considered as putative candidates or key players in the defense response, given the possibility of post-translational regulation and the complexity of regulatory networks. In addition, the study of a particular host-pathotype interaction may result in the identification of defense mechanisms specific for that interaction. One way to identify robust candidate genes involved in resistance is by finding common responses across different host-pathotype

combinations. Once common regulators have been found, functional validation, such as via mutation analysis (e.g., via gene editing) is needed to confirm their effectiveness in conferring clubroot resistance.

Some studies have found common gene expression patterns, and these genes may represent good candidates for further study as potential modifiers of resistance. In addition to genes for pathogen recognition during PTI and ETI, such as those encoding PRRs and R proteins (Chen et al., 2016a; Zhang et al., 2016; Luo et al., 2018; Ciaghi et al., 2019; Mei et al., 2019; Wang et al., 2019), other genes have been generally recognized as playing roles in defense against *P. brassicae*. These include genes encoding RBOH proteins (Chen et al., 2016a; Zhang et al., 2016; Jia et al., 2017; Luo et al., 2018; Ciaghi et al., 2019; Wang et al., 2019), genes associated with SA-signaling pathways (e.g., pathogenesis-related 1) (Chen et al., 2016a; Luo et al., 2018; Ciaghi et al., 2019), cell wall modification (Zhang et al., 2016; Luo et al., 2018; Wang et al., 2019) and calcium signaling (Zhang et al., 2016; Jia et al., 2017; Luo et al., 2018; Mei et al., 2019). Wang et al., (2019) reported *PRPS4* (resistant to *Pseudomonas syringae* 4) and pectin methylesterase 44 (*PME44*) as involved in the defense response of *B. oleracea* to *P. brassicae*. Homologs of *PRPS4* and *PME44* were also upregulated in the clubroot resistance response of *B. rapa* carrying the *CRb* gene (Chen et al., 2016a). These types of candidate genes can be further studied by gene editing to validate their function and/or try to increase host resistance.

2.7 Need for an integrated omics approach to identify candidate genes for clubroot resistance

Candidate genes may be grouped into two categories: positional and functional, which can be detected by various genetic, genomic and transcriptomic approaches. Positional candidate genes are identified based on their genomic locations, while functional candidate genes are identified through functional genomics, like gene expression analyses, to determine their biological roles in specific traits or processes (Varshney et al., 2005). With the help of omics approaches, knowledge regarding clubroot resistance is increasing rapidly. Many *R* genes encoding TIR-NBS-LRR proteins have been identified as candidate CR genes in mapping-based studies (Table 1). Since there may be multiple genes in a CR locus, differential expression analysis can help to target specific candidates in a locus of interest, as well as to identify candidate genes beyond major *R* genes. For instance, a recent RNA-seq study found 151 putative CR genes in a *B. rapa* line carrying the *CRb* locus, including genes encoding PRRs, R proteins, RBOHs, Ca^{2+} influx coding proteins, mitogen-activated protein kinases (MAPKs), WRKYs, chitinases and pathogenesis-related proteins, and genes related to SA/JA/ET metabolism and cell wall modification (Chen et al., 2016a). Li et al., (2016) used GWAS to identify nine CR QTLs in *B. napus*, and predicted 30 CR candidate genes residing in these QTLs using the data from Chen et al., (2016). Such reports highlight the importance of transcriptomic approaches for complementing studies where resistance has been associated to phenotype through screening.

The integration of *P. brassicae* genomics and transcriptomics may provide additional layers of information for understanding *Brassica-P. brassicae* interactions and

detecting candidate genes for resistance. The availability of *P. brassicae* genomic sequences and the possibility to analyze the pathogen transcriptome has enabled studies on population diversity and the identification of candidate secreted effector proteins (Pérez-López et al., 2018; Sedaghatkish et al., 2019). An RNA-seq analysis of *P. brassicae* identified 33 expressed secretory protein genes during the primary infection stage, of which 28 could suppress plant cell death to benefit pathogen propagation and colonization (Chen et al., 2019). Similarly, Pérez-López et al., (2020) used RNA-seq to identify 32 small secreted *P. brassicae* proteins that were highly expressed during secondary infection, seven of which were annotated with predicted functions (one cyclin, one serine protease, one cysteine protease inhibitor, one translocase, one 1,3(4)-beta-D-glucanase and two kinases). Another study used the same RNA-seq library data but focused on host transcriptomic profiles, identifying 17 *R* genes that were significantly upregulated following *P. brassicae* infection (Irani et al., 2018). While individual studies of either the pathogen or host can help to elucidate mechanisms of infection and defense, the ideal studies will show co-expression of both parties, allowing inferences regarding molecular interactions. For example, co-expression of pathogen effectors and host *R* genes could be validated via studies of effector-receptor interactions (e.g., two-hybrid experiments), which can point to specific *R* genes involved in resistance.

Since proteins and metabolites are the actual interacting molecules in defense responses, proteomics and metabolomics approaches should be used to complement transcriptomics data (Mehta et al., 2008; Castro-moretti et al., 2020). Proteomics, which allows the high-throughput detection of protein expression profiles, has been used to identify key proteins in plant-pathogen interactions (Mehta et al., 2008). Song et al.,

(2016) analyzed differentially accumulated proteins in response to clubroot by a shotgun label-free proteomics approach, to better understand *Rcr1*-mediated resistance mechanisms previously studied using transcriptomics (Chu et al., 2014), and found specific post-transcriptional modifications that could regulate protein metabolism without a concomitant change in gene expression levels. A novel calcium-independent signaling pathway, which may consist of a unique MAPK cascade and the ubiquitin-26S proteasome, was also identified in *Rcr1*-mediated clubroot resistance. Metabolomics approaches are also useful to study the changes occurring during host-pathogen interactions (Castro-moretti et al., 2020). Yahaya et al., (2017) used a non-target metabolomics approach to obtain results that were combined with previous transcriptomic data from Malinowski et al., (2016) to improve understanding of the interaction between *A. thaliana* and *P. brassicae*. The study suggested that *P. brassicae* may manipulate plant metabolism to accumulate components such as free Beta-L-arabinose, glucoronate-1P, galacturonate, amino acids (glutamate, aspartate and alanine), vitamin B6 and folates, in order to promote host colonization (Yahaya et al., 2017). In contrast, the plant may increase the abundance of the amino acid proline to defend itself against the pathogen.

2.8 Genome-editing technology as a tool to validate and use CR candidate genes

As more and more candidate genes for clubroot resistance are identified through omics approaches, the next consideration is how to effectively validate and utilize these candidates in clubroot resistance breeding. Conventionally, the validation of candidate genes is often achieved by mutagenesis. Unlike the model plant *A. thaliana*, for which most mutations are commercially available (Alonso et al., 2003), custom screening of

random mutations of non-model *Brassica* crops is often needed (Figure 2.2). For example, Ueno et al., (2012) obtained several *CRa* mutants from numerous random mutants induced by UV light, and successfully characterized the *CRa* gene for clubroot resistance. The molecular characterization of the *CRa* mutants showed that mutations in each of the TIR, NBS, and LRR domains accounted for the absence of *CRa*-mediated resistance, indicating the importance of each domain for the proper function of the *CRa* gene (Ueno et al., 2012). Random mutagenesis by irradiation or chemical treatments can, however, generate a large number of background mutations, making the selection process long and laborious (Ueno et al., 2012; Braatz et al., 2017). Moreover, while breeders place an emphasis on the identification and application of molecular markers associated with clubroot resistance, and MAS has been introduced as the main approach to accelerate resistance breeding, it can still take many years to introgress CR genes into canola (Figure 2.3) (Hirani et al., 2016). The speed of candidate gene validation through random mutagenesis and the application of CR genes through MAS is unlikely to keep pace with the increasing demand for CR cultivars. Given the rapid spread of clubroot (Strelkov and Hwang, 2014; Gossen et al., 2015) and the emergence of new pathotypes of *P. brassicae* (Strelkov et al., 2018), it is important to accelerate the breeding process.

A promising strategy that could revolutionize the speed of candidate gene validation and deployment is targeted genome-editing, wherein specific changes can be induced in the genome using sequence-specific nucleases (Hsu et al., 2014). The clustered regularly interspaced palindromic repeat (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) system (Figure 2.4a and 4b) is the latest method allowing efficient genome editing in a broad array of organisms (Sander and Joung, 2014). Application of

the CRISPR/Cas9 system has already resulted in the inexpensive and efficient validation of candidate genes (Joung et al., 2017). More importantly, in the context of the current review, CRISPR/Cas9 also has significant potential as an alternative to traditional breeding and transgenic methods for achieving clubroot resistance in *B. napus* (Figure 2.1). With this system, specific mutations can be obtained by specific deletions or insertions in the target genomic region (Sander and Joung, 2014) (Figure 2.4b). The flexibility of CRISPR/Cas9 in modifying target genes facilitates the alteration and validation of candidate *CR* genes (Figures 2.4b and 2.4c).

Homozygous lines with desirable resistance traits can be obtained after fewer generations with CRISPR/Cas9 than via traditional backcrossing. For instance, CRISPR/Cas9 was used to produce blast-resistant rice (*Oryza. sativa* L.) in the T_2 generation (Wang et al., 2016), powdery mildew resistant-tomato (*Solanum lycopersicum* L.) in the T_1 generation (Nekrasov et al., 2017), and broad virus resistant-cucumber (*Cucumis sativus* L.) in the T_3 generation (Chandrasekaran et al., 2016). Ongoing case studies suggest that CRISPR/Cas9 is promising for the acceleration of basic and applied crop improvement research (Mishra and Zhao, 2018). There are already several reports of the application of the CRISPR/Cas9 system in *Brassica* breeding (Lawrenson et al., 2015; Kirchner et al., 2017; Yang et al., 2017; Murovec et al., 2018; Okuzaki et al., 2018; Sun et al., 2018), and a transgene-free CRISPR/Cas9 protocol for site-directed mutagenesis of different *Brassica* species has been published (Murovec et al., 2018) this latter protocol may facilitate the application of this technology in crop improvement.

2.9 Conclusions

Genomics approaches, including GBS, high-density SNP arrays and GWAS, along with the transcriptomics approach BSR-seq, are efficient in mapping candidate *CR* regions and identifying genes associated with clubroot resistance, especially *R* genes. While *R* genes are important for resistance to *P. brassicae*, other genes may also be key regulators of resistance. Gene expression analysis through RNA-seq may help to speed up clubroot resistance breeding by improving understanding of host-*P. brassicae* interactions, and identifying candidate genes that contribute to resistance in addition to the traditionally targeted *R* genes. As more candidate genes are identified, genome-editing technologies such as the CRISPR/Cas9 system may further accelerate the development of CR canola and other Brassicas.

2.10 Tables

Table 2.1 Candidate clubroot resistance genes identified by various genomic approaches and bulked segregant RNA-seq analysis.

Host plant	Candidate gene	Classification of gene	Resistance to *P. brassicae*	Method	References
B. oleracea	paralogous gene of *CRc* in the R block[a] of chromosome C03	*R* genes[b]	Race 2 and 9	GBS	(Lee et al., 2016)
B. rapa	genes encoding TNL-class disease resistance proteins in the loci of *Rcr4*, *Rcr8*, and *Rcr9*	*R* genes	genes in *Rcr4* for pathotypes 2, 3, 5, 6 and 8; genes in *Rcr8* and *Rcr9* for pathotype 5x	GBS	(Yu et al., 2017)
B. napus	30 genes in nine loci 10 genes in *SCR-C6* 18 genes in *MCR-C9*	DEGs[c] *R* genes *R* genes	Pathotype 4	GWAS	(Li et al., 2016)
B. rapa	*Bra019409* and *Bra019410* in the locus *Rcr1*	*R* genes	Pathotypes 2, 3, 5 and 6	BSR-seq	(Yu et al., 2016)
B. rapa	*Bra019410* and *Bra019413* in the locus *Rcr2*	*R* genes	Pathotype 3	BSR-seq	(Huang et al., 2017)
B. rapa	*Bra001160*, *Bra001161*, and *Bra001175* in the locus *CRd*	*R* genes	Race 4	BSR-seq	(Pang et al., 2018)
B. nigra	*BniB015819* for *Rcr6*	*R* genes	Pathotype 3	BSR-seq	(Chang et al., 2019)
B. rapa	*Bra010552, Bra010588, Bra010589, Bra010590,* and *Bra010663* in the locus *Rcr6*	*R* genes	Pathotype 3	BSR-seq	(Chang et al., 2019)
B. oleracea	*Bo7g108760* and *Bo7g109000* in the locus *Rcr7*	*R* genes	Pathotype 3	BSR-seq	(Dakouri et al., 2018)

[a] R block refers to a syntenic region with Arabidopsis from chromosome 5

[b] *R* genes represent major resistance genes that produce resistance (R) proteins.

[c] DEGs refer to differentially expressed genes previously identified in *B. rapa* (Chen et al., 2016a).

Table 2.2 Differential expression analysis of *Arabidopsis thaliana* challenged with *Plasmodiophora brassicae* based on microarrays.

P. brassicae source[a]	Arabidopsis ecotype/mutant and susceptibility	Tissue	Time point	Summary of studies	References
e	Col-0[b] (susceptible)	Whole root	10 and 23 dai[c]	● DEGs identified in both time points were associated with defense, sugar phosphate metabolism, growth and cell cycle. ● Highlighted the significance auxin and cytokinin in clubroot development.	(Siemens et al., 2006)
16/19/31	Col-0 (susceptible)	Whole root	4, 7 and 10 dai	● More DEGs were identified at 4 dai, and induced genes were important for signal transduction and pathogen recognition. ● Suggested the importance of SA in enhancing clubroot resistance.	(Agarwal et al., 2011)
eH/e_2	Bur-0[d] (partially resistant to eH; susceptible to e_2)	Whole plant	1, 2 and 7 dai	● Observed reduced or delayed metabolic diversion, faster and/or stronger activation of classical defense responses, and repression of cell enlargement and division in the partial resistant host compared to the susceptible one.	(Jubault et al., 2013)
e3	Col-0 (susceptible)	Individual cells at varied disease stages	14 and 21 dai	● Confirmed roles of auxin and cytokinin metabolism and signaling in clubroot development. ● Identified novel hormone pathways in clubroot development: brassinosteroid synthesis and signal perception. ● Showed the success of combining laser microdissection and pressure catapulting to obtain pools of homogeneous population cell types with transcriptomic analysis to explore host responses to *P. brassicae.*	(Schuller et al., 2014)
16/2/12	Col-0 (ipt1;3;5;7 mutant[e])	Whole root and hypocotyl	16 dai	● *P. brassica*e inoculation increased the expression of cytokinin-responsive genes, but the host phenotype was not affected. ● A small amount cytokinin synthesized by *P. brassicae* had little impact on clubroot development.	(Malinowski et al., 2016)

[a] *P. brassicae* source is as listed in each study.

[b] Col-0, Arabidopsis ecotype Columbia.

[c] Dai, days after inoculation.

[d] Bur-0, Arabidopsis ecotype Burren.

[e] Ipt1;3;5;7 mutant results in reduced cytokinin content.

Table 2.3 Differential expression analysis of *Arabidopsis thaliana* challenged with *Plasmodiophora brassicae* based on RNA-seq.

P. brassicae source[a]	Arabidopsis ecotype/mutant and susceptibility	Tissue	Time point	Summary of studies	References
16/2/12	Col-0[b] (susceptible)	Hypocotyl	16 and 26 dai[c]	● Observed strong repression of genes involved in host cytokinin metabolism, biosynthesis, signaling, degradation and conjugation during gall formation. ● Decreased cytokinin content during gall formation may enhance gall development.	(Malinowski et al., 2016)
P3	Col-0 (susceptible)	Shoot and root	17, 20 and 24 dai	● Genes associated with the metabolism of lipid, cell wall compounds, and shikimate pathway metabolites, were highly regulated in both tissue types. ● Several genes related to biosynthesis of JA were up-regulated in both tissues. ● Genes associated with cell wall modification, sucrose and starch biosynthesis, and several transcription factor classes were regulated differentially between the two tissues. ● Described the similarities and differences in responses of above- and below-ground tissues during clubroot disease development.	(Irani et al., 2018)
ZJ-1	Col-0 (susceptible)	Whole root	24 and 48 hai[d]	● The pathways of lignin, flavonoid, glucosinolates and terpenoids synthesis were enhanced. ● Many genes associated with hormone and receptor kinases were differentially regulated. ● Suggested the importance of early responses of host to *P. brassicae* in the entire infection process.	(Zhao et al., 2017)

[a] *P. brassicae* source is as listed in each study.

[b] Col-0*, Arabidopsis* ecotype Columbia.

[c] dai, days after inoculation.

[d] hai, hours after inoculation.

Table 2.4 Differential expression analysis of Brassica spp. challenged with *Plasmodiophora brassicae* based on RNA-seq.

P. brassicae source[a]	Host and/or treatment/response	Tissues	Time point	Summary of studies	References
Pathotype 3	*B. rapa* F_1 populations: CR[b] population (carrying *Rcr1*); CS[c] population (not carrying *Rcr1*)	Whole root	15 dai[d]	● Genes associated with JA- and ET- related pathways, and biosynthesis of callose and indole-containing compounds were up-regulated in CR plants. ● Genes associated with auxin biosynthesis and cell growth/development were down-regulated in CR plants. ● Highlighted *Rcr1*-mediated mechanisms in clubroot resistance.	(Chu et al., 2014)
Pathotype 4	*B. rapa* near-isogenic lines: BJN3-2 (CR, carrying *CRb*); BJN3-2 (CS, not carrying *CRb*)	Whole root	0, 12, 72 and 96 hpi[e]	● Most DEGs (between inoculated CR and CS plants) were associated with transport, metabolism, signal transduction, and defense. ● Showed stronger ETI responses, especially SA signaling, in *CRb*-mediated clubroot resistance.	(Chen et al., 2016a)
Field population, Kunming, China	*B. napus*: Huashuang 3 (CR); Zhongshuangyou 8 (CS)	0.5-1 cm from the main root	20 dai	● DEGs related to broad-spectrum and clubroot-specific (*Crr1* and *Cra*) disease resistance were enhanced, but genes related to IAA signal transduction, and cytokinin and myrosinase synthesis were repressed in CR plants. ● Reported two *Crr1* homologous genes that were induced in CR plants.	(Chen et al., 2016b)
Pathotype ECD16/4/0	Cabbage lines (*Brassica oleracea*) : CR21(CR); CS54 (CS)	Whole root	3 dai	● 541 genes were specifically up-regulated in the CR line, of which most were involved in the metabolism or disease resistance responses. ● Resistance-related genes were identified, including those involved in pathogen recognition, cell wall modification, plant hormone signaling, generation of reactive oxygen species and transcriptional regulation. ● Identified several CR candidate genes.	(Wang et al., 2019)

Field population, Fuling, Chongqing, China	*B. napus* lines: 'ZHE-226' (CR); 'Zhongshuang 11' (CS)	Whole root	0, 3, 6, 9 and 12 dai	• Fast and strong responses in CR plants, including activation of receptor kinases and guanine nucleotide-binding (G) proteins, enhanced Ca^{2+} signaling, production of reactive oxygen species and cell death, and homeostasis of auxin and cytokinin. • Showed an effective signaling network activated by receptor kinases, G proteins and Ca^{2+} signaling in rapeseed conferring clubroot resistance.	(Mei et al., 2019)
Pathotype 4	CR wild Cabbage (*B. macrocarpa*.); CS Broccoli (*B. oleracea*)	Whole root	0,7 and 14 dai	• Genes associated with cell wall, glucosinolate biosynthesis, and plant hormone signal transduction were induced at 7 dai but repressed at 14 dai. • Genes associated with NBS-LRR proteins and chitinase encoding, SA and Ca^{2+} signal transduction, cell wall, phytoalexins biosynthesis, and respiratory burst oxidase homolog proteins were mainly up-regulated in CR plants compared with CS plants. • Described early activation of transcriptional changes of host after *P. brassicae* inoculation and stronger disease defense response in CR plants.	(Zhang et al., 2016)
Pathotype 4	Chinese cabbage (*B. rapas*) lines: R635–10 (CR); S177–47 (CS)	Whole root	30 dai	• DEGs associated with metabolic process, response to stimulus, biological regulation, plant-pathogen interaction, glucosinolate biosynthesis, cell wall thickening, SA homeostasis, Ca^{2+} influx, chitin metabolism, pathogenesis-related pathways, and glucosinolate synthesis were significantly up-regulated in CR plants. • DEGs associated with DNA replication, citrate cycle, oxidative phosphorylation, cell wall expansion, nodulin-relatedness, indole acetic acid and cytokinin synthesis were largely up-regulated in CS plants. • Root swelling of CS plants may be caused by uncontrollable root cell division, which is well controlled in CR plants.	(Jia et al., 2017)

Field population, Austria	Kohlrabi (*B. oleracea*)	SL, YG and OG[f]	Harvested from a field	• Many genes associated with host cell wall synthesis and reinforcement, cytokinin metabolism and signaling, and SA-defense processes were up-regulated in symptomless roots. • *P. brassicae* secreted SA methyl transferase gene may counteract the plant SA-defense by converting SA to methyl-salicylate.	(Ciaghi et al., 2019)
Field population, Chongqing, China	*B. juncea* H; P; P+B[g]	Whole root	15 dai	• Resistance-related DEGs were associated with pathogenesis-related protein synthesis, PTI, and ETI signaling pathways, calcium influx, SA pathway, reactive oxygen intermediates, mitogen-activated protein kinase cascades, and cell wall modification. • Stated the molecular mechanism of a potential biocontrol strain against *P. brassicae*.	(Luo et al., 2018)

[a] *P. brassicae* source is as listed in each study.

[b] CR, clubroot resistant.

[c] CS, clubroot susceptible.

[d] dai, days after inoculation.

[c] Hai, hours after inoculation.

[f] SL, symptomless roots; YG, young white spindle galls with waxy appearance; OG, old brownish spindle galls.

[g] H, no inoculation; P, only inoculated with *P. brassicae*; P+B, inoculated with *P. brassica* and the biocontrol agent *Zhihengliuella aestuari*

2.11 Figures

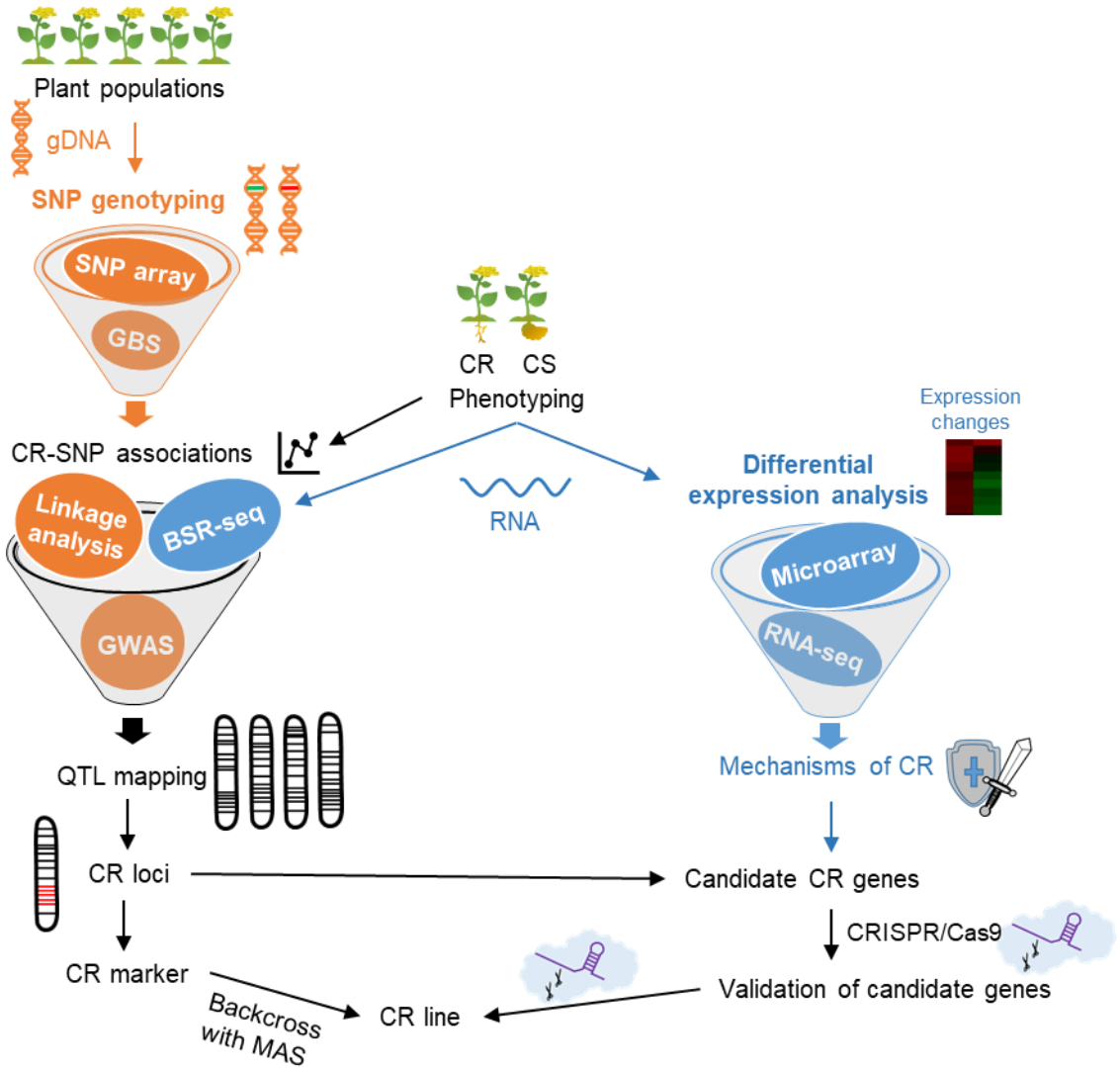

Figure 2.1 Potential applications of integrated genomics and transcriptomics technologies to clubroot resistance breeding. Genomics-based analyses are represented in orange (color online); transcriptomics-based analyses are represented in blue; CR, clubroot resistant; CS, clubroot susceptible. gDNA, genomic DNA; SNP, single nucleotide polymorphism; GBS, genotyping by sequencing; GWAS, genome-wide association study; BSR-seq, bulked segregant RNA-seq analysis; QTL, quantitative trait locus; CRISPR/Cas9, the CRISPR/Cas9 gene-editing system (see Figure 2.4).

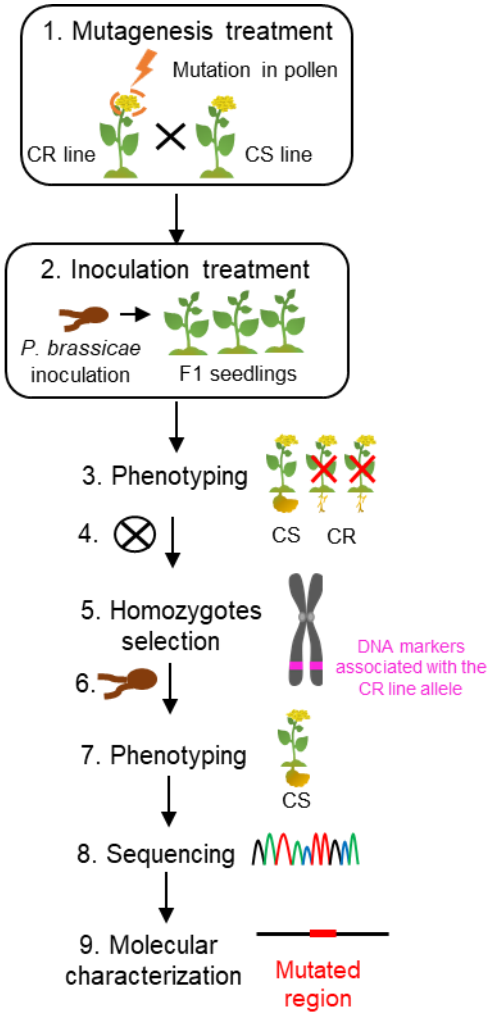

Figure 2.2 An example of candidate resistance gene validation by random mutagenesis. (1) A CR (clubroot resistant) line is mutated and its pollen used to inoculate a susceptible (CS) line; (2) seedlings obtained from the cross are inoculated with *Plasmodiophora brassicae*; (3) phenotypic resistance test to select susceptible plants; (4) self-fertilization of selected susceptible plants; (5) offspring plants are screened using DNA markers associated with the CR line allele; (6-7) the selected offspring mutants are subjected to inoculation and phenotyping again; (8) the mutated region related to the marker is sequenced; (9) mutations can now be related to the phenotype and potential candidate genes are identified. This diagram is based on the validation of a clubroot resistance gene *Cra* (Ueno et al., 2012).

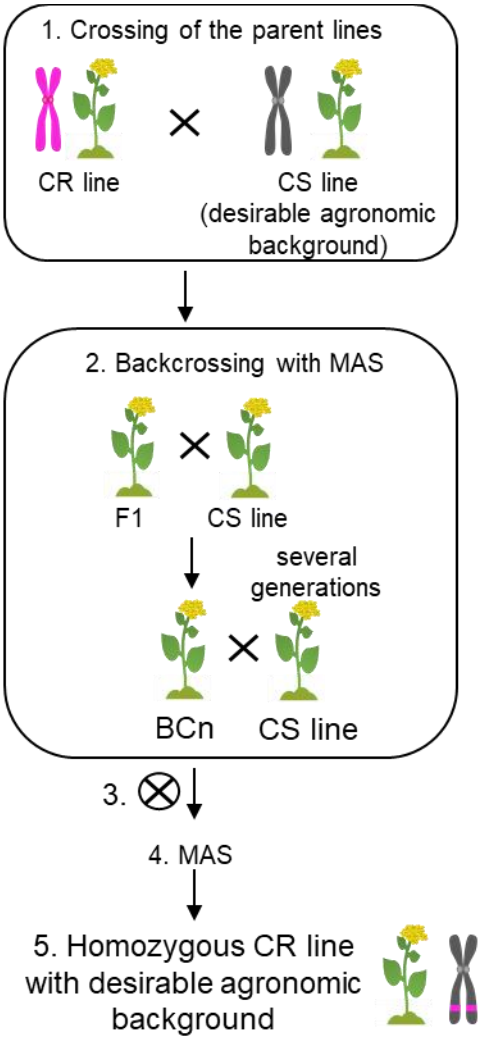

Figure 2.3 Breeding of homozygous clubroot resistant (CR) lines using traditional backcrossing methods. (1) A clubroot susceptible (CS) line, which has a desirable agronomic background, is crossed with a CR line carrying a CR gene/locus; (2) several cycles of backcrossing to the CS line to introgress the CR gene/locus into a desirable background; marker assisted selection (MAS) is applied for CR screening; (3) offspring seedlings are self-fertilized; (4) MAS for CR screening; (5) lines with introgression of a homozygous CR gene/locus and desirable agronomic background. BCn, offspring plants after several cycles of backcrossing. The diagram is modified from Hirani et al., (2016).

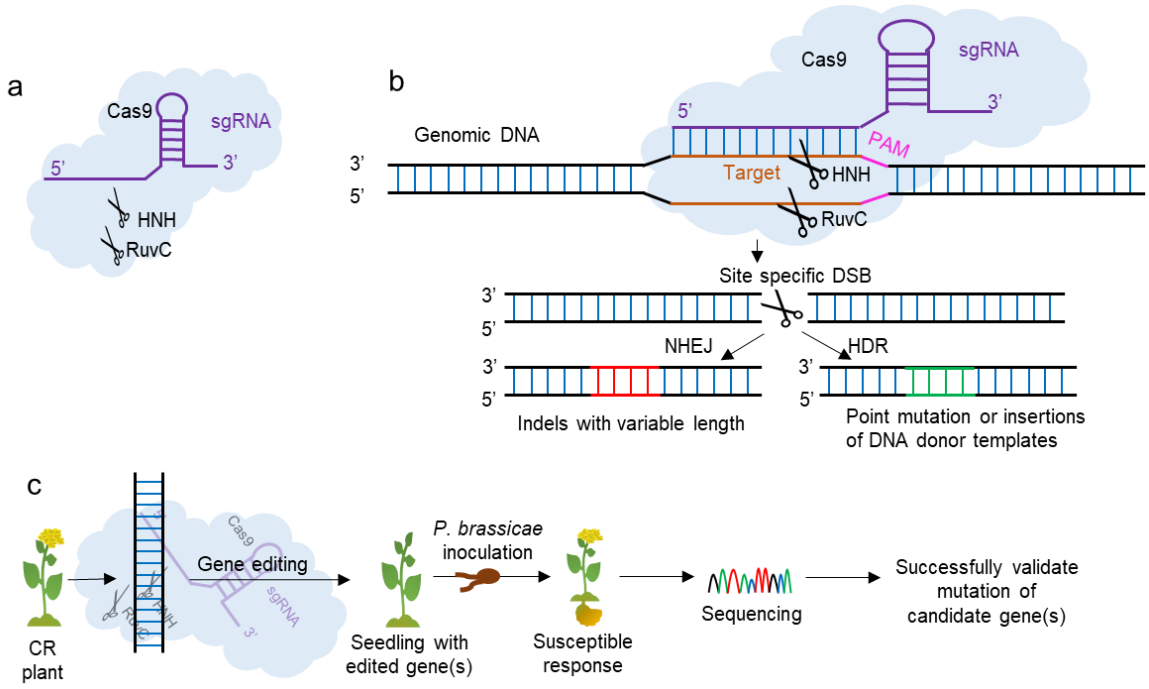

Figure 2.4 Application of the CRISPR/Cas9 gene-editing system to validate candidate clubroot resistance (CR) genes. (a) The CRISPR/Cas9 system. The system consists of two components, a Cas9 nuclease and single guide RNA (sgRNA). The sgRNA will guide the enzyme to a DNA target sequence next to the protospacer adjacent motif (PAM). Cas9 contains two domains homologous to RuvC and NHN nucleases. The RuvC domain breaks the non-complementary strand while the HNH domain breaks the complementary strand. (b) The principles of target gene editing through CRISPR/Cas9 system. The target sequence of the genomic DNA is cleaved by CRISPR/Cas9, leading to site specific double strand DNA break (DSB). The DSB can be repaired by the cell's non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms, leading to alteration of the target gene(s). The NHEJ mechanism generates deletion/insertion (indel) mutations of variable length specific nucleotides in target sites, while the HDR mechanism leads point mutations or insertions from DNA donor templates. (c) Validation of CR candidate genes using the CRISPR/Cas9 system. Altering key genes for clubroot resistance, in theory, results in plants with a susceptible response to *Plasmodiophora brassicae* infection. This reaction can be confirmed by a phenotypic resistance test and DNA sequencing to validate alteration of the sequence of the candidate gene.

Chapter 3: Comparative transcriptome analysis of rutabaga (*Brassica napus*) cultivars indicates activation of salicylic acid and ethylene-mediated defenses in response to *Plasmodiophora brassicae*

3.1 Introduction

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is an important soilborne disease of the Brassicaceae. Susceptible plants develop characteristic root galls following infection, which interrupt water and nutrient uptake and result in significant yield and quality losses. Globally, losses from clubroot have been estimated at 10-15% (Dixon, 2009b). In Canada, the disease has long been an issue on cruciferous vegetables (Howard et al., 2010), and since the early 2000s has emerged as an important constraint to the production of canola (oilseed rape; *Brassica napus* L.) (Strelkov and Hwang, 2014). As canola is one of the most valuable crops for Canadian farmers, contributing $26.7 billion CAD annually to the national economy (LMC International, 2016), there have been significant efforts to improve the understanding and management of this disease. While numerous control strategies have been evaluated, including long rotations out of susceptible hosts and the application of soil amendments to reduce disease pressure (Strelkov et al., 2011; Hwang et al., 2014), the deployment of genetically resistant canola cultivars remains the backbone of clubroot management (Peng et al., 2014b).

The first clubroot resistant (CR) canola cultivars were introduced to Canada in 2009-2010, and at present there are about 30 CR varieties from various seed companies on the market (Canola Council of Canada, 2019). The basis of this resistance, however, appears to be similar across most cultivars, and is derived from the European winter *B.*

napus ‘Mendel’ (Fredua-Agyeman et al., 2018). Although ‘Mendel’-type resistance initially provided excellent protection against all pathotypes of *P. brassicae* known in Canada, it was first overcome in 2013 (Strelkov et al., 2016), just four years after its introduction. Subsequent studies have documented the loss or erosion of resistance in an increasing number of fields, likely because of selection pressure imposed by CR canola on *P. brassicae* populations (Strelkov et al., 2018, 2020a). This has resulted in the emergence of multiple ‘novel’ pathotypes of *P. brassicae* that are highly virulent on CR canola; among these, pathotype 3A, as defined on the Canadian Clubroot Differential (CCD) set, is predominant in western Canada, where most canola is grown (Strelkov et al., 2018). New sources of clubroot resistance, combined with other management strategies and an enhanced understanding of resistance mechanisms, will enhance long-term management of this disease.

Plants have a two-layer immune system for defense against pathogen attack. Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) is the first line of defense to generic pathogen signals (Dodds and Rathjen, 2010). This first line of defense is initiated by pattern recognition receptors (PRRs), usually receptor kinases and receptor-like proteins (RLPs), which recognize evolutionarily conserved PAMPs or endogenous damage-associated molecular patterns (DAMPs) (Zipfel, 2014). Pathogens can, however, suppress PTI and facilitate virulence via the production of specific effectors. These effectors can be detected by specific resistance (*R*) genes in the plant in a “gene-for-gene” type interaction, activating the second layer of immunity, called effector-triggered immunity (ETI) (Dodds and Rathjen, 2010). The *R*-gene response has been studied more extensively in the clubroot pathosystem and has proven important for

resistance to this disease. For example, two clubroot resistance genes, *CRa* and *Crr1*, cloned in *B. rapa*, encode Toll-interleukin receptor nucleotide-binding site leucine-rich repeat (TIR-NBS-LRR) proteins, characterized as *R* genes (Ueno et al., 2012; Hatakeyama et al., 2013). In each of *B. napus*, *B. oleracea* and *B. rapa*, around 10-20 QTL have been mapped for clubroot resistance (Neik et al., 2017). Various additional *R* genes have been identified in clubroot resistance loci in *B. rapa*, including *Crd*, *Rcr1*, *Rcr2*, *Rcr4*, *Rcr6*, *Rcr8* and *Rcr9* (Yu et al., 2016, 2017; Huang et al., 2017; Pang et al., 2018; Chang et al., 2019), which could be important resources for resistance breeding. Recently, there has been increasing interest in identifying and utilizing PTI-related genes in quantitative resistance loci (QTL) to achieve long-term resistance to many diseases (Boyd et al., 2013). Therefore, key resistance regulators beyond *R* genes also have potential for use in clubroot resistance breeding programs.

Transcriptomic analyses have been conducted with increasing frequency in the study of *P. brassicae*-host interactions. For instance, recent transcriptomic studies of the responses of *B. rapa* and *B. juncea* to the clubroot pathogen have suggested the involvement of PTI and ETI in resistant reactions. These responses included the activation of genes encoding PRRs, R proteins, mitogen-activated protein kinases (MAPK), transcriptional factors (TFs), pathogenesis-related (PR) proteins, as well as genes involved in cell wall modification, calcium and hormone signalling, and the production of reactive oxygen species (ROS) (Chen et al., 2016a; Luo et al., 2018). In another study comparing the transcriptomes of clubroot susceptible (CS) and CR *B. napus* lines carrying resistance introgressed from rutabaga (*B. napus* subsp. *rapifera* Metzg), long noncoding RNAs appeared to be involved in regulating target genes

involved in the plant-pathogen interaction, hormone signaling and primary/secondary metabolism in response to *P. brassicae* (Summanwar et al., 2019). Studies with rutabaga are particularly relevant for understanding the interaction between the clubroot pathogen and canola, since rutabaga is a source of resistance for the latter (Ayers and Lelacheur, 1972; Rahman et al., 2014; Hasan and Rahman, 2016; Fredua-Agyeman et al., 2020).

A recent study investigating the transcriptomes of *B. napus* cultivars with differential resistance to *P. brassicae* pathotype 5X indicated the involvement of salicylic acid (SA)-mediated immunity in the resistance expressed by the cultivar 'Laurentian' (Galindo-González et al., 2020). This cultivar, however, is susceptible to pathotype 3A, the predominant resistance-breaking pathotype in western Canada (Strelkov et al., 2018). In the current study, the transcriptomic profiles of the rutabagas 'Wilhelmsburger' and 'Laurentian' were compared by RNA sequencing (RNA-seq) at multiple time-points during secondary infection, following inoculation with pathotype 3A of *P. brassicae*. Both the resistant ('Wilhemsburger') and susceptible ('Laurentian') cultivars activated *RLP* genes, *R* genes and genes involved in SA synthesis and signaling in response to the pathogen. The resistant host, however, also appeared to coordinate the activity of genes involved in various additional pathways, including ethylene (ET) signaling. Several key genes were identified that may serve as good candidates for future clubroot resistance breeding studies, including functional validation and increased resistance through gene editing.

3.2 Materials and methods

3.2.1 Pathogen material

Plasmodiophora brassicae field isolate F3-14, originally collected from the CR canola 'L135C' and classified as pathotype 3A on the Canadian Clubroot Differential set (Strelkov et al., 2018), was used as the inoculum for this study. The isolate was stored as frozen (-20 °C) root galls and resting spore suspensions were prepared following Strelkov et al. (2006). Briefly, 100 g of the root galls were homogenized in 1 L distilled water (dH_2O) in a blender for 2 min, with the resulting homogenate filtered through eight layers of cheesecloth to remove any debris. The spore concentration was estimated with a haemocytometer and adjusted to about 1×10^7 spores/mL with dH_2O.

3.2.2 Plant material and inoculation

All experiments were conducted with the rutabagas 'Wilhelmsburger' and 'Laurentian', which are resistant and susceptible, respectively, to pathotype 3A of *P. brassicae* (Strelkov et al., 2018). The universally susceptible Chinese cabbage (*Brassica rapa* L. var. *pekinensis*) 'Granaat' was also included as a check in all inoculations, to ensure that the inoculum was viable and conditions were favorable for clubroot development. Eight-day-old seedlings, germinated in Petri dishes on moistened filter paper, were inoculated by the root dip method following Strelkov et al. (2006). The seedlings were briefly (10 s) dipped in the resting spore suspension and planted in pots (6 cm × 6 cm × 6 cm) filled with water-saturated Sunshine LA4 potting mix (SunGro Horticulture, Vancouver, BC, Canada). An additional 1 mL of inoculum was added to the base of each seedling with a micropipette to ensure strong disease pressure. Non-inoculated control plants were transferred directly from the Petri dishes to the potting

mix. Plants were placed in insect cages (47.5 cm × 47.5 cm × 93.0 cm) to avoid potential insect infestations that could interfere with plant responses, and the experiment was conducted in a greenhouse under long day conditions (16 h) at 22°C. Roots were harvested at 7, 14 and 21 days after inoculation (dai), washed with tap water and briefly dried on paper towels before being collected in Falcon tubes (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and flash-frozen in liquid nitrogen. Five independent biological replicates were assigned for each treatment, with 27 pooled plants in each biological replicate. Clubroot symptom severity was evaluated at 45 dai on a 0-3 scale following Kuginuki et al. (1999), where: 0 = no visible galls, 1 = a few small galls, 2 = moderate galling, 3 = severe galling. Then, the severity rating results were used to calculate a disease index (DI) using the formula of Horiuchi & Hori (1980) as modified by Strelkov et al. (2006): DI (%) = $[(n_1 \times 1 + n_2 \times 2 + n_3 \times 3)/(N \times 3)] \times 100$, where n_1, n_2 and n_3 refer to the number of plants in each symptom severity class and N refers to the total number of plants tested.

3.2.3 RNA extraction

RNA was extracted from whole-root tissues of each host genotype at each time-point. Pooled tissues of all 27 plants of each biological replicate were ground to a fine powder in a mortar with a pestle in the presence of liquid nitrogen. The RNA was extracted from the 0.1 mL tissue homogenates using 1 mL Trizol (Ambion-Life Technologies, Carlsbad, CA, U.S.A.), 0.2 mL chloroform (Fisher Chemical, Fair Lawn, NJ, U.S.A.), and precipitated with 0.5 mL 2-propanol (Fisher Chemical, Fair Lawn, NJ, U.S.A.), followed by a cleanup step using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The RNA was treated with DNAse (Qiagen,

Hilden, Germany) for 15 min at room temperature to remove any DNA contamination, and the quantity, purity and quality of the RNA were assessed with a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and Agilent 2200 TapeStation system (Agilent, Santa Clara, CA, U.S.A.).

3.2.4 RNA-seq analysis

Three RNA samples (biological replicates) per treatment with RNA Integrity Numbers (RIN) ≥ 8.0 were sent to Oklahoma State Genomics for library preparation and sequencing. Library preparation was performed using the KAPA mRNA HyperPrep Kit (KAPA Biosystems, Wilmington, MA, U.S.A.) following the manufacturer's instructions. Products were sequenced using a NextSeq 500 system (Illumina, San Diego, CA, U.S.A.) to generate 75-bp single-end reads. Reads were filtered with Trimmomatic (Bolger et al., 2014) to remove low quality reads (phred score < 33), adapters, leading/trailing low quality or unknown bases, and reads shorter than 36 bases. The quality of the filtered reads was checked using fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and multiqc (Ewels et al., 2016) prior to further analysis. The sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA641167.

Filtered reads from each fastq file were aligned to the *B. napus* reference genome (AST_PRJEB5043_v1) (Chalhoub et al., 2014) using Tophat v. 2.11 (Trapnell et al., 2012). Files of the mapped reads and reference genome were used as input for Cufflinks v. 2.2.1 (Trapnell et al., 2012), to detect differentially expressed transcripts between inoculated and non-inoculated samples. The Cufflinks analysis was performed with the

option of GTF-guide and --frag-bias-correct using the downloaded reference genome structural annotation; the multi-read-correct option was also used to weigh read mapping to various genomic locations more accurately. The resulting assembly files from all treatments and biological replicates were merged with Cuffmerge. The number of transcripts per sample was quantified using Cuffquant with the merged consensus transcripts file as a reference. Finally, differentially expressed transcript levels between inoculated and non-inoculated plants at each of the three time-points were detected with Cuffdiff. Expression levels were measured and normalized as reads per kb of transcript per million mapped reads (RPKM). Changes in expression with an absolute $\log_2$ fold-change ($\log_2$FC) > 1 and false discovery rate (q-value, controlled by Benjamini-Hockberg) < 0.05 were considered significant. When calculating $\log_2$FC, a pseudo-count of RPKM (0.5) was added to each value to decrease the noise from genes with zero or very low expression.

3.2.5 Validation of RNA-seq data by quantitative Real-Time PCR (qRT-PCR)

To validate differential gene expression identified via RNA-seq, qRT-PCR analysis was performed on 10 genes across all treatments and samples (Table 3.1). These selected genes showed significant expression changes in RNA-seq in at least four of six comparison sets of inoculated vs. non-inoculated samples. Four biological replicates per treatment and time-point were used for cDNA synthesis. Oligo dT (18) (Thermo Fisher Scientific, Waltham, MA, U.S.A)-primed cDNA was synthesized from 500 ng of total RNA using the RevertAid H Minus Reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, U.S.A.) according to the manufacturer's protocol. The absence of genomic DNA contamination was confirmed by end-point PCR, using a 20 µL reaction

with 2.5 ng cDNA and 0.2 µM for each forward and reverse primer of a clathrin adaptor complex (*CAC)* gene (Table 3.1). PCR analysis was performed with an initial denaturation step of 3 min at 95 °C followed by 35 cycles of 30 sec at 95 °C, 30 sec at 60 °C, and 1 min at 72 °C, ending with an extension of 10 min at 72 °C. Amplified products were subjected to agarose gel electrophoresis, which resulted in two distinct bands of 125 bp (for cDNA) and 288 bp (for the control genomic DNA).

Quantitative real-time PCR was performed on a ViiA 7 Real-Time PCR System (Applied Biosystems-Life Technologies, Singapore). Each reaction consisted of 5 µL of in-house SYBR-green, 2.5 µL of cDNA (0.25 ng/µL) and 2.5 µL of paired primers (3.2 µM). Reaction conditions included a denaturation step at 95 °C for 5 min followed by 40 cycles at 95 °C for 30 sec and 60 °C for 1 min; melting curves were generated using a cycle of 15 sec at 95 °C, 1 min at 60 °C and 15 sec at 95 °C. All qRT-PCR assays were conducted with four biological replicates and three technical replicates per biological replicate.

Fold changes between *P. brassicae*-inoculated samples and non-inoculated samples were calculated using the $2^{(-\Delta\Delta Ct)}$ method (Livak and Schmittgen, 2001). To select suitable housekeeping genes for normalization, primers from six previously published housekeeping genes were tested (Chandna et al., 2012; Yang et al., 2014; Han et al., 2017): *CAC*, guanosine nucleotide diphosphate dissociation inhibitor 1 (*GDI1*), ubiquitin conjugating enzyme 9 (*UBC9*), ubiquitin conjugating enzyme (*UBC11*), tubulin alpha-5 (*TUA5*) and vacuolar ATP synthase subunit E1 (*VHA-E1*).The stability of the genes across all samples was determined with Bestkeeper (Pfaffl et al., 2004). The most stable housekeeping genes were *GDI1*, *UBC9* and *TUA5*. Relative expression of the

target genes was quantified using the geometric mean of the Ct values of the three selected housekeeping genes (Table 3.1). To compare results obtained from RNA-seq and qRT-PCR analysis, Pearson correlations of log_2FC values were obtained from the two methods for each combination of treatment and time-point.

3.2.6 Bioinformatic analyses

Transcripts were annotated using BLASTX (E value $\leq 1e^{-10}$) against the *B. napus* (Chalhoub et al., 2014) and Arabidopsis (*Arabidopsis thaliana*) (TAIR10) (Lamesch et al., 2012) databases. Venn diagrams of differentially expressed genes (DEGs) were generated using the online tool jvenn (http://jvenn.toulouse.inra.fr/app/example.html) (Bardou et al., 2014). Principal component analysis (PCA) of all samples was performed with the 'ggplot2' package in R.

Differentially expressed genes were analysed using Agrigo 2 (http://systemsbiology.cau.edu.cn/agriGOv2/) (Tian et al., 2017) to obtain enriched functional categories per time-point and cultivar. Arabidopsis gene IDs matching DEGs of each cultivar at each time-point were used as the input gene list, and Arabidopsis gene IDs matching all identified genes were used as the background reference for the 'Plant GO slim' analysis. Biological processes with adjusted *p*-values (Fisher, adjust by Yekutieli) < 0.01 were considered as significantly enriched.

MAPMAN (Thimm et al., 2004) was used to display gene sets onto diagrams of metabolic pathways or other relevant processes. The *B. napus* gene IDs matching differentially expressed transcripts in each cultivar at each time-point were used as the input gene list and the gene IDs from the reference genome were used as the background

reference. The distribution of genes showing opposite regulation patterns between the two cultivars was displayed using FunRich (Pathan et al., 2015).

3.3 Results and discussion

3.3.1 Disease assessment

Clubroot development in both host cultivars following *P. brassicae* inoculation was evaluated based on the severity of root galling. Noticeable galls appeared 14 dai in 'Laurentian', but were not visible in 'Wilhelmsburger' until 21 dai (Figure 3.1). At 45 dai, the DI on 'Laurentian' was 99%, indicating complete susceptibility, while on 'Wilhelmsburger' the DI was 48%. This suggested that disease development progressed more slowly, and was not as severe, in 'Wilhelmsburger'. These results are consistent with the previously reported reactions of these hosts to *P. brassicae* pathotype 3A (Strelkov et al., 2018). As expected, the susceptible test with Chinese cabbage 'Granaat', developed severe clubroot (DI = 100% at 45 dai).

3.3.2 RNA-seq analysis

RNA sequencing was used to assess transcriptional changes between control and inoculated plants at 7, 14 and 21 dai. On average, 38 million single-end reads were generated from 36 cDNA libraries. From these reads, 82.14%~90.76% were aligned to the reference genome of *B. napus*. Principal component analysis showed consistency among replicates and good separation between inoculated and non-inoculated samples (Figure 3.2).

In total, 110,069 transcripts were identified across samples, which were annotated based on similarity to *B. napus* and Arabidopsis genes. Among these transcripts, 20,466

transcripts showed significant expression changes in at least one of six comparison sets of inoculated vs. non-inoculated samples (Table 3.2, Dataverse file – Transcripts with significant expression changes.xlsx - https://doi.org/10.7939/DVN/N4ZP5Y). Thousands of transcripts were significantly differentially expressed at each time-point. At 7 dai, when no disease symptoms were visible yet in either host, more genes were significantly regulated in 'Wilhelmsburger' (3839) than 'Laurentian' (2863). At that same time-point, more upregulated genes than downregulated genes were identified in both hosts (Figure 3.3). Similarly, when challenging two *B. napus* hosts with *P. brassicae* 5X, more genes were significantly regulated in the resistant host than in the susceptible host at 7 dai (Galindo-González et al., 2020). At 14 dai, although less genes were significantly regulated in 'Wilhelmsburger' (2136) than 'Laurentian' (5717), the patterns of regulation were different in the two hosts. Around two-thirds of the genes were upregulated in the former, but more than half were downregulated in the latter (Figure 3.3). At 21 dai, more significantly regulated genes were identified in 'Laurentian' (14519) than 'Wilhelmsburger' (7388), with more downregulated genes than upregulated genes in both hosts (Figure 3.3). A similar trend was reported by Galindo-González et al. (2020) in susceptible and resistant hosts at 21 dai.

3.3.3 Validation of RNA-seq data by qRT-PCR

The expression of 10 target genes from each cultivar at each time-point (*P. brassicae* inoculated samples vs. non-inoculated samples) was evaluated by qRT-PCR analysis to validate the RNA-seq results. The resulting $\log_2$FC from RNA-seq and qRT-PCR indicated a high correlation among the selected genes (Figure 3.4).

3.3.4 Enrichment analysis of DEGs

To understand general changes in the molecular mechanisms associated with the host responses to *P. brassicae*, DEGs were subjected to enrichment analysis with Agrigo2 (http://systemsbiology.cau.edu.cn/agriGOv2/) (Tian et al., 2017). We identified enriched biological processes for upregulated and downregulated genes in each host at each time-point. Generally, more enriched categories were identified from upregulated genes in 'Laurentian' than in 'Wilhelmsburger', especially at 14 and 21 dai. Moreover, 10 categories showed an earlier induction in 'Laurentian' (14 dai) compared with 'Wilhelmsburger' (21 dai), including "biological regulation", "photosynthesis", "regulation of biological/cellular process" and several metabolic-related processes (Figure 3.5). This is consistent with previous studies suggesting the induction of photosynthesis as a mechanism to provide nutrients that are transported to the root galls (Siemens et al., 2006, 2011; Ludwig-Müller et al., 2009). Similarly, at 21 dai, 17 categories were enriched only in 'Laurentian', including cell cycle and multiple reproductive and metabolic-related processes. "Secondary metabolic process" was enriched for downregulated genes in 'Laurentian' at 14 dai and showed the highest significance among all enriched categories. In clubroot susceptible *B. oleracea*, *B. rapa* and *B. napus*, genes and proteins involved in the synthesis of some secondary metabolites (e.g., aliphatic glucosinolates and lignin) were downregulated during clubroot development (Cao et al., 2008; Jia et al., 2017; Ciaghi et al., 2019). The functional categories "response to stress", "response to stimulus" and stimulus-related categories ("response to abiotic/biotic/endogenous/external/extracellular stimulus") were enriched by DEGs in both hosts throughout the time-course and showed higher significance than

most of the other functional categories (Figure 3.5), confirming a general stress-activated response to *P. brassicae* inoculation. The increasing number of downregulated, stress-related genes over time in 'Laurentian' seemed to be associated with disease development following inoculation with the compatible pathotype 3A. This is in contrast to the sustained immune response observed over the entire time-course when this same cultivar was challenged with the incompatible pathotype 5X (Galindo-González et al., 2020).

3.3.5 Overview of biotic stress-related pathways

Regulation of various biotic stress-associated responses is important for the host response to *P. brassicae* infection (Song et al., 2016; Mei et al., 2019; Summanwar et al., 2019). Our results from Agrigo2 showed that "response to biotic stimulus" was enriched significantly over time in both 'Laurentian' and 'Wilhelmsburger' (Figure 3.5). Therefore, we further investigated DEGs related to this functional category using MapMan (Thimm et al., 2004), to visualize the regulation of genes in major pathways and processes related to biotic stress (Figures 3.6 to 3.8).

At 7 dai, more DEGs assigned to biotic stress were identified in 'Wilhelmsburger' than in 'Laurentian' (Figure 3.6). Among the categories designated in MapMan for biotic stress, most *WRKY* TFs were upregulated in both hosts. In 'Wilhelmsburger', most of the DEGs in the ET category were upregulated, while these were mostly downregulated in 'Laurentian'. Although less jasmonic acid (JA)-related DEGs were identified in 'Laurentian' than in 'Wilhelmsburger', all DEGs in 'Laurentian' were upregulated, while most genes in 'Wilhelmsburger' were downregulated. At 14 dai, although fewer DEGs in 'Wilhelmsburger' than in 'Laurentian' were assigned to biotic stress, most were

upregulated (Figure 3.7). The largest number of DEGs assigned to various biotic stress related categories were identified in both cultivars at 21 dai; most of these genes were downregulated, with few evident differences between cultivars (Figure 3.8). Collectively, the results suggest that the DEGs related to biotic stress identified at 7 and 14 dai show a clearer distinction related to clubroot resistance, than genes regulated at 21 dai. Therefore, we further analyzed DEGs involved in some major categories related to biotic stress and concentrated on differences at 7 and 14 dai (Figure 3.9).

3.3.6 Genes related to SA, ET and JA metabolism

Salicylic acid, JA and ET are important pathogen-responsive plant hormones. In general, a dichotomy has been established for SA vs. JA/ET in response to biotrophic and necrotrophic pathogens, respectively (Berens et al., 2017). This dichotomy is not always clear cut, however, and JA- or ET-mediated resistance to some biotrophic pathogens, including *P. brassicae*, has been found (Jubault et al., 2013; Guerreiro et al., 2016; Fu et al., 2019b; Wang et al., 2020). In Arabidopsis, genes involved in both the SA and ET pathways were upregulated at 7 dai during a partially resistant response to *P. brassicae*, while genes involved in the JA pathways were downregulated (Jubault et al., 2013). In *B. rapa*, the clubroot resistance reaction involved activation of JA, ET and SA signaling pathways (Fu et al., 2019b).

The activation of SA-mediated pathways have been reported widely in resistant reactions following *P. brassicae* inoculation (Lemarié et al., 2015; Jia et al., 2017; Fu et al., 2019a; Galindo-González et al., 2020). Isochorismate synthase 1 (*ICS1*) and *ICS2* are two genes redundantly involved in SA synthesis (Garcion et al., 2008). In our study, two

transcripts corresponding to *ICS1* (BnaA07g22090D and BnaC06g22820D) were upregulated in both 'Wilhelmsburger' and 'Laurentian' across the three time-points; one transcript corresponding to gene *ICS2* (BnaC08g18420D) was upregulated in both hosts at 7 and 14 dai (Table 3.2). The marker gene for SA-mediated responses, pathogenesis-related gene 1 (*PR1*, BnaC03g45470D), showed high upregulation in both 'Laurentian' and 'Wilhelmsburger' over time, with the exception of no significant regulation in 'Laurentian' at 7 dai ($\log_2$ FC = 2.6, q value > 0.05) (Table 3.2). Upregulation of these genes confirmed the involvement of SA-triggered immunity in both hosts. The same *ICS2* and *PR1* genes were also upregulated in 'Laurentian' at 7, 14 and 21 dai, when it was challenged with *P. brassicae* pathotype 5X (Galindo-González et al., 2020). In both hosts, most genes related to SA metabolism corresponded to downregulated transcripts belonging to the SABATH methyltransferase gene family (Figure 3.9). Members of this family are important for the methylation of phytohormones (Qu et al., 2010), which can inactivate SA by converting it to methyl salicylate (Dempsey et al., 2011). At 7 dai, three of four transcripts belonging to the SABATH methyltransferase gene family were downregulated in 'Laurentian', and seven of nine transcripts of the same family were downregulated in 'Wilhelmsburger', including one that was upregulated in 'Laurentian' (*BSMT1*, BnaA03g31730D) (Figure 3.9; Table 3.3, Dataverse file – Expression changes and annotations of transcripts matched to Figure 3.9.xlsx - https://doi.org/10.7939/DVN/N4ZP5Y). The clubroot pathogen can manipulate host SA levels to weaken host defenses, by secreting methyltransferase PbBSMT, which leads to strong conversion of SA to methyl salicylate at infection sites; overexpression of *BSMT1* in Arabidopsis reduced SA levels by half, although this manipulation alone did not alter

susceptibility to *P. brassicae* (Djavaheri et al., 2019). Our results suggest stronger repression of SA methylation in 'Wilhelmsburger' than in 'Laurentian' at 7 dai, but an SA-mediated response is likely involved in both cultivars.

Ethylene-mediated responses are part of clubroot defense mechanisms in plants with various backgrounds. For example, genes related to signaling and ET metabolism were upregulated in resistant plants carrying the CR gene *Rcr1* relative to susceptible plants that lacked this gene (Chu et al., 2014). Similarly, in a Chinese cabbage inbred line carrying a CR gene *CRd*, ET signaling-related genes were upregulated when challenged with an avirulent pathotype of *P. brassicae*, but were not regulated when challenged with a virulent pathotype (Fu et al., 2019b). Several Arabidopsis mutants of genes within the ET signaling pathway showed increased susceptibility to *P. brassicae* infection (Knaust and Ludwig-Müller, 2013). In our study, regulation of genes involved in the ET category showed the greatest differences between the two cultivars at 7 dai, as 33 of 38 transcripts in 'Wilhelmsburger' were upregulated and 23 of 30 significantly regulated transcripts in 'Laurentian' were downregulated (Figure 3.9; Table 3.3). Ethylene response factors (*ERFs*) are important in activating other defense genes in response to *P. brassicae* (Knaust and Ludwig-Müller, 2013). In our study, 'Wilhelmsburger' had more upregulated transcripts annotated as *ERFs* (11) than 'Laurentian' (two) at 7 dai, a trend that was also observed at 14 dai (Figure 3.9). For example, at 7 dai, three transcripts corresponding to the ethylene response factor 104 (*ERF104*) were upregulated in 'Wilhelmsburger', two of which were downregulated in 'Laurentian' (BnaC07g31350D and BnaA03g40380D). *ERF104* is activated by MAP kinase 6 (*MPK6*) upon perception of bacterial flagellin peptide flg22 in Arabidopsis, which alters plant susceptibility to

Pseudomonas syringae (Bethke et al., 2009). A transcript matching the gene *MPK6* (BnaC03g24500D) was activated only in 'Wilhelmsburger' but not in 'Laurentian' at 7 dai (Table 3.2), suggesting that upregulation of *ERF104* and *MPK6* is involved in clubroot resistance. In addition, three transcripts matching *ERF11* were upregulated in 'Wilhelmsburger' and not in 'Laurentian' at 7 and/or 14 dai (Table 3.3). The activation of *ERF11* in apple enhanced SA accumulation and increased resistance to the biotrophic fungus *Botryosphaeria dothidea* (Wang et al., 2020), suggesting possible cross-talk between ET and SA in some biotrophic interactions

Jasmonic acid-related genes also showed the greatest differences in expression at 7 dai, when 22 of 31 DEGs in 'Wilhelmsburger' were downregulated and all nine DEGs in 'Laurentian' were upregulated (Figure 3.9). Six transcripts involved in JA biosynthesis, including 12-oxophytodienoate reductase 1 (*OPR1*, BnaC09g41020D and BnaA10g17650D), allene oxide cyclase 2 (*AOC2*, BnaA06g33410D, BnaC09g52570D and BnaA09g19550D) and allene oxide synthase (*AOS*, BnaA02g23180D), were downregulated in 'Wilhelmsburger' and upregulated in 'Laurentian' (Table 3.3). This contrasting pattern of expression is consistent with the regulation of JA biosynthesis genes in CR and CS responses, which was reported in other studies at early stages of infection (Jubault et al., 2013; Su et al., 2018). At 14 and 21 dai, genes in the JA category showed general downregulation in both hosts (Figure 3.9). Collectively, our results indicated that JA does not seem central to defense in the resistant cultivar, while it may be a mechanism that is activated in this susceptible interaction.

3.3.7 Pathogenesis-related genes

The most notable differences in regulation of *PR* genes between 'Wilhelmsburger' and 'Laurentian' were detected at 14 dai. At this time-point, while less *PR* genes were identified in the resistant vs. the susceptible host, most were upregulated in the former (46 of 47), while 45 of 98 genes were downregulated in the latter (Figure 3.7).

Transcripts encoding RLP and TIR-NBS-LRR proteins showed general upregulation in both hosts at 14 dai (Figure 3.9). All 19 genes encoding RLPs were upregulated in 'Wilhelmsburger', while 20 of 21 were upregulated in 'Laurentian'. Receptor-like proteins are key components of PRRs, which recognize PAMPs or endogenous DAMPs to activate PTI-mediated responses (Zipfel, 2014). For example, the protein RLP30 is required for perception of a fungal PAMP known as sclerotinia culture filtrate elicitor 1 (SCFE1) (Zhang et al., 2013). Another protein RLP23 binds to a conserved 20 amino acid fragment from necrosis and ethylene-inducing peptide 1-like proteins (NLPs) produced by multiple bacterial, oomycete and fungal microbes, and mediates plant resistance to diverse pathogens such as *Phytophthora infestans* and *Sclerotinia sclerotorium* (Albert et al., 2015). In our study, transcripts encoding RLP23 and RLP30 were upregulated in both hosts (Table 3.3), indicating their potential role in basal resistance to clubroot. In addition, 10 upregulated transcripts corresponding to TIR-NBS-LRR proteins were identified in 'Wilhelmsburger' and nine were identified in 'Laurentian'. Only four of these, however, were found to be upregulated in common between the two hosts (Figure 3.9, Table 3.3). TIR-NBS-LRR genes are *R* genes linked to ETI responses (Dodds and Rathjen, 2010) and are important for clubroot resistance,

representing one of the main sources of candidate CR genes (Huang et al., 2017; Pang et al., 2018). We identified a gene BnaA03g29300D which is the homolog of an important candidate gene of *CRd* (Bra001175) in *B. rapa* (Pang et al., 2018). This gene was upregulated in 'Wilhelmsburger' and 'Laurentian' at 14 dai and 21 dai, respectively. Another gene BnaAnng17440D, which was upregulated in 'Wilhelmsburger' but not regulated in 'Laurentian' at 7 dai, was similar to another candidate gene of *CRd* (Bra001160) according to sequence alignment (Pang et al., 2018). In addition, the resistance associated with *CRd* involved the activation of both SA and ET signaling pathways (Fu et al., 2019b), consistent with the upregulation of genes related to these hormones in this study.

In addition, *PR* genes involved in SA-mediated defense also showed differential regulation between the two hosts at 7 dai, including *PR1* which was only upregulated in 'Wilhelmsburger' and nonexpresser of PR genes 1 (*NPR1*)-like protein 3 (*NPR3*), which were only upregulated in 'Laurentian' (Table 3.2). The upregulation of *PR1* in clubroot resistance responses has been reported widely (Chen et al., 2016a; Luo et al., 2018; Fu et al., 2019b). *PR1* is a marker gene for SA-mediated resistance, which is positively regulated by TGACG motif-binding protein (*TGA*) and *NPR1* genes (Vlot et al., 2009). In contrast, *NPR3* is a co-repressor of SA-induced defense gene expression; it interacts with *TGAs* to inhibit expression of defense-related genes under low SA levels, while its repression is inhibited when SA is high (Ding et al., 2018). In addition, *NPR3* may activate JA synthesis by promoting the degradation of a group of JA transcriptional repressor jasmonate-zim-domain proteins (JAZs) (Liu et al., 2016). Therefore, the

upregulation of *NPR3* in 'Laurentian' may be associated with upregulation of multiple JA synthesis genes in this host.

Expression of transcripts belonging to the dirigent-like protein family was most divergent between 'Laurentian' and 'Wilhelmsburger' at 14 dai. Twenty-seven of these transcripts were downregulated in 'Laurentian' at this time, while two were upregulated in 'Wilhelmsburger' (Figure 3.9). Genes belonging to this family are thought to participate in biotic and abiotic defense by increasing lignan and lignin synthesis (Paniagua et al., 2017). Lignin synthesis positively regulates clubroot resistance (Lahlali et al., 2017; Ciaghi et al., 2019). Our results showed that, at 14 dai, more genes involved in lignin biosynthesis were downregulated in 'Laurentian' (23 of 26 genes) than in 'Wilhelmsburger' (four of eight genes) (Figure 3.10). At this time-point, two transcripts matching genes encoding dirigent protein 6 (*DIR6*, BnaAnng27090D and BnaC01g15510D) were downregulated in 'Laurentian', but were not regulated in 'Wilhelmsburger'. These genes contain the TIR-NBS-LRR domain and their sequences showed high similarity to Bo7g109000 in *B. oleracea*, a gene that is located in the target region of a major clubroot resistance gene *Rcr7* (Dakouri et al., 2018). This indicates that greater downregulation of genes in the dirigent-like protein family in 'Laurentian' may be associated with more rapid galling of the roots.

3.3.8 Signaling

Signaling networks are important for the activation of plant defenses against clubroot (Mei et al., 2019). As with the *PR* genes, genes involved in signaling pathways showed notable differences in expression at 14 dai, with a greater proportion of these

genes upregulated in ‘Wilhelmsburger’ vs. ‘Laurentian’ (Figure 3.7). This was especially evident for calcium regulated genes and LRR receptor kinases (Figure 3.9). A transcript encoding the LRR receptor kinase pep 1 receptor 2 (PEPR2, BnaC05g49970D) was upregulated in ‘Wilhelmsburger’ at 7 dai but downregulated in ‘Laurentian’ at 7 and 14 dai (Table 3.3). The protein PEPR2 perceives Arabidopsis DAMP Pep1/2 peptide, and cooperates with ET to amplify resistance to *Botrytis cinerea* (Liu et al., 2013; Zipfel, 2013). In addition, three transcripts encoding a protein suppressor of BIR1–1 (*SOBIR1*) were upregulated in ‘Wilhelmsburger’ at 14 dai, of which only one was upregulated in ‘Laurentian’. The protein SOBIR1 interacts with various RLPs, such as RLP23 and RLP30 (discussed above), to enhance plant immunity upon fungal pathogen challenge (Liebrand et al., 2013; Zhang et al., 2013; Albert et al., 2015).

In the calcium signaling subcategory, most upregulated transcripts in ‘Wilhelmsburger’ at 14 dai encoded calcium binding proteins (CBPs; all eight transcripts upregulated) and calmodulin-binding proteins (CaMBPs; all 17 transcripts upregulated). At the same time-point, a large portion of *CBP* transcripts (14 of 26) were downregulated in ‘Laurentian’, and only seven transcripts encoding CaMBPs were upregulated in this cultivar (Figure 3.9). This is consistent with previous transcriptomics studies suggesting a Ca^{2+} influx in the clubroot resistance response (Chen et al., 2016a; Luo et al., 2018). Several members of the CaMBP family are important in plant defense. For example, CaMBP 60-like G (*CBP60g*) and the closely related SAR deficient 1 (*SARD1*) gene are involved in SA biosynthesis and pathogen defense responses (Dempsey et al., 2011). These two genes showed upregulation in the resistant interaction at 14 dai when challenged with *P. brassicae* pathotype 5X (Galindo-González et al., 2020). Our results

showed that at 14 dai, all three transcripts matching *CBP60g* were upregulated only in 'Wilhelmsburger'. At that same time, five transcripts annotated as *SARD1* also were upregulated in 'Wilhelmsburger', of which three were upregulated in 'Laurentian' (Table 3.3). Ca^{2+} also activates burst oxidase homolog (RBOH) proteins which are key factors in enhancing production of ROS during the plant immunity response (Stael et al., 2015). This type of response has been well studied in clubroot interactions (Chen et al., 2016a; Zhang et al., 2016; Jia et al., 2017; Su et al., 2018; Mei et al., 2019; Ning et al., 2019). In our analysis, three transcripts annotated as *RBOHs* (*RBOHA*, *RBOHC* and *RBOHG*) were upregulated in 'Wilhelmsburger' but not in 'Laurentian' at 14 dai (Table 3.2). The homologs of the same three genes were upregulated in clubroot resistant wild cabbage (*B. macrocarpa*) following *P. brassicae* infection (Zhang et al., 2016). Collectively, our results support calcium-dependent activation of defense responses against clubroot.

3.3.9 Transcription factors

Transcription factors play important roles in modulating the host immune responses (Birkenbihl et al., 2017). The activation of *WRKY* TFs in plants in response to *P. brassicae* has been reported widely (Jubault et al., 2013; Chen et al., 2016a; Jia et al., 2017; Luo et al., 2018; Fu et al., 2019b; Ning et al., 2019). In the present study, most *WRKY* TFs were upregulated in both hosts over the entire time-course (Figures 3.6 to 3.8). Some transcripts were upregulated in 'Wilhelmsburger' but not regulated in 'Laurentian' at 7 or 14 dai, including *WRKY22*, *WRKY29*, *WRKY33,* and *WRKY46* at 7 dai, and *WRKY46* and *WRKY53* at 14 dai. *WRKY46*, *WRKY53* and *WRKY70* are involved in the SA-signaling pathway and play overlapping and synergistic roles in plant resistance to *P. syringae* (Hu et al., 2012). In our study, transcripts corresponding to at

least five genes of *WRKY70* were upregulated in both hosts at all three time-points, except in 'Laurentian' at 7 dai (three genes). These results suggest that the activation of *WRKY46*, *WRKY53* and *WRKY70* is associated with SA-mediated defense responses to clubroot, and that regulation of WRKY46 and WRKY70 at 7 dai may be related to enhanced SA-mediated responses in 'Wilhelmsburger'. *WRKY22* and *WRKY29* are activated in PTI and regulate resistance to *P. syringae* and *B. cinerea* (Asai et al., 2002). While *WRKY33* typically has been associated with resistance to necrotrophic fungal pathogens (Zheng et al., 2006), its upregulation in response to *P. brassicae* has also been reported and is believed to be modulated by *MPK6* (Jia et al., 2017; Luo et al., 2018; Ning et al., 2019). In our study, a transcript matching *MPK6* and another matching the ET synthesis gene 1-amino-cyclopropane-1-carboxylate synthase 2 (*ACS2*), which is activated by *MPK6-WKRY33* (Li et al., 2012), exhibited an expression pattern similar to *WRKY33* in both 'Wilhelmsburger' and 'Laurentian' at 7 dai (Table 3.3). This suggests that *MPK6-WKRY33* may contribute to ET synthesis to enhance clubroot resistance. In addition to *WRKY33*, *WRKY22*, *WRKY29* and *WRKY46* are also activated by *MPK6* following pathogen challenge (Asai et al., 2002; Li et al., 2012; Sheikh et al., 2016). For example, in cabbage showing resistance to *P. brassicae,* the activation of *MEKK1-MKK4*/*MKK5-MPK3*/MPK6 resulted in upregulation of *WRKY22*/*WRKY29*/*WRKY33* (Ning et al., 2019). Collectively, these results indicate a central role of *WRKYs* in regulatory defense responses to clubroot.

Members of the basic leucine zipper (*bZIP*) TF family are important regulators of many key developmental and physiological processes, including biotic stress responses (Alves et al., 2013). The upregulation of some *bZIPs* have been associated with CR

responses (Jubault et al., 2013; Jia et al., 2017). *TGAs*, a type of *bZIP* TF, are important for activating SA-regulated genes such as *PR1* (Vlot et al., 2009). In our study, 'Wilhelmsburger' showed a higher proportion of upregulated *bZIP* TFs than 'Laurentian' at 7 dai (Figure 3.9). Three transcripts annotated as *TGA10* and five encoding *TGA1* were upregulated only in 'Wilhelmsburger' at 7 dai (Table 3.3), which may be associated with the upregulation of *PR1* observed in this host.

3.3.10 Protein degradation

Proteolysis-related genes also showed distinct differences in expression between 'Laurentian' and 'Wilhelmsburger'. While numerous genes involved in protein degradation were regulated in both hosts, proportionally more genes were upregulated in 'Wilhelmsburger' and downregulated in 'Laurentian' at 7 and 14 dai (Figures 3.6 and 3.7). At 7 dai, five transcripts encoding E2 ubiquitin-conjugating enzymes (E2) and 57 transcripts encoding E3 ubiquitin ligase (E3) RING proteins were upregulated only in 'Wilhelmsburger' (Figure 3.9), of which two WAV3 homolog 1 (*WAVH1*) genes (BnaC04g35190D and BnaA04g13100D) and two BCA2Â zinc finger ATL 10 (*BTL10*) genes (BnaA06g17960D and BnaCnng37520D) were downregulated in 'Laurentian' (Table 3.3). At 14 dai, proportionally more transcripts encoding E2 and E3 RING proteins were upregulated in 'Wilhelmsburger' (38 of 50 transcripts) than in 'Laurentian' (49 of 82 transcripts) (Figure 3.9). The E2 ubiquitin-conjugating enzymes and E3 ubiquitin ligase proteins are key components of the ubiquitin–proteasome system. These bind to form a multimer that attaches to proteins, targeting them for degradation by 26S proteasomes. This ubiquitin–proteasome system interacts with key components of plant immunity to positively or negatively regulate resistance to plant pathogens (Mandal et al.,

2018). Genes encoding RING proteins in the E3 ubiquitin pathway were upregulated in *Rcr1*-mediated clubroot resistance and downregulated in susceptible Arabidopsis following *P. brassicae* infection (Song et al., 2016; Zhao et al., 2017). Several genes in the Arabidopsis Tóxicos en Levadura (*ATL*) family of E3 RING proteins are involved in plant defense against pathogens (Mandal et al., 2018). In our study, two transcripts matching *ATL2* and three transcripts matching *ATL31* were upregulated in 'Wilhelmsburger' at both 7 and 14 dai, of which one of each were also upregulated in 'Laurentian' at 14 dai. (Table 3.3). *ATL2* and *ATL31* are induced by pathogens or PAMPs (Guzmán, 2012). The expression of *PR1* was induced in Arabidopsis mutants constitutively expressing *ATL2* (Serrano and Guzmán, 2004). Overexpression of *ATL31* in Arabidopsis increased resistance to *P. syringae*, while knock-out of these genes decreased resistance (Maekawa et al., 2012). One of our *ATL31* genes (BnaA09g03720D) was upregulated in the resistant 'Laurentian' but downregulated in susceptible 'Brutor' (*B. napus*) when inoculated with *P. brassicae* pathotype 5X (Galindo-González et al., 2020), suggesting resistance via an increase in SA levels.

3.3.11 Analysis of genes with opposite regulation in the resistant vs. susceptible hosts

Two hundred ninety-eight, 25 and 18 transcripts showed opposite patterns of regulation in the two hosts at 7, 14 and 21 dai, respectively (Figure 3.11). These genes can be key regulators of clubroot resistance or susceptibility. Therefore, we further investigated their expression and putative functions to select good candidates for gene editing-based functional validation.

We first divided genes identified at 7 dai into two lists: genes upregulated in 'Wilhelmsburger' but downregulated in 'Laurentian' (List A, Table 3.4) and genes downregulated in 'Wilhelmsburger' but upregulated in 'Laurentian' (List B, Table 3.5). Genes on each list were then grouped based their functional categories in Mapman (Thimm et al., 2004). The majority of genes on both lists (33.6% in List A and 27.3% in List B) belonged to the "not assigned" category (i.e., did not match any Mapman classification), followed by "RNA" (23% in List A and 12.7% in List B) and "hormone metabolism" (10.7% in List A and 11.3% in List B) (Figures 3.12A and 3.12B). These results supported the importance of transcriptional regulation and hormone metabolism in the *B. napus*-*P. brassicae* interaction at 7 dai. The functional category "lipid metabolism" was identified only on List B (6.7%). Five of 10 transcripts involved in lipid metabolism were related to lipid synthesis (Table 3.5), which is consistent with the upregulation of lipid synthesis genes in *P. brassicae*-infected roots and the accumulation of lipid droplets in the parasite as a nutrient sink for *P. brassicae* survival (Bi et al., 2016; Irani et al., 2018). In addition, JA is a lipid-derived signal (Weber, 2002; Wasternack et al., 2006), consistent with the similar regulation patterns of JA and lipid synthesis related genes in this study. A transcript matching gene fatty acid desaturase 7 (*FAD7*, BnaA03g31600D) was downregulated in 'Wilhelmsburger' ($log_2FC = -1.21$) and upregulated in 'Laurentian' ($log_2FC = 1.02$). *FAD7* is involved in the synthesis of both fatty acid and JA, but it inhibits SA accumulation and signaling (Avila et al., 2012), suggesting that this gene is an important candidate of susceptible factors.

Most transcripts assigned to the "RNA" functional category matched the APETALA2 (*AP2*)/ethylene-responsive element binding protein (*EREBP*) (10 on List A

and 9 on List B) (Figure 3.12C). Members of the *ERF* subfamily of AP2 TFs are involved in the regulation of disease resistance pathways, and some *ERFs* have been shown to be regulated by plant hormones and pathogen challenge (Gutterson and Reuber, 2004). The high proportion of additional regulated *ERFs* in the AP2/EREBP gene family in 'Wilhelmsburger' and 'Laurentian' at 7 dai (Tables 3.4 and 3.5) supports the importance of *ERFs* in the host response to *P. brassicae*. In addition, two of the transcripts on List A matched *MYB15*, which is consistent with upregulation of this gene in a CR rapeseed accession but not in a CS accession upon *P. brassicae* infection (Li et al., 2020). *MYB15* contributes to resistance to *P. syringae* in Arabidopsis (Chezem et al., 2017). Moreover, a *MYB15* promoter in Chinese wild grape (*Vitis quinquangularis*) is involved in multiple defense mechanisms during PTI (Luo et al., 2019). This suggests that *MYB15* may be a good candidate for functional validation in clubroot resistance.

On List A, the transcript showing the most distinct regulation in the two hosts at 7 dai matched the gene plastidic type I signal peptidase 2A (*PLSP2A*, BnaC05g04750D); this transcript showed the greatest upregulation in 'Wilhelmsburger' ($log_2FC = 4.86$) and the greatest downregulation in 'Laurentian' ($log_2FC = -3.52$). *PLSP2A* corresponds to a thylakoidal processing peptidase usually expressed in both photosynthetic tissues and roots and is important for thylakoid membrane organization (Hsu et al., 2011). In cabbage (*B. oleracea*), a large portion of differentially modulated proteins in resistant vs. susceptible interactions with *P. brassicae* were localised to the thylakoid (Moon et al., 2020). These results suggest that *PLSP2A* is an important candidate for resistance to *P. brassicae*. Indeed, a gene involved in phosphorylation of the thylakoid membrane has been suggested to be a candidate for resistant to *Leptosphaeria maculans* in *B. napus* (Fu

et al., 2019a). On list B, the two transcripts showing the greatest downregulation in 'Wilhelmsburger' matched two copies of "cytochrome P450, family 94, subfamily C, polypeptide 1" (CYP94C1, BnaC04g16670D and BnaA07g13320D, log_2FC = ~-4), both of which were upregulated in 'Laurentian' (log_2FC = ~1.7). In addition, transcripts matching three copies of *CYP94B1* (BnaC03g50910D, BnaA06g38770D and BnaA09g06580D) were also identified on List B. Both *CYP94C1* and *CYP94B1* are involved in the catabolism of jasmonoyl-L-isoleucine (JA-Ile), a major bioactive form of JA, and their expression was induced by JA treatment (Koo and Howe, 2012). This is consistent with the regulation of JA synthesis genes in the two hosts, and suggests an interruption of the JA-mediated response in 'Laurentian' at 7 dai. Expression of *CYP94C1* also increased in early galling tissues in the Chinese sumac (*Rhus javanica*) infested by aphid (*Schlechtendalia chinensis*) (Hirano et al., 2020). Collectively, these findings suggest that *CYP94C1* and *CYP94B1* are good candidate susceptibility factors during clubroot development.

Transcripts showing opposite regulation patterns in 'Wilhelmsburger' and 'Laurentian' at 14 dai are listed in Table 3.6. At this time-point, the transcript showing the greatest upregulation in 'Wilhelmsburger' corresponded to an LRR transmembrane protein kinase (BnaC05g27810D) (log_2FC = 4.12), which was downregulated in 'Laurentian' (log_2FC = -1.25). Its orthologous gene in Arabidopsis encodes a protein localized to the plasma membrane, where a large portion of upregulated gene products were identified in clubroot resistant reaction in *B. rapa* (Chu et al., 2014). Considering the possible roles of LRR protein kinases in mediating resistance to pathogens, this gene may be another candidate of resistance. A transcript matching cytochrome p450 79f1

(*CYP79F1*) was downregulated in 'Laurentian' ($log_2FC = -2.52$) and upregulated in 'Wilhemsburger' ($log_2FC = 1.15$). An Arabidopsis mutant of *CYP79F1* had reduced aliphatic glucosinolate and increased indole glucosinolate content (Chen et al., 2003). Higher aliphatic glucosinolates and lower indole glucosinolates have been associated with clubroot resistance in previous studies (Ludwig-Müller et al., 2009; Jia et al., 2017). Transcripts matching two other key genes involved in aliphatic glucosinolate synthesis (*CYP83A1* and bile acid transporter 5 (*BAT5*)) (Hemm et al., 2003; Gigolashvili et al., 2009) showed a pattern of regulation similar to *CYP79F1*. Recently, *CYP83A1* has been screened as a candidate gene for clubroot resistance in rapeseed, by combining functional enrichment analysis, co-expression network analysis and haplotype analysis (Li et al., 2020). These results suggest that the accumulation of aliphatic glucosinolates may be important for clubroot resistance.

At 21 dai, the most upregulated transcript in 'Wilhelmsburger' and the most downregulated transcript in 'Laurentian' did not match any *B. napus* or Arabidopsis gene annotations. A transcript matching BnaA04g25230D/AT2G43610 belonging to the chitinase family protein, however, showed the second highest level of upregulation in 'Wilhelmsburger' ($log_2FC = 1.53$), contrasting with downregulation in 'Laurentian' ($log_2FC = -1.53$) (Table 3.7). Chitinases are a subgroup of PR proteins which attack pathogens directly by hydrolyzing chitin, a component of *P. brassicae* and many fungal cell walls (Bishop et al., 2000; Schwelm et al., 2015). The differential regulation of chitinase genes has been described in the defense response to *P. brassicae* (Chen et al., 2016a, 2018; Ciaghi et al., 2019).

3.3.12 A model of the resistance response in 'Wilhemsburger' to *P. brassicae*

Based on the discussion above, we propose a model of the major defense mechanisms induced by *P. brassicae* pathotype 3A in its interaction with the resistant *B. napus* 'Wilhelmsburger' (Figure 3.13). Upon infection, PRRs (e.g., *RLP23*, *RLP30*, *SOBIR1*, *PEPR2*) on the host cell surface recognize extracellular PAMPs and DAMPs, leading to PTI. In parallel, *R* proteins (e.g., *TIR-NBS-LRR*) recognize specific effectors from the pathogen, triggering ETI. The two-layer immunity of PTI and ETI have overlapping roles in the defense network, such as activating MAPKs (Peng et al., 2018b). Activated MAPKs can phosphorylate TFs to enhance their transcriptional activity (Turjanski et al., 2007). For example, regulation of *MPK6* resulted in the activation of multiple *WRKY* TFs, including *WRKY22*, *WRKY29*, *WRKY33* and *WRKY46*, in the resistant host in our study. Furthermore, some WRKY TFs may mediate resistance by regulating plant hormone metabolism. *WRKY33* activates an ET biosynthesis gene *ACS2*, while *WRKY46*, *WRKY53*, *WKRY70*, and some *bZIP* TFs (e.g., *TGA1* and *TGA10*) positively regulate SA signaling. In parallel, *ERF11* and *ERF104* are involved in ET signaling. Our results also suggest the activation of calcium-dependent defenses, triggering a cascade that helps in the activation of *RBOHs* to increase ROS levels, leading to an increase in SA levels. Infection by *P. brassicae* also may induce expression of RING-type ubiquitin ligase genes. In particular, *ATL2* and *ATL31* in turn increase clubroot resistance by enhancing SA-mediated responses. The involvement of SA-mediated responses and their antagonistic effect on JA-mediated clubroot resistance mechanisms is consistent with a recent report by Galindo-González et al. (2020). Our results suggesting a role for ET, however, contrast with the findings of Galindo-González

et al. (2020). This apparent contradiction may reflect specific pathotype by host interactions, and the evaluation of multiple pathotypes with similar hosts may help to identify common defense and susceptibility genes across the clubroot pathosystem. Ultimately, an improved understanding of *P. brassicae*/Brassica interactions will aid in the development of novel strategies for clubroot resistance breeding.

3.4 Tables

Table 3.1 Genes used for PCR and qRT-PCR

Gene alias	Gene annotation	Primer sequences (forward/reverse 5' – 3')	References
ERF6	Ethylene responsive element binding factor 6	GAGGTTGGGATGTGGGAAC/ TCTCCTCCGTCTTTACAACTTTC	Designed in our lab
ORA47	Integrase-type DNA-binding superfamily protein	TTCAACCTCCGTGTCTGAAG/ CCCTGGAAACATCCCAAAGT	Designed in our lab
SUS1	Sucrose synthase 1	AGTCAGGCTTCCACATTGAC/ AGACGGATCCTCCTTACACTTA	Designed in our lab
PMIP	Plastid movement impaired protein	AGGAATCCAGATGAGTGCAAAG/ CTCCTTGTTCTTCATTACCGAGAG	Designed in our lab
GATL10	Galacturonosyltransferase-like 10	ACGACGCGTCTTCAGATTT/ GTCGCAAGCTAACAAGAGTTTG	Designed in our lab
Cal	Calcium-binding endonuclease/exonuclease/phosphatase family	ATACTCTGCGGAGATTGGAATG/ CCGATGAGCAGTGTCGTAAG	Designed in our lab
GH3.12	Auxin-responsive GH3 family protein	ACAATACGTTCCCACCATGAA/ TTGATCCCGAACGTTGTCTC	Designed in our lab
Mee14	Maternal effect embryo arrest 14	TCTGTTACTCTTCGTCCGTTTC/ CTCAAGCTTAGGAACGTCTCTC	Designed in our lab
WRKY18	WRKY DNA-binding protein 18	CAAGATCCGGCAGACTTCTTAG/ CTGCTCATATTGTTGATGGTGATG	Designed in our lab
ERP	Ethylene-responsive nuclear protein-like protein	AGATTCAGACCGCTCCAAAC/ CTTACTCCGAACGGATTTCCTC	Designed in our lab
UBC9	Ubiquitin conjugating enzyme 9	CAACATCAACAGCAACGGAAG/ GGTCAACAATGAACAGATCGATAAC	Designed in our lab
GDI1	Guanosine nucleotide diphosphate dissociation inhibitor 1	CACTCGGTGCAGGTCATC/ CAACGTTGTGGGAATATGAACAG	modified from (Yang et al., 2014)

TUA5	Tubulin alpha-5	TTATGGGAAGAAGTCTAAGCT/ TCGATGTCTAGTGATCTACG	modified from (Chandna et al., 2012)
CAC	Clathrin adaptor complex	GCTAAATACAACCCATCAAT/ GTCCAAGATTTCTTCTCTCC	modified from (Chandna et al., 2012)

Table 3.2 Dataverse file – Transcripts with significant expression changes.xlsx - https://doi.org/10.7939/DVN/N4ZP5Y

Table 3.3 Dataverse file – Expression changes and annotations of transcripts matched to Figure 3.9.xlsx - https://doi.org/10.7939/DVN/N4ZP5Y

Table 3.4 Transcripts showing upregulation in 'Wilhelmsburger' and downregulation in 'Laurentian' at 7 dai (List A)

Transcript ID	*B. napus* ID	Arabidopsis ID (Tair 10)	Arabidopsis annotation (Tair 10)	R_7 log_2FC	S_7 log_2FC
XLOC_083125	---NA---	---NA---	---NA---	1.77	-1.89
XLOC_099473	---NA---	---NA---	---NA---	2.56	-2.48
XLOC_066498	BnaA05g16430D	AT1G32100	pinoresinol reductase 1	2.14	-1.71
XLOC_071579	BnaC08g42050D	AT1G11530	C-terminal cysteine residue is changed to a serine 1	2.08	-1.25
XLOC_056803	BnaC06g01490D	AT1G47960	cell wall / vacuolar inhibitor of fructosidase 1	1.05	-1.76
XLOC_090577	BnaCnng22160D	AT4G12390	pectin methylesterase inhibitor 1	1.05	-1.38
XLOC_045377	BnaC05g04750D	AT1G06870	Peptidase S24/S26A/S26B/S26C family protein	4.86	-3.52
XLOC_086244	BnaA05g10880D	AT2G32430	Galactosyltransferase family protein	1.08	-1.16
XLOC_065249	BnaA02g09840D	AT5G54130	Calcium-binding endonuclease/exonuclease/phosphatase family	1.57	-1.76
XLOC_079191	BnaC02g13790D	AT5G54130	Calcium-binding endonuclease/exonuclease/phosphatase family	1.72	-2.28
XLOC_015492	BnaC02g21950D	AT1G73165	CLAVATA3/ESR-RELATED 1	2.76	-1.44
XLOC_085986	BnaC02g21950D	AT1G73165	CLAVATA3/ESR-RELATED 1	3.53	-1.67
XLOC_076272	BnaA02g17260D	AT1G75500	Walls Are Thin 1	1.90	-1.20
XLOC_032146	BnaC04g41820D	AT2G31085	CLAVATA3/ESR-RELATED 6	1.73	-1.18
XLOC_060401	BnaC03g42890D	AT3G22550	Protein of unknown function (DUF581)	1.14	-1.06
XLOC_033857	BnaC01g10920D	AT4G18510	CLAVATA3/ESR-related 2	1.08	-1.54
XLOC_046030	BnaC01g14980D	AT4G23410	tetraspanin5	1.02	-1.78
XLOC_004472	BnaC09g26970D	AT5G50820	NAC domain containing protein 97	1.07	-1.27
XLOC_040706	BnaA10g05960D	AT5G50820	NAC domain containing protein 97	2.06	-1.40
XLOC_070194	BnaA06g10910D	AT1G16310	Cation efflux family protein	1.33	-1.01
XLOC_088297	BnaCnng18870D	AT1G33440	Major facilitator superfamily protein	1.26	-1.90
XLOC_046822	BnaC06g30900D	AT1G69850	nitrate transporter 1:2	1.17	-1.07
XLOC_048250	BnaA09g13380D	AT1G62280	SLAC1 homologue 1	1.41	-2.38
XLOC_021907	BnaA06g26560D	AT5G24030	SLAC1 homologue 3	1.04	-2.17
XLOC_015461	BnaC08g35570D	AT2G21560	unknown protein	1.23	-2.95
XLOC_049118	BnaA09g43070D	AT2G21560	unknown protein	1.03	-2.52
XLOC_063543	BnaA09g36180D	AT3G56880	VQ motif-containing protein	1.06	-1.22

XLOC_049739	BnaA10g06980D	AT5G53730	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.17	-1.57
XLOC_056009	BnaC09g30130D	AT5G53730	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	1.94	-1.39
XLOC_030420	BnaC02g14040D	AT5G53830	VQ motif-containing protein	1.34	-1.15
XLOC_041653	BnaA10g04540D	AT1G07090	Protein of unknown function (DUF640)	1.60	-1.32
XLOC_056875	BnaA08g24590D	AT1G12805	nucleotide binding	1.92	-2.41
XLOC_067863	BnaC08g15750D	AT1G12805	nucleotide binding	1.61	-2.18
XLOC_027865	BnaA09g23980D	AT1G33055	unknown protein	1.81	-2.39
XLOC_035803	BnaA09g23980D	AT1G33055	unknown protein	1.20	-2.04
XLOC_075965	BnaA07g28300D	AT1G69760	unknown protein	2.05	-1.35
XLOC_000951	BnaA07g23310D	AT1G71970	unknown protein	1.14	-1.09
XLOC_023592	BnaC03g45960D	AT2G15890	maternal effect embryo arrest 14	1.78	-1.54
XLOC_042648	BnaA03g17960D	AT2G38640	Eukaryotic translation initiation factor 2B (eIF-2B) family protein	1.28	-1.37
XLOC_098173	BnaC03g72170D	AT2G38640	Eukaryotic translation initiation factor 2B (eIF-2B) family protein	1.55	-1.17
XLOC_103659	BnaCnng57370D	AT3G20898	unknown protein	1.20	-1.22
XLOC_103661	BnaCnng57370D	AT3G20898	unknown protein	1.12	-1.26
XLOC_060400	BnaC03g42880D	AT3G22540	Protein of unknown function (DUF1677)	1.23	-1.57
XLOC_040960	BnaA09g38160D	AT3G59900	auxin-regulated gene involved in organ size	1.25	-1.15
XLOC_102962	BnaCnng55150D	AT3G62990	unknown protein	1.95	-1.91
XLOC_094228	BnaA09g55710D	AT3G63210	Fasciclin-like arabinogalactan family protein	1.04	-1.43
XLOC_082407	BnaC09g22280D	AT4G05070	Wound-responsive family protein	1.02	-1.18
XLOC_100109	BnaA01g35330D	AT4G17350	CONTAINS InterPro DOMAIN/s	1.02	-1.12
XLOC_038166	BnaC01g15700D	AT4G23880	unknown protein	1.08	-1.37
XLOC_028581	BnaC01g17670D	AT4G25760	glutamine dumper 2	1.29	-2.68
XLOC_031635	BnaC08g12600D	AT4G27657	unknown protein	2.19	-2.64
XLOC_031636	BnaC08g12610D	AT4G27657	unknown protein	1.18	-1.04
XLOC_049687	BnaC08g12600D	AT4G27657	unknown protein	1.96	-1.65
XLOC_066395	BnaA03g48750D	AT4G27657	unknown protein	1.06	-1.33
XLOC_005198	BnaC07g45080D	AT4G34560	unknown protein	1.83	-1.23
XLOC_024292	BnaA08g16650D	AT4G39190	unknown protein	1.47	-1.03

XLOC_003017	BnaA10g26700D	AT5G03230	Protein of unknown function, DUF584	1.52	-1.45
XLOC_002759	BnaA10g22030D	AT5G10210	CONTAINS InterPro DOMAIN/s	1.89	-1.39
XLOC_075568	BnaA10g17360D	AT5G17350	unknown protein	1.53	-1.14
XLOC_101683	BnaC02g47530D	AT5G44350	ethylene-responsive nuclear protein -related	1.58	-1.16
XLOC_087226	BnaA02g10260D	AT5G53486	unknown protein	1.63	-1.66
XLOC_037557	BnaC01g17670D	AT5G57685	glutamine dumper 3	1.13	-1.20
XLOC_095769	BnaC01g17670D	AT5G57685	glutamine dumper 3	1.80	-1.85
XLOC_049990	BnaC03g49100D	AT5G65207	unknown protein	1.04	-1.43
XLOC_074935	BnaAnng04790D	AT5G65300	unknown protein	1.20	-1.16
XLOC_100323	BnaCnng46560D	AT5G66440	unknown protein	1.18	-1.71
XLOC_014791	BnaC09g07570D	AT5G66985	unknown protein	1.20	-2.89
XLOC_042763	BnaA03g17390D	---NA---	--NA---	1.15	-1.17
XLOC_069234	BnaA09g51380D	---NA---	--NA---	1.18	-1.96
XLOC_097217	BnaAnng36730D	---NA---	--NA---	1.33	-1.77
XLOC_036814	BnaA03g38910D	AT2G15880	Leucine-rich repeat (LRR) family protein	1.40	-1.53
XLOC_011035	BnaC03g53100D	AT1G02460	Pectin lyase-like superfamily protein	1.30	-1.06
XLOC_023766	BnaA10g01160D	AT1G02460	Pectin lyase-like superfamily protein	1.05	-1.07
XLOC_048369	BnaA09g17560D	AT5G45340	cytochrome P450, family 707, subfamily A, polypeptide 3	1.13	-1.47
XLOC_059116	BnaC09g18860D	AT5G45340	cytochrome P450, family 707, subfamily A, polypeptide 3	1.02	-1.18
XLOC_089988	BnaA01g37200D	AT3G13730	cytochrome P450, family 90, subfamily D, polypeptide 1	2.50	-1.98
XLOC_010672	BnaA07g06300D	AT3G23630	isopentenyltransferase 7	1.02	-1.02
XLOC_022993	BnaA04g00130D	AT3G63110	isopentenyltransferase 3	1.42	-1.06
XLOC_028015	BnaC04g20940D	AT3G63110	isopentenyltransferase 3	1.27	-1.28
XLOC_098733	BnaA09g55640D	AT3G63110	isopentenyltransferase 3	1.34	-1.37
XLOC_036738	BnaA03g40380D	AT5G61600	ethylene response factor 104	1.37	-1.34
XLOC_059027	BnaC07g31350D	AT5G61600	ethylene response factor 104	1.37	-1.38
XLOC_015651	BnaA03g35890D	AT3G20640	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	1.55	-1.13
XLOC_065953	BnaC05g32220D	AT3G20640	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	2.08	-1.08
XLOC_069150	BnaC03g41710D	AT3G20640	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	1.78	-1.22
XLOC_101684	BnaA02g22670D	AT5G44350	ethylene-responsive nuclear protein -related	1.38	-1.37

XLOC_102923	BnaAnng24300D	AT2G29970	SMAX1-LIKE 7	1.33	-1.08
XLOC_054925	BnaC05g14050D	AT1G18300	nudix hydrolase homolog 4	1.51	-1.13
XLOC_043736	BnaC03g54940D	AT3G44260	Polynucleotidyl transferase, ribonuclease H-like superfamily protein	1.26	-1.00
XLOC_032868	BnaC05g37890D	AT5G22250	Polynucleotidyl transferase, ribonuclease H-like superfamily protein	1.21	-1.25
XLOC_044248	BnaA02g05510D	AT5G22250	Polynucleotidyl transferase, ribonuclease H-like superfamily protein	1.31	-1.01
XLOC_072661	BnaC02g09390D	AT5G22250	Polynucleotidyl transferase, ribonuclease H-like superfamily protein	1.54	-1.13
XLOC_088457	BnaCnng19040D	AT1G66140	zinc finger protein 4	1.11	-1.02
XLOC_055613	BnaA09g04660D	AT5G25160	zinc finger protein 3	1.41	-2.04
XLOC_096372	BnaC05g51600D	AT1G49560	Homeodomain-like superfamily protein	1.69	-1.64
XLOC_010702	BnaA07g06740D	AT3G23250	myb domain protein 15	1.61	-1.56
XLOC_018647	BnaC07g08320D	AT3G23250	myb domain protein 15	1.19	-1.13
XLOC_031633	BnaC07g26040D	AT5G54145	DNA binding	1.86	-1.80
XLOC_015404	BnaC08g36830D	AT1G19210	Integrase-type DNA-binding superfamily protein	1.50	-1.53
XLOC_017278	BnaC08g18660D	AT1G19210	Integrase-type DNA-binding superfamily protein	1.82	-1.82
XLOC_030849	BnaA08g22160D	AT1G19210	Integrase-type DNA-binding superfamily protein	1.19	-1.33
XLOC_057548	BnaA09g44290D	AT1G19210	Integrase-type DNA-binding superfamily protein	1.30	-1.28
XLOC_047832	BnaC06g00880D	AT1G44830	Integrase-type DNA-binding superfamily protein	1.05	-1.68
XLOC_000880	BnaA07g21980D	AT1G74930	Integrase-type DNA-binding superfamily protein	1.79	-2.45
XLOC_005795	BnaC06g35730D	AT1G74930	Integrase-type DNA-binding superfamily protein	1.75	-1.85
XLOC_019467	BnaC06g22710D	AT1G74930	Integrase-type DNA-binding superfamily protein	1.81	-2.16
XLOC_083102	BnaA07g31860D	AT1G74930	Integrase-type DNA-binding superfamily protein	1.66	-1.77
XLOC_026901	BnaC07g39680D	AT4G25470	C-repeat/DRE binding factor 2	1.76	-1.07
XLOC_014907	BnaC02g18730D	AT1G68880	basic leucine-zipper 8	1.34	-2.71
XLOC_015733	BnaA03g34220D	AT3G16500	phytochrome-associated protein 1	1.16	-1.96
XLOC_080664	BnaCnng10960D	AT1G31050	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	1.33	-1.21
XLOC_005245	BnaC07g45960D	AT4G36060	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	1.20	-1.05
XLOC_033624	BnaC05g34400D	AT3G18710	plant U-box 29	1.21	-1.67
XLOC_073471	BnaC03g48440D	AT2G18500	ovate family protein 7	1.19	-1.72
XLOC_026562	BnaA10g14110D	AT5G22240	Ovate family protein	1.44	-2.08
XLOC_032943	BnaC05g37880D	AT5G22240	Ovate family protein	1.53	-1.27

XLOC_023313	BnaC04g35190D	AT2G22680	Zinc finger (C3HC4-type RING finger) family protein	1.42	-1.08
XLOC_061811	BnaA04g13100D	AT2G22680	Zinc finger (C3HC4-type RING finger) family protein	1.47	-1.01
XLOC_033436	BnaA06g17960D	AT3G46620	zinc finger (C3HC4-type RING finger) family protein	1.63	-1.47
XLOC_097354	BnaCnng37520D	AT3G46620	zinc finger (C3HC4-type RING finger) family protein	1.52	-1.29
XLOC_097200	BnaAnng17320D	AT3G47160	RING/U-box superfamily protein	1.07	-1.25
XLOC_099500	BnaA01g34500D	AT4G36550	ARM repeat superfamily protein	1.70	-1.47
XLOC_018903	BnaC07g25020D	AT3G28340	galacturonosyltransferase-like 10	1.70	-1.65
XLOC_019927	BnaA06g31630D	AT3G28340	galacturonosyltransferase-like 10	1.67	-1.37
XLOC_034535	BnaC09g01660D	AT3G28340	galacturonosyltransferase-like 10	1.77	-1.63
XLOC_055761	BnaA09g02250D	AT3G28340	galacturonosyltransferase-like 10	1.82	-1.64
XLOC_109309	BnaCnng76560D	AT1G72300	Leucine-rich receptor-like protein kinase family protein	1.11	-1.28
XLOC_108369	BnaC05g49970D	AT1G17750	PEP1 receptor 2	1.24	-1.06
XLOC_098599	BnaA02g37150D	AT2G01660	plasmodesmata-located protein 6	1.10	-1.11
XLOC_087413	BnaA05g32910D	AT3G04530	phosphoenolpyruvate carboxylase kinase 2	1.36	-1.71

Table 3.5 Transcripts showing downregulation in 'Wilhelmsburger' and upregulation in 'Laurentian' at 7 dai (List B)

Transcript ID	*B. napus* ID	Arabidopsis ID (Tair 10)	Arabidopsis annotation (Tair 10)	R_7 log_2FC	S_7 log_2FC
XLOC_109981	---NA---	AT4G23160	cysteine-rich RLK (RECEPTOR-like protein kinase) 8	-1.63	1.14
XLOC_063189	---NA---	---NA---	---NA---	-1.99	1.38
XLOC_063190	---NA---	---NA---	---NA---	-1.83	1.32
XLOC_063191	---NA---	---NA---	---NA---	-1.61	1.24
XLOC_063192	---NA---	---NA---	---NA---	-1.34	1.21
XLOC_107194	---NA---	---NA---	---NA---	-2.26	1.09
XLOC_085537	BnaC05g18860D	AT1G23730	beta carbonic anhydrase 3	-1.41	1.16
XLOC_097534	BnaA09g29930D	AT1G23730	beta carbonic anhydrase 3	-1.92	1.10
XLOC_105275	BnaA06g40800D	AT4G38400	expansin-like A2	-1.32	1.06
XLOC_109988	BnaCnng78620D	AT4G38400	expansin-like A2	-2.12	1.26
XLOC_033275	BnaC09g48180D	AT5G07010	sulfotransferase 2A	-2.42	1.79
XLOC_106611	BnaAnng33290D	AT5G07010	sulfotransferase 2A	-1.81	2.32
XLOC_005592	BnaC07g47700D	AT4G38580	farnesylated protein 6	-3.02	1.53
XLOC_099984	BnaA06g40860D	AT4G38580	farnesylated protein 6	-1.28	1.39
XLOC_078031	BnaAnng05970D	AT5G08730	IBR domain-containing protein	-1.38	1.08
XLOC_045292	BnaC05g04720D	AT1G06830	Glutaredoxin family protein	-1.06	1.00
XLOC_004158	BnaC04g16670D	AT2G27690	cytochrome P450, family 94, subfamily C, polypeptide 1	-4.20	1.70
XLOC_039386	BnaA07g13320D	AT2G27690	cytochrome P450, family 94, subfamily C, polypeptide 1	-3.72	1.65
XLOC_018027	BnaC03g50910D	AT5G63450	cytochrome P450, family 94, subfamily B, polypeptide 1	-2.48	1.18
XLOC_067995	BnaA06g38770D	AT5G63450	cytochrome P450, family 94, subfamily B, polypeptide 1	-2.31	1.36
XLOC_077131	BnaA09g06580D	AT5G63450	cytochrome P450, family 94, subfamily B, polypeptide 1	-2.45	1.52
XLOC_099662	BnaA09g06580D	AT5G63450	cytochrome P450, family 94, subfamily B, polypeptide 1	-2.29	1.35
XLOC_099663	BnaA06g38770D	AT5G63450	cytochrome P450, family 94, subfamily B, polypeptide 1	-2.54	1.28
XLOC_066435	BnaC07g50320D	AT4G27520	early nodulin-like protein 2	-2.62	1.54
XLOC_094153	BnaC07g50320D	AT4G27520	early nodulin-like protein 2	-1.95	1.18
XLOC_024489	BnaA03g43760D	AT4G18340	Glycosyl hydrolase superfamily protein	-1.07	1.08
XLOC_103954	BnaA06g38320D	AT1G15125	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	-1.03	1.26

XLOC_051951	BnaA02g12280D	AT1G65840	polyamine oxidase 4	-1.66	1.16
XLOC_075088	BnaC05g07890D	AT1G10370	Glutathione S-transferase family protein	-2.08	1.64
XLOC_046164	BnaA03g46710D	AT4G24440	transcription initiation factor IIA gamma chain / TFIIA-gamma (TFIIA-S)	-2.30	1.26
XLOC_036941	BnaA09g25880D	AT1G30640	Protein kinase family protein	-1.11	1.07
XLOC_006907	BnaA03g29080D	AT3G05580	Calcineurin-like metallo-phosphoesterase superfamily protein	-1.09	1.00
XLOC_050767	BnaC05g01110D	AT1G02340	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-1.41	1.21
XLOC_097769	BnaAnng17910D	AT1G01470	Late embryogenesis abundant protein	-2.12	1.27
XLOC_056968	BnaC06g29620D	AT1G13245	ROTUNDIFOLIA like 17	-1.54	1.22
XLOC_064630	BnaA01g06620D	AT4G30430	tetraspanin9	-1.28	1.08
XLOC_080529	BnaA03g11960D	AT5G54200	Transducin/WD40 repeat-like superfamily protein	-1.20	1.15
XLOC_017992	BnaC03g51560D	AT5G62680	Major facilitator superfamily protein	-1.57	1.05
XLOC_059193	BnaA04g05540D	AT3G52310	ABC-2 type transporter family protein	-1.56	1.15
XLOC_029500	BnaC02g01960D	AT5G06530	ABC-2 type transporter family protein	-1.67	1.02
XLOC_077928	BnaA03g53200D	AT4G35180	LYS/HIS transporter 7	-1.47	2.30
XLOC_047619	BnaA06g19930D	AT1G66760	MATE efflux family protein	-1.19	1.25
XLOC_018562	BnaC05g21520D	AT1G27760	interferon-related developmental regulator family protein	-2.02	1.01
XLOC_004002	BnaC07g06130D	AT2G17120	lysm domain GPI-anchored protein 2 precursor	-1.06	1.51
XLOC_068025	BnaA07g03170D	AT2G17120	lysm domain GPI-anchored protein 2 precursor	-1.29	1.06
XLOC_040027	BnaA05g31720D	AT3G05500	Rubber elongation factor protein (REF)	-1.06	1.07
XLOC_064150	BnaA05g31720D	AT3G05500	Rubber elongation factor protein (REF)	-1.74	1.14
XLOC_106827	BnaCnng68620D	AT3G05500	Rubber elongation factor protein (REF)	-1.47	1.15
XLOC_027539	BnaC02g30820D	AT4G29090	Ribonuclease H-like superfamily protein	-1.98	1.15
XLOC_026522	BnaA10g15950D	AT5G19110	Eukaryotic aspartyl protease family protein	-1.81	1.20
XLOC_049344	BnaC09g38640D	AT5G19110	Eukaryotic aspartyl protease family protein	-2.44	1.22
XLOC_105072	BnaC09g38640D	AT5G19110	Eukaryotic aspartyl protease family protein	-1.73	1.00
XLOC_105073	BnaA10g15950D	AT5G19110	Eukaryotic aspartyl protease family protein	-1.65	1.43
XLOC_075922	BnaC03g15270D	AT5G53050	alpha/beta-Hydrolases superfamily protein	-1.62	2.28
XLOC_109803	BnaCnng78120D	AT5G54170	Polyketide cyclase/dehydrase and lipid transport superfamily protein	-1.41	1.11
XLOC_067857	BnaC08g15550D	AT1G12320	Protein of unknown function (DUF1442)	-1.01	1.04
XLOC_039479	BnaC05g17150D	AT1G21790	TRAM, LAG1 and CLN8 (TLC) lipid-sensing domain containing protein	-1.74	1.02
XLOC_064848	BnaC07g12600D	AT1G27030	unknown protein	-2.01	1.04
XLOC_104871	BnaA07g36440D	AT1G29640	AtS40-3	-1.58	1.34

XLOC_069304	BnaC02g17680D	AT1G67920	unknown protein	-2.07	1.61
XLOC_005930	BnaC06g33640D	AT1G72450	jasmonate-zim-domain protein 6	-2.33	1.41
XLOC_010002	BnaC08g34610D	AT2G23120	Late embryogenesis abundant protein, group 6	-1.22	1.13
XLOC_087505	BnaA09g42180D	AT2G23120	Late embryogenesis abundant protein, group 6	-1.24	1.15
XLOC_071738	BnaA09g41660D	AT2G24100	unknown protein	-1.09	1.32
XLOC_006971	BnaA03g27740D	AT3G02140	AFP2 (ABI five-binding protein 2) family protein	-1.72	1.47
XLOC_026069	BnaC03g32780D	AT3G02140	AFP2 (ABI five-binding protein 2) family protein	-2.04	1.40
XLOC_088098	BnaC03g34540D	AT3G06070	unknown protein	-3.27	1.84
XLOC_096643	BnaAnng16590D	AT3G10120	unknown protein	-1.28	1.15
XLOC_061613	BnaA05g18720D	AT3G20340	Expression of the gene is downregulated in the presence of paraquat	-3.13	2.10
XLOC_066370	BnaC05g31700D	AT3G20340	Expression of the gene is downregulated in the presence of paraquat	-3.02	2.26
XLOC_041576	BnaC04g43000D	AT3G29785	unknown protein	-1.72	1.22
XLOC_037573	BnaC04g25850D	AT4G23160	cysteine-rich RLK (RECEPTOR-like protein kinase) 8	-2.60	1.36
XLOC_001594	BnaC01g02040D	AT4G36820	Protein of unknown function (DUF607)	-1.22	2.51
XLOC_011601	BnaA03g04250D	AT5G13220	jasmonate-zim-domain protein 10	-1.25	1.27
XLOC_019357	BnaA02g01520D	AT5G13220	jasmonate-zim-domain protein 10	-1.33	1.21
XLOC_025177	BnaC02g04570D	AT5G13220	jasmonate-zim-domain protein 10	-1.80	1.30
XLOC_002661	BnaA10g20060D	AT5G13220	jasmonate-zim-domain protein 10	-1.49	1.19
XLOC_092028	BnaC03g71460D	AT5G13220	jasmonate-zim-domain protein 10	-1.54	1.29
XLOC_019273	BnaA02g03120D	AT5G16550	unknown protein	-3.08	1.46
XLOC_075245	BnaC02g06670D	AT5G16550	unknown protein	-2.16	1.22
XLOC_049283	BnaC09g38790D	AT5G19060	CONTAINS InterPro DOMAIN/s	-1.34	1.00
XLOC_088623	BnaA10g28920D	AT5G56980	unknown protein	-1.84	1.29
XLOC_010427	BnaC08g03050D	---NA---	---NA---	-2.16	1.81
XLOC_031791	BnaC04g13640D	---NA---	---NA---	-1.08	1.20
XLOC_086702	BnaC02g09940D	---NA---	---NA---	-1.94	1.01
XLOC_095793	BnaA03g55600D	---NA---	---NA---	-1.35	1.17
XLOC_002866	BnaA10g24060D	AT5G06860	polygalacturonase inhibiting protein 1	-2.13	1.70
XLOC_002867	BnaA10g24070D	AT5G06860	polygalacturonase inhibiting protein 1	-1.98	1.41
XLOC_033252	BnaC09g48680D	AT5G06860	polygalacturonase inhibiting protein 1	-1.53	1.08
XLOC_001369	BnaC01g06700D	AT4G31780	monogalactosyl diacylglycerol synthase 1	-1.49	1.05
XLOC_008185	BnaA01g05080D	AT4G31780	monogalactosyl diacylglycerol synthase 1	-1.83	1.04

XLOC_048759	BnaA03g51720D	AT4G31780	monogalactosyl diacylglycerol synthase 1	-1.96	1.14
XLOC_007066	BnaA03g31600D	AT3G11170	fatty acid desaturase 7	-1.21	1.02
XLOC_077062	BnaA09g10340D	AT2G20900	diacylglycerol kinase 5	-1.58	1.26
XLOC_005797	BnaC06g35790D	AT1G75000	GNS1/SUR4 membrane protein family	-1.70	1.02
XLOC_000903	BnaA07g22450D	AT1G73920	alpha/beta-Hydrolases superfamily protein	-1.11	1.35
XLOC_056720	BnaC05g26220D	AT1G49660	carboxyesterase 5	-1.20	1.39
XLOC_019892	BnaA06g30990D	AT3G29200	chorismate mutase 1	-1.41	1.06
XLOC_007619	BnaC04g31100D	AT5G38710	Methylenetetrahydrofolate reductase family protein	-1.12	1.17
XLOC_000560	BnaA05g28470D	AT3G53260	phenylalanine ammonia-lyase 2	-2.13	1.22
XLOC_048637	BnaA05g28470D	AT3G53260	phenylalanine ammonia-lyase 2	-1.90	1.07
XLOC_021004	BnaA07g35280D	AT1G80820	cinnamoyl coa reductase	-1.53	1.56
XLOC_077237	BnaC06g40190D	AT1G80820	cinnamoyl coa reductase	-1.49	1.63
XLOC_039749	BnaC02g02220D	AT5G05600	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	-1.75	2.18
XLOC_046470	BnaA03g01650D	AT5G05600	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	-2.83	2.14
XLOC_049506	BnaC02g02220D	AT5G05600	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	-1.39	1.98
XLOC_007383	BnaA04g09150D	AT5G39050	HXXXD-type acyl-transferase family protein	-1.70	1.11
XLOC_084789	BnaA02g32910D	AT5G23350	GRAM domain-containing protein / ABA-responsive protein-related	-1.01	1.67
XLOC_078109	BnaC02g41650D	AT5G23370	GRAM domain-containing protein / ABA-responsive protein-related	-1.21	1.65
XLOC_084788	BnaA02g32920D	AT5G23370	GRAM domain-containing protein / ABA-responsive protein-related	-1.09	1.26
XLOC_084790	BnaA02g32900D	AT5G23370	GRAM domain-containing protein / ABA-responsive protein-related	-1.25	1.37
XLOC_050478	BnaA10g08770D	AT1G44350	IAA-leucine resistant (ILR)-like gene 6	-1.95	1.36
XLOC_027642	BnaA06g03580D	AT1G23160	Auxin-responsive GH3 family protein	-1.29	1.16
XLOC_057890	BnaC09g00690D	AT4G03400	Auxin-responsive GH3 family protein	-1.90	1.47
XLOC_086583	BnaA09g01540D	AT4G03400	Auxin-responsive GH3 family protein	-1.05	1.01
XLOC_077894	BnaC06g02210D	AT5G13320	Auxin-responsive GH3 family protein	-1.45	1.50
XLOC_003215	BnaC03g07520D	AT5G16010	3-oxo-5-alpha-steroid 4-dehydrogenase family protein	-1.87	1.20
XLOC_037072	BnaA02g23180D	AT5G42650	allene oxide synthase	-1.69	1.02
XLOC_038927	BnaA09g19550D	AT3G25770	allene oxide cyclase 2	-1.42	1.86
XLOC_048933	BnaA06g33410D	AT3G25770	allene oxide cyclase 2	-2.39	1.01
XLOC_096458	BnaC09g52570D	AT3G25770	allene oxide cyclase 2	-1.25	1.65
XLOC_002087	BnaA10g17650D	AT1G76680	12-oxophytodienoate reductase 1	-2.37	1.62
XLOC_052540	BnaC09g41020D	AT1G76680	12-oxophytodienoate reductase 1	-2.62	1.32

XLOC_091671	BnaA03g31730D	AT3G11480	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	-1.85	1.13
XLOC_058757	BnaA06g02800D	AT1G50460	hexokinase-like 1	-1.67	1.16
XLOC_039282	BnaA03g59290D	AT1G58170	Disease resistance-responsive (dirigent-like protein) family protein	-2.58	1.00
XLOC_043339	BnaA09g11650D	AT1G63730	Disease resistance protein (TIR-NBS-LRR class) family	-1.08	1.12
XLOC_017752	BnaC04g10350D	AT2G34930	disease resistance family protein / LRR family protein	-1.27	1.30
XLOC_015991	BnaA03g32930D	AT3G13650	Disease resistance-responsive (dirigent-like protein) family protein	-1.15	1.06
XLOC_100075	BnaAnng20120D	AT1G73260	kunitz trypsin inhibitor 1	-1.04	1.19
XLOC_025600	BnaC02g15270D	AT5G52300	CAP160 protein	-1.45	1.30
XLOC_063402	BnaA02g10970D	AT5G52300	CAP160 protein	-1.57	1.17
XLOC_031110	BnaC03g56530D	AT1G13930	Involved in response to salt stress.	-1.03	1.22
XLOC_071486	BnaC03g56530D	AT1G13930	Involved in response to salt stress.	-1.41	1.41
XLOC_082747	BnaA02g04970D	AT5G20630	germin 3	-3.62	1.13
XLOC_080577	BnaCnng10890D	AT3G20330	PYRIMIDINE B	-1.29	1.10
XLOC_009262	BnaA04g04610D	AT3G53620	pyrophosphorylase 4	-1.13	1.06
XLOC_067486	BnaA04g13360D	AT2G07760	Zinc knuckle (CCHC-type) family protein	-1.51	1.55
XLOC_050893	BnaC04g41990D	AT2G31180	myb domain protein 14	-2.03	1.90
XLOC_050894	BnaC04g41980D	AT2G31180	myb domain protein 14	-1.93	1.33
XLOC_083710	BnaA09g12950D	AT1G12630	Integrase-type DNA-binding superfamily protein	-1.65	1.69
XLOC_060478	BnaCnng04580D	AT1G25560	AP2/B3 transcription factor family protein	-1.48	1.11
XLOC_073070	BnaA08g19490D	AT1G25560	AP2/B3 transcription factor family protein	-1.62	1.42
XLOC_107022	BnaCnng69090D	AT1G75490	Integrase-type DNA-binding superfamily protein	-1.46	1.67
XLOC_083186	BnaC09g12830D	AT5G52020	Integrase-type DNA-binding superfamily protein	-1.82	2.00
XLOC_014601	BnaC09g06610D	AT5G64750	Integrase-type DNA-binding superfamily protein	-1.89	2.19
XLOC_054458	BnaC09g06610D	AT5G64750	Integrase-type DNA-binding superfamily protein	-2.73	2.20
XLOC_084629	BnaC02g43290D	AT5G64750	Integrase-type DNA-binding superfamily protein	-1.35	1.54
XLOC_035955	BnaA07g12050D	AT5G67180	target of early activation tagged (EAT) 3	-1.52	1.00
XLOC_009010	BnaA04g02550D	AT2G40740	WRKY DNA-binding protein 55	-1.15	1.05
XLOC_001978	BnaC01g02130D	AT4G36730	G-box binding factor 1	-1.06	1.02
XLOC_070736	BnaC06g05090D	AT1G51950	indole-3-acetic acid inducible 18	-1.40	1.24
XLOC_056986	BnaC01g39910D	AT3G04730	indoleacetic acid-induced protein 16	-2.09	1.18
XLOC_038036	BnaC09g10990D	AT1G62975	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-2.65	1.55

XLOC_107028	BnaC09g10990D	AT1G62975	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-2.60	1.85
XLOC_109501	BnaC09g10990D	AT1G62975	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-2.25	1.27
XLOC_066766	BnaA03g49880D	AT4G29930	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-3.59	1.42
XLOC_084524	BnaC07g42240D	AT4G29930	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-3.20	1.31
XLOC_093973	BnaC06g30840D	AT1G69790	Protein kinase superfamily protein	-2.44	2.09
XLOC_096144	BnaA07g28320D	AT1G69790	Protein kinase superfamily protein	-1.64	1.00
XLOC_007184	BnaA03g29200D	AT3G05870	anaphase-promoting complex/cyclosome 11	-1.71	1.09
XLOC_059635	BnaC05g40420D	AT3G12920	SBP (S-ribonuclease binding protein) family protein	-1.17	1.09
XLOC_033708	BnaC05g34330D	AT3G18773	RING/U-box superfamily protein	-1.13	1.38
XLOC_022087	BnaC06g38620D	AT1G78100	F-box family protein	-1.62	1.15
XLOC_104776	BnaC09g01760D	AT3G28600	P-loop containing nucleoside triphosphate hydrolases superfamily protein	-2.73	1.50
XLOC_007359	BnaA04g09770D	AT5G40000	P-loop containing nucleoside triphosphate hydrolases superfamily protein	-1.37	1.84
XLOC_071582	BnaC08g42100D	AT1G11300	protein serine/threonine kinases	-2.36	1.17
XLOC_101890	BnaCnng51650D	AT1G09932	Phosphoglycerate mutase family protein	-1.56	1.23

Table 3.6 Transcripts showing contrasting patterns of expression between 'Wilhelmsburger' and 'Laurentian' at 14 dai.

Transcript ID	*B. napus* ID	Arabidopsis ID (Tair 10)	Arabidopsis annotation (Tair 10)	R_7 log_2FC	S_7 log_2FC
XLOC_009697	BnaC08g32430D	AT3G62720	xylosyltransferase 1	1.28	-1.07
XLOC_056879	BnaA08g24500D	AT1G12940	nitrate transporter2.5	1.06	-1.28
XLOC_039522	BnaC05g16810D	AT1G21528	unknown protein	1.09	-1.73
XLOC_080308	BnaC03g24030D	AT2G43110	unknown protein	2.97	-2.47
XLOC_057263	BnaA09g30050D	AT1G23200	Plant invertase/pectin methylesterase inhibitor superfamily	1.04	-1.03
XLOC_083144	BnaA05g12620D	AT5G65940	beta-hydroxyisobutyryl-CoA hydrolase 1	1.34	-1.18
XLOC_072011	BnaA06g11010D	AT1G16410	cytochrome p450 79f1	1.15	-2.52
XLOC_035753	BnaA04g06630D	AT4G13770	cytochrome P450, family 83, subfamily A, polypeptide 1	1.08	-2.42
XLOC_024721	BnaA03g24950D	AT4G12030	bile acid transporter 5	1.27	-1.64
XLOC_035386	BnaC09g52780D	---NA---	---NA---	1.04	-1.06
XLOC_067172	BnaAnng03830D	AT3G50060	myb domain protein 77	1.17	-1.17
XLOC_094824	BnaC08g48630D	AT3G50060	myb domain protein 77	1.07	-1.42
XLOC_032217	BnaA06g06570D	AT1G10720	BSD domain-containing protein	2.26	-1.79
XLOC_006228	BnaC05g27810D	AT1G29720	Leucine-rich repeat transmembrane protein kinase	4.12	-1.25
XLOC_009213	---NA---	---NA---	---NA---	1.03	-1.61
XLOC_104808	---NA---	---NA---	---NA---	1.12	-3.85
XLOC_038163	BnaC01g15840D	AT4G24015	RING/U-box superfamily protein	-1.06	1.33
XLOC_107368	BnaAnng35440D	AT4G24015	RING/U-box superfamily protein	-1.04	1.18
XLOC_067486	BnaA04g13360D	AT2G07760	Zinc knuckle (CCHC-type) family protein	-1.62	1.83
XLOC_100818	---NA---	---NA---	---NA---	-5.86	7.77
XLOC_035803	BnaA09g23980D	AT1G33055	unknown protein	-1.08	1.15
XLOC_035715	BnaA04g06090D	AT4G14270	Protein containing PAM2 motif	-1.04	1.07
XLOC_091276	BnaC03g77840D	AT1G50820	Aminotransferase-like, plant mobile domain family protein	-1.96	1.30
XLOC_049476	BnaC02g01610D	AT5G07440	glutamate dehydrogenase 2	-1.54	1.53
XLOC_093868	BnaAnng13920D	AT5G01320	Thiamine pyrophosphate dependent pyruvate decarboxylase family protein	-1.23	1.05

Table 3.7 Transcripts showing contrasting patterns of expression between 'Wilhelmsburger' and 'Laurentian' at 21 dai.

Transcript ID	*B. napus* ID	Arabidopsis ID (Tair 10)	Arabidopsis annotation (Tair 10)	R_7 log_2FC	S_7 log_2FC
XLOC_075448	---NA---	---NA---	---NA---	1.20	-1.23
XLOC_079252	---NA---	---NA---	---NA---	2.46	-2.16
XLOC_067025	BnaA04g25230D	AT2G43610	Chitinase family protein	1.53	-1.53
XLOC_011994	BnaA03g06690D	AT5G17690	like heterochromatin protein (LHP1)	1.22	-1.02
XLOC_070016	BnaC01g36910D	AT3G14440	nine-cis-epoxycarotenoid dioxygenase 3	1.38	-1.55
XLOC_009539	BnaA08g10360D	AT4G22620	SAUR-like auxin-responsive protein family	1.39	-1.13
XLOC_028084	BnaC04g20700D	AT3G63370	Tetratricopeptide repeat (TPR)-like superfamily protein	1.49	-1.01
XLOC_109380	---NA---	---NA---	---NA---	-3.64	3.61
XLOC_047629	BnaC08g08350D	AT4G14090	UDP-Glycosyltransferase superfamily protein	-1.04	1.93
XLOC_091276	BnaC03g77840D	AT1G50820	Aminotransferase-like, plant mobile domain family protein	-1.69	1.10
XLOC_004467	BnaC09g26890D	---NA---	---NA---	-1.03	1.06
XLOC_004525	BnaC02g36110D	---NA---	---NA---	-1.84	2.03
XLOC_106545	BnaAnng22160D	---NA---	---NA---	-4.81	2.88
XLOC_107887	BnaAnng36730D	---NA---	---NA---	-2.22	1.17
XLOC_029328	BnaC09g17150D	AT5G42800	dihydroflavonol 4-reductase	-1.46	3.98
XLOC_096372	BnaC05g51600D	AT1G49560	Homeodomain-like superfamily protein	-1.28	2.93

3.5 Figures

Figure 3.1 Phenotypes of Plasmodiophora brassicae-inoculated roots of the rutabagas ‘Wilhelmsburger’ (R) and ‘Laurentian’ (S) at 7, 14 and 21 days after inoculation.

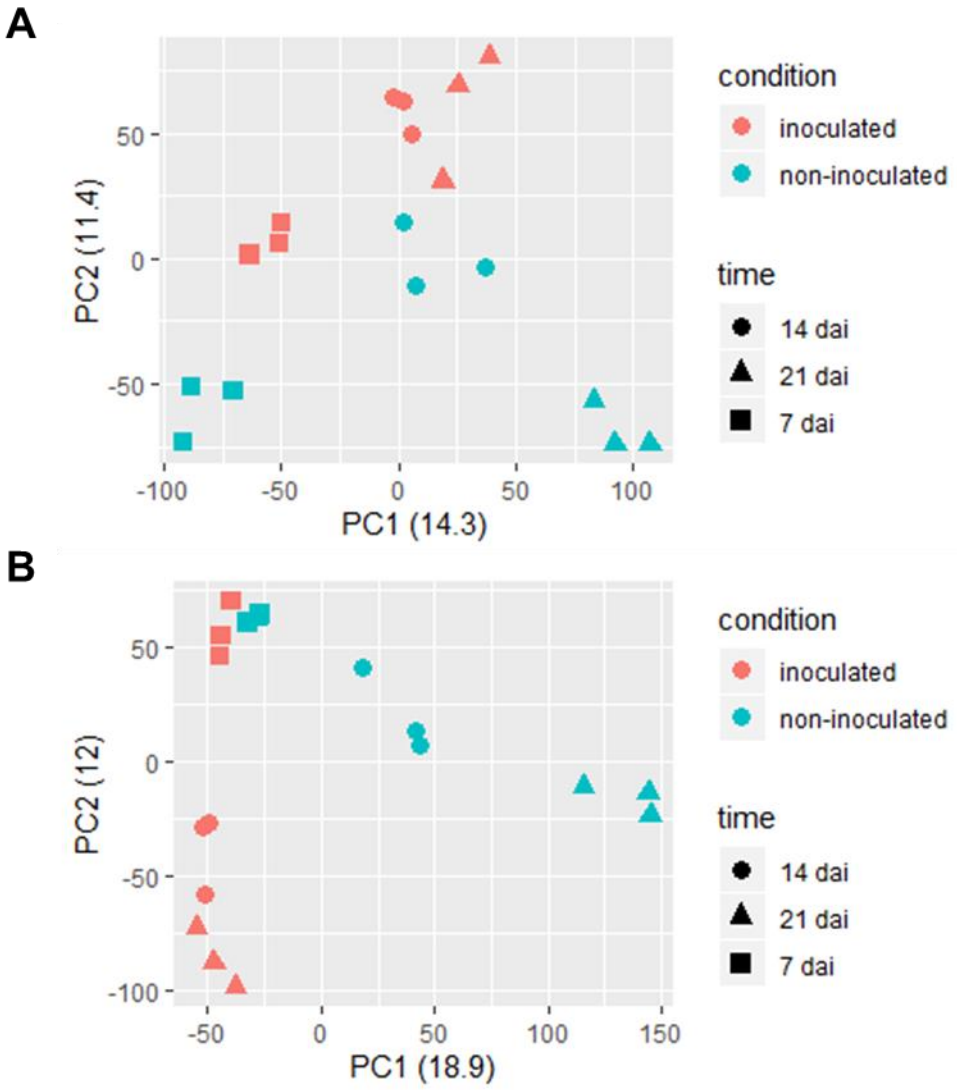

Figure 3.2 PCA plots of variation between inoculated and control samples. (A) The PCA plot of 'Wilhelmsburger'; (B) The PCA plot of 'Laurentian'. Each condition has three biological replicates.

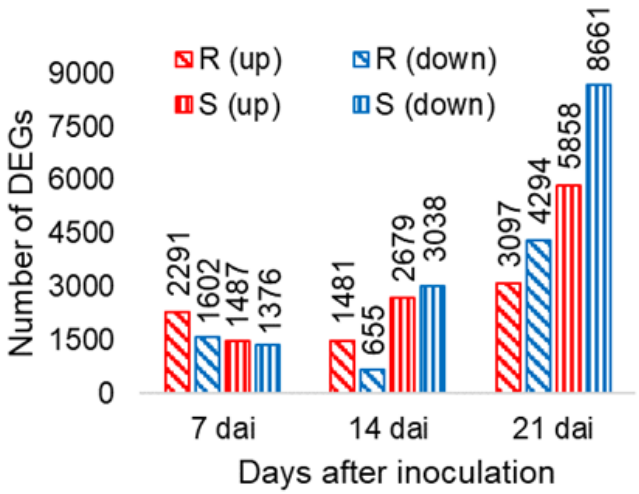

Figure 3.3 Numbers of differentially expressed transcripts in each rutabaga cultivar and time-point. Up, upregulation; down, downregulation; R, 'Wilhelmsburger'; S, 'Laurentian'; dai, days after inoculation.

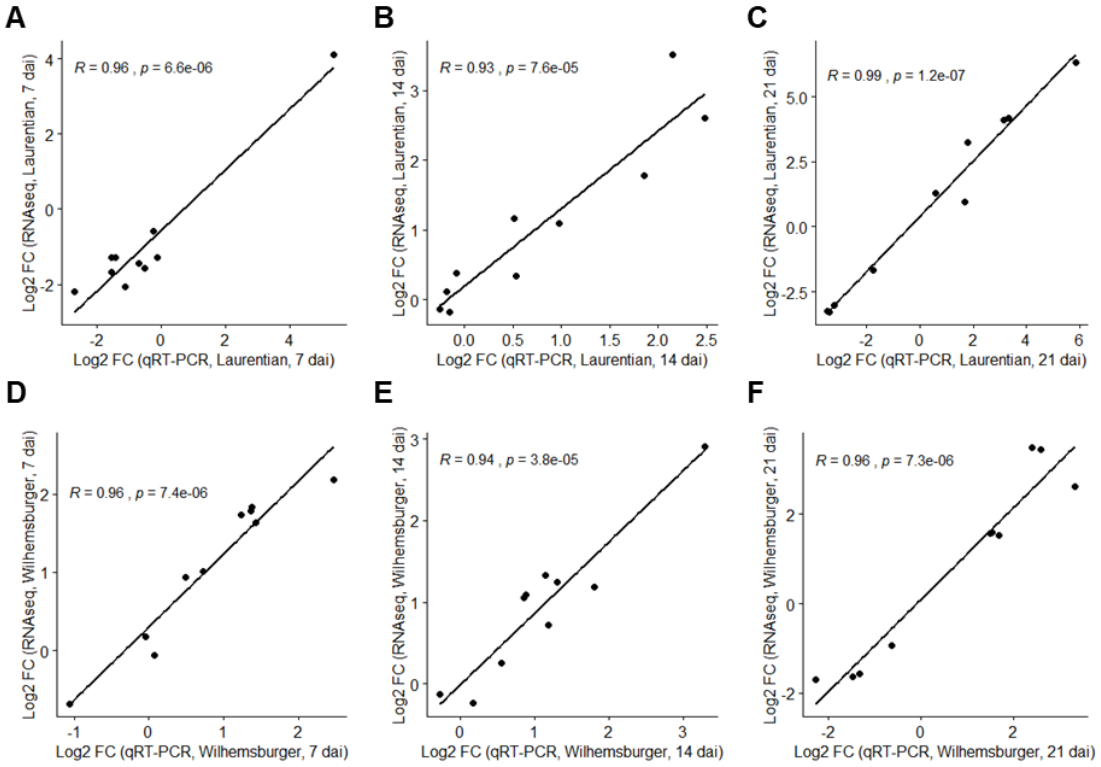

Figure 3.4 Correlation of log2 (fold-change) of 10 selected genes based on RNA-seq and qRT-PCR analyses (inoculated versus non-inoculated). The R values indicate the correlation coefficient between the two methods in each host and time-point, and the p-values indicate the significance level of the t-test. (A) 'Laurentian', 7 dai; (B) 'Laurentian', 14 dai; (C) 'Laurentian', 21 dai; (D) 'Wilhelmsburger', 7 dai; (E) 'Wilhelmsburger', 14 dai; (F) 'Wilhelmsburger', 21 dai.

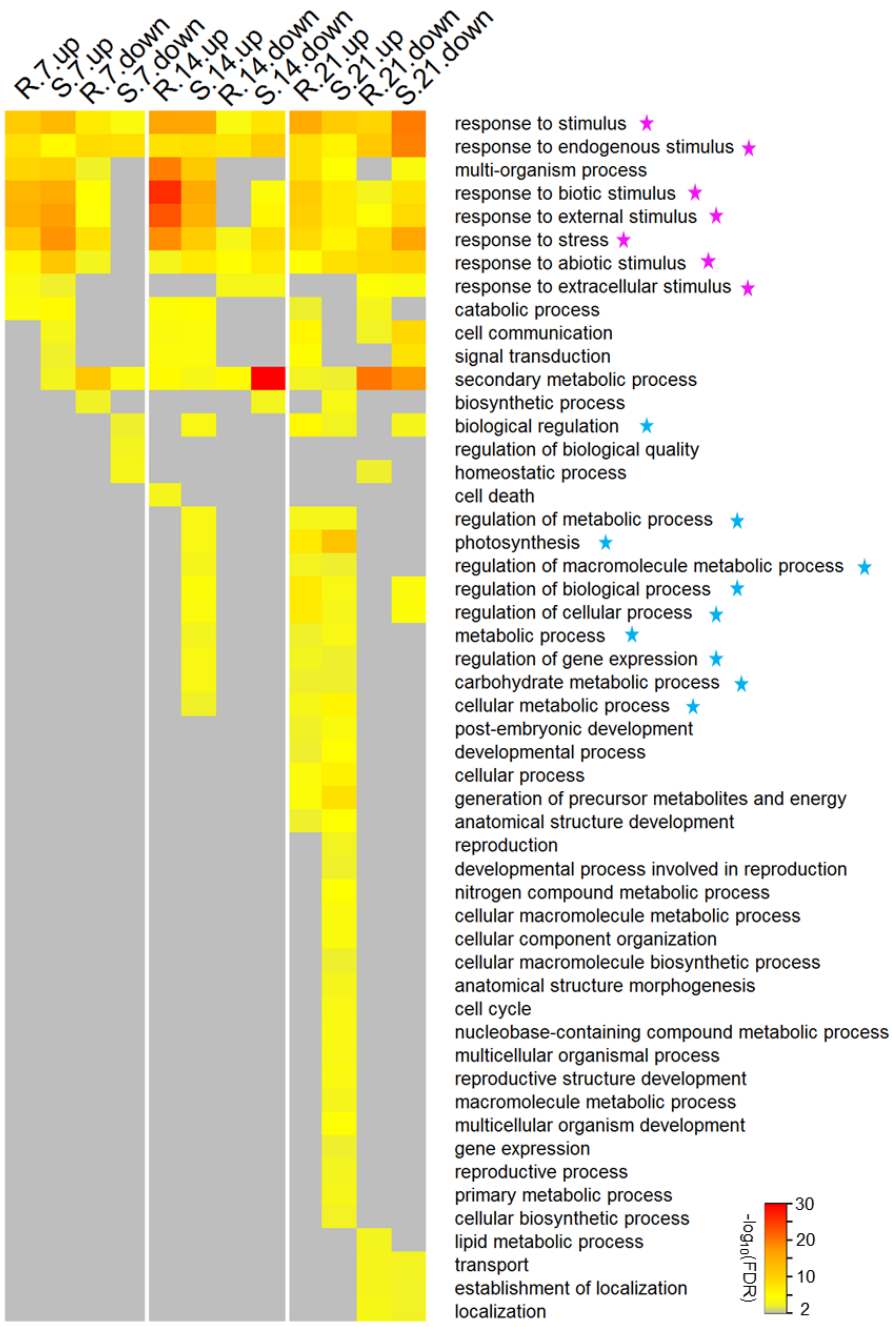

Figure 3.5 Enriched biological processes in the rutabagas 'Wilhelmsburger' (R) and 'Laurentian' (S) for upregulated and downregulated genes through a time course after inoculation with *Plasmodiophora brassicae*. The significance of enrichment is scaled using -log10 (false discovery rate, FDR). Grey indicates no significant enrichment, yellow indicates low significant enrichment, and red indicates high significant enrichment. Terms marked with blue stars are enriched in 'Laurentian' at both 14 and 21 dai, but only at 21 dai in 'Wilhelmsburger'. Terms marked with pink stars are related to the stress and stimulus categories.

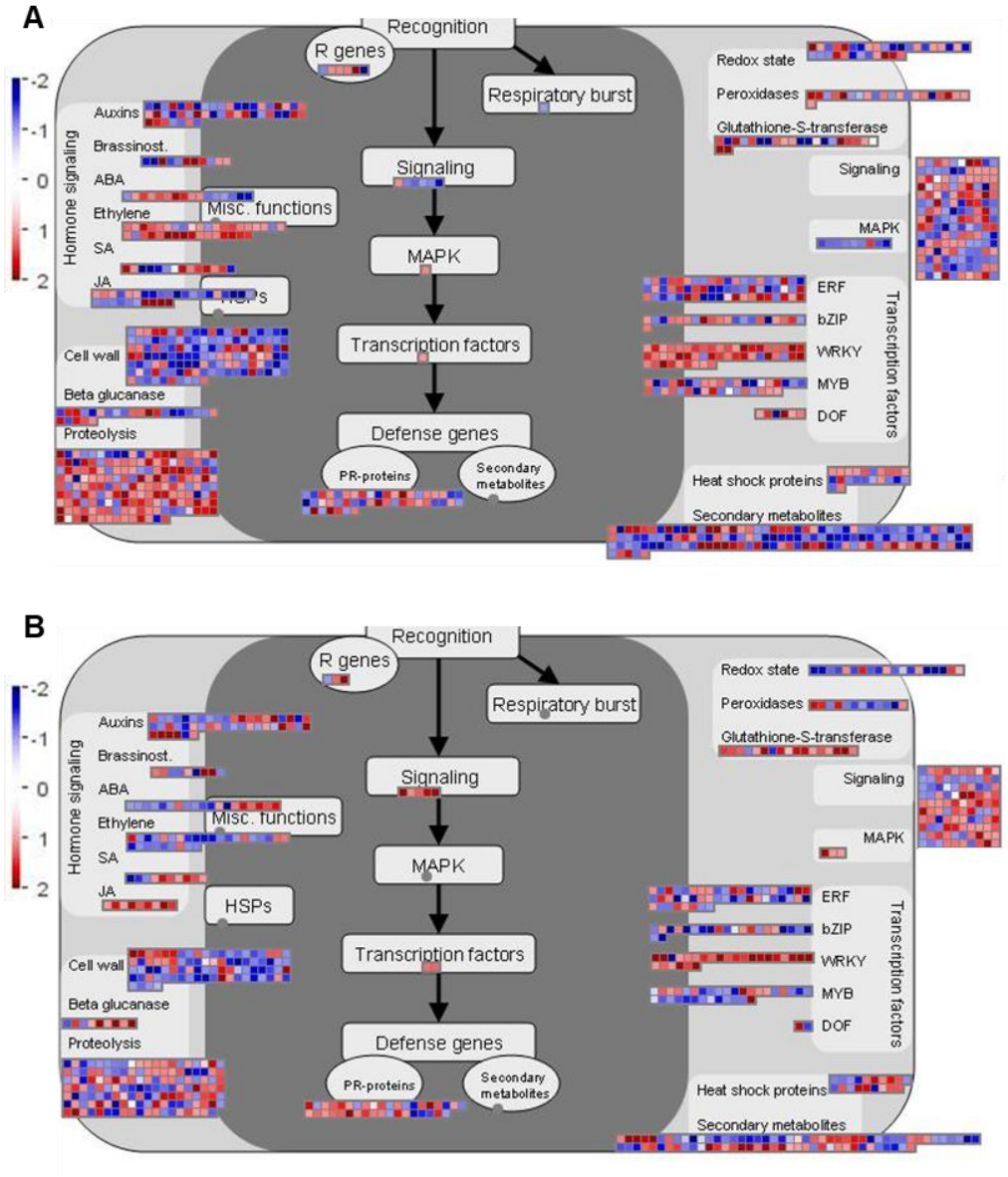

Figure 3.6 Distribution of differentially expressed genes involved in the biotic stress response in two rutabaga hosts at 7 days after inoculation with *Plasmodiophora brassicae*. (A) 'Wilhemsburger' and (B) 'Laurentian'. The log2 fold-changes are presented on a scale where red represents upregulation and blue represents downregulation. JA, jasmonic acid; SA, salicylic acid; bZIP, basic region-leucine zipper; ERF, APETALA2/Ethylene-responsive element binding protein family; MAPK, mitogen-activated protein kinase; PR-protein, pathogenesis-related protein; R genes, resistance genes.

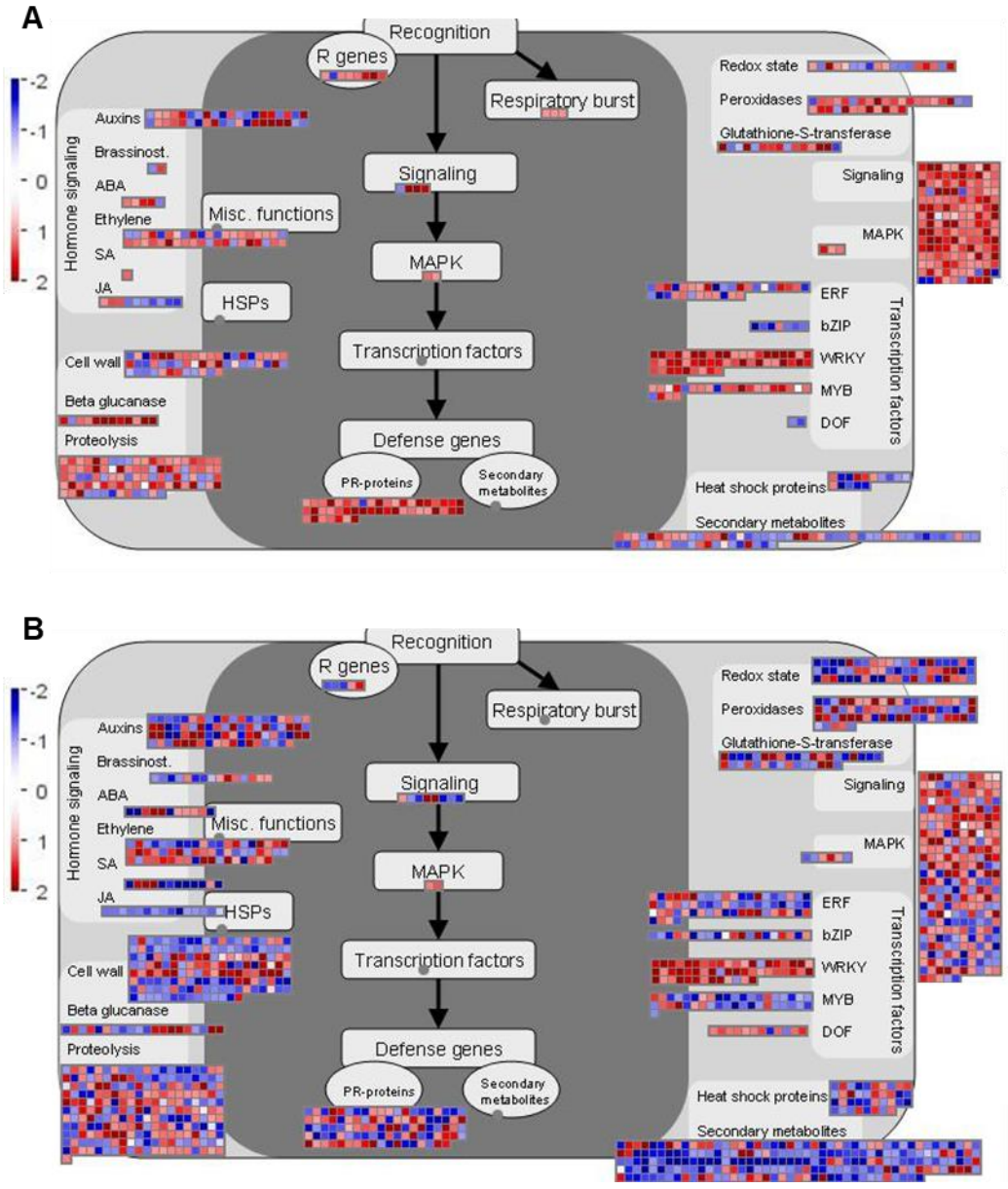

Figure 3.7 Distribution of differentially expressed genes involved in the biotic stress response in two rutabaga hosts at 14 days after inoculation with *Plasmodiophora brassicae*. (A) 'Wilhemsburger' and (B) 'Laurentian'. The log2 fold-changes are presented on a scale where red represents upregulation and blue represents downregulation. JA, jasmonic acid; SA, salicylic acid; bZIP, basic region-leucine zipper; ERF, APETALA2/Ethylene-responsive element binding protein family; MAPK, mitogen-activated protein kinase; PR-protein, pathogenesis-related protein; R genes, resistance genes.

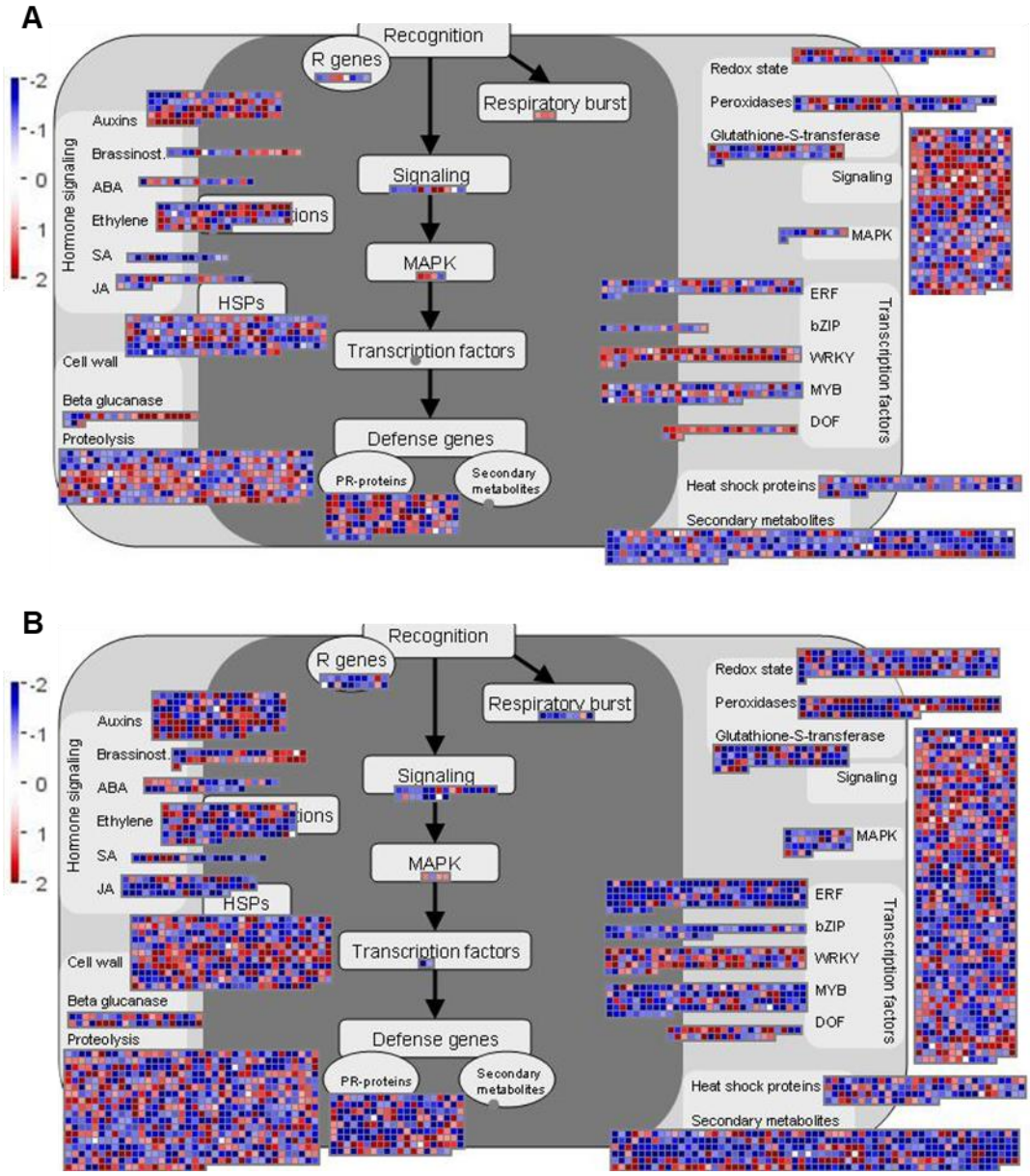

Figure 3.8 Distribution of differentially expressed genes involved in the biotic stress response in two rutabaga hosts at 21 days after inoculation with *Plasmodiophora brassicae*. (A) 'Wilhemsburger' and (B) 'Laurentian'. The log2 fold-changes are presented on a scale where red represents upregulation and blue represents downregulation. JA, jasmonic acid; SA, salicylic acid; bZIP, basic region-leucine zipper; ERF, APETALA2/Ethylene-responsive element binding protein family; MAPK, mitogen-activated protein kinase; PR-protein, pathogenesis-related protein; R genes, resistance genes.

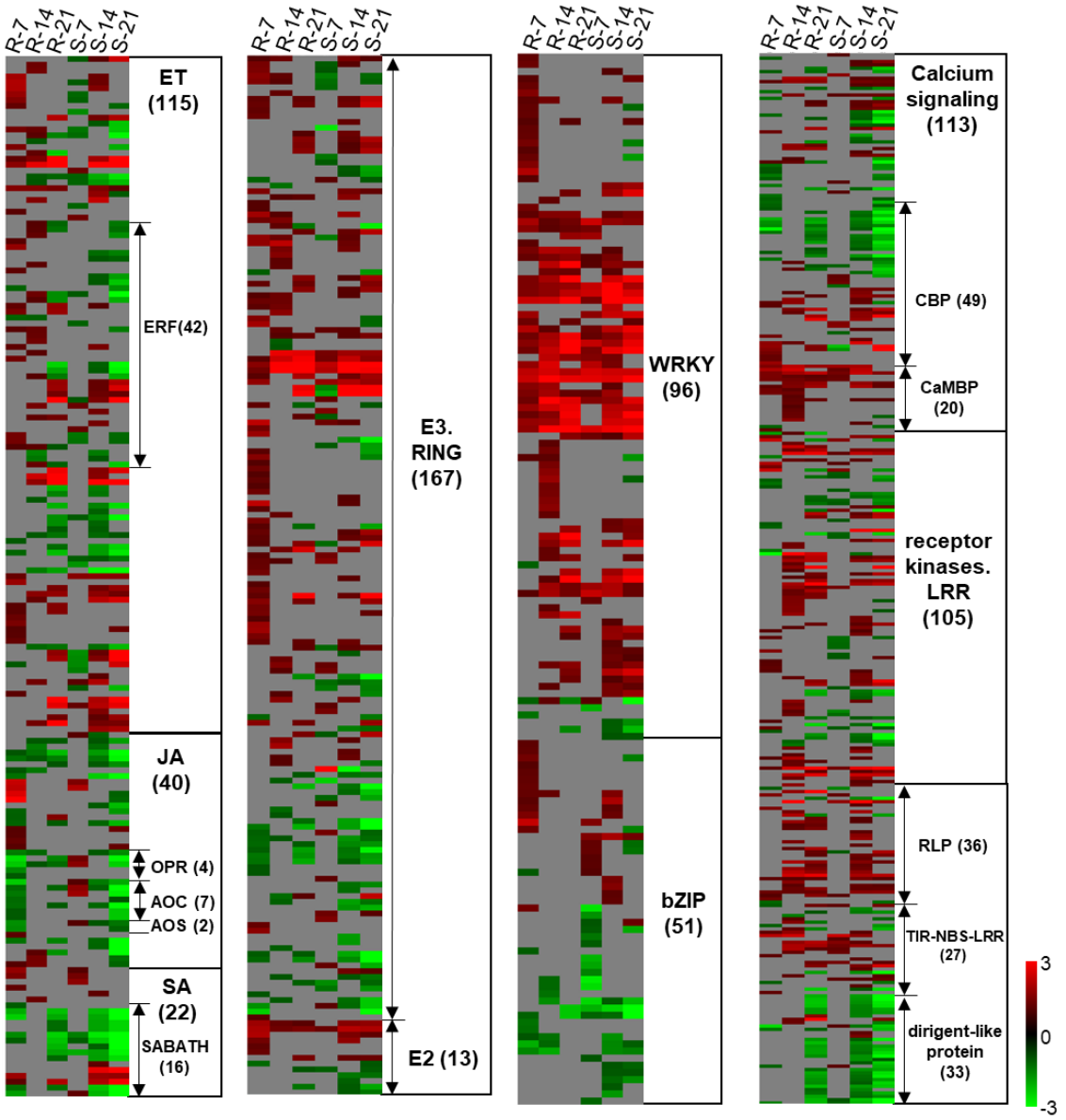

Figure 3.9 Heatmaps of differentially expressed genes in the rutabagas 'Wilhelmsburger' (R) and 'Laurentian' (S) in response to *Plasmodiophora brassicae* through a time course. Only genes that showed significant differential expression in each host at 7 or 14 dai were selected. In the heatmap scale used in this diagram, red indicates upregulation, green indicates downregulation, and gray indicates no significant regulation. ERF, ethylene response factor; JA, jasmonic acid; OPR, oxophytodienoate reductase; AOC, allene oxide cyclase; AOS, allene oxide synthase; SA, salicylic acid; SABATH, SABATH methyltransferase gene family; E2, E2 ubiquitin-conjugating enzymes, E3, E3 ubiquitin ligase; CBP, calcium binding protein; CaMBP, calmodulin-binding protein; LRR, leucine-rich repeat; RLP, receptor like protein. The number of transcripts for each term are indicated in parentheses.

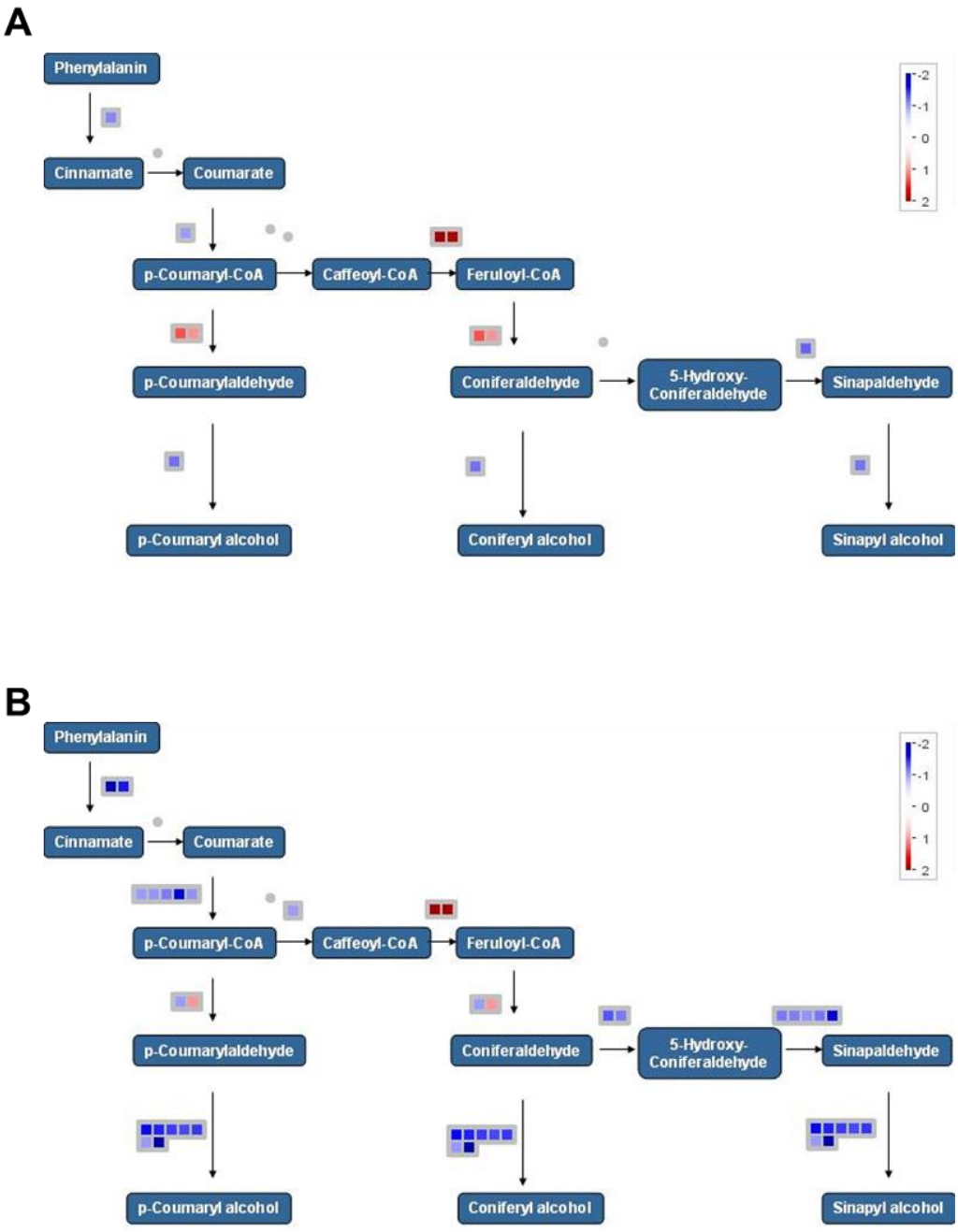

Figure 3.10 Distribution of DEGs involved in lignin synthesis in the two hosts at 14 dai. (A) 'Wilhemsburger' and (B) 'Laurentian'. Values of log2 fold change for each gene are represented in a scale where red represents upregulation and blue represents downregulation.

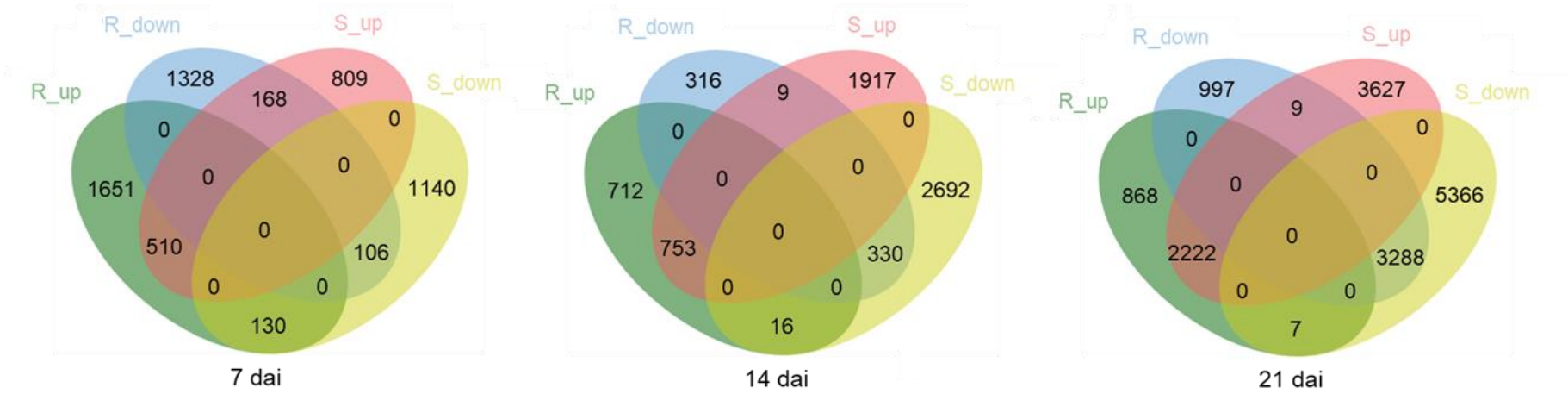

Figure 3.11 Venn diagrams of differentially expressed transcripts in each rutabaga cultivar and time-point. Up, upregulation; down, downregulation; R, 'Wilhelmsburger'; S, 'Laurentian'; dai, days after inoculation.

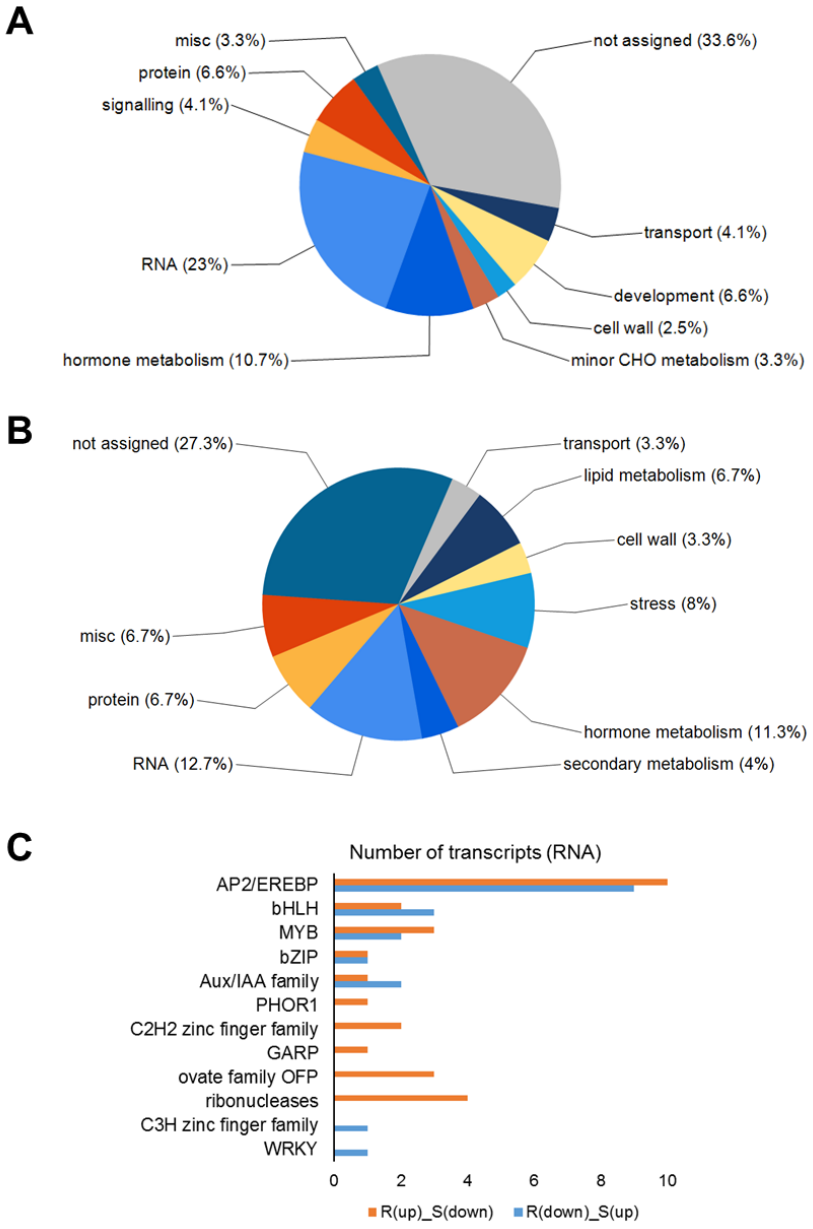

Figure 3.12 Distribution of the number of transcripts showing opposite regulation patterns in the rutabagas 'Wilhelmsburger' (R) and 'Laurentian' (S) at 7 days after inoculation with *Plasmodiophora brassicae*, using Mapman annotation. (A) Pie diagram generated from transcripts upregulated in 'Wilhlemsburger' and downregulated in 'Laurentian'. (B) Pie diagram generated from transcripts downregulated in 'Wilhelmsburger' and upregulated in 'Laurentian'. (C) Number of transcripts related to RNA regulation.

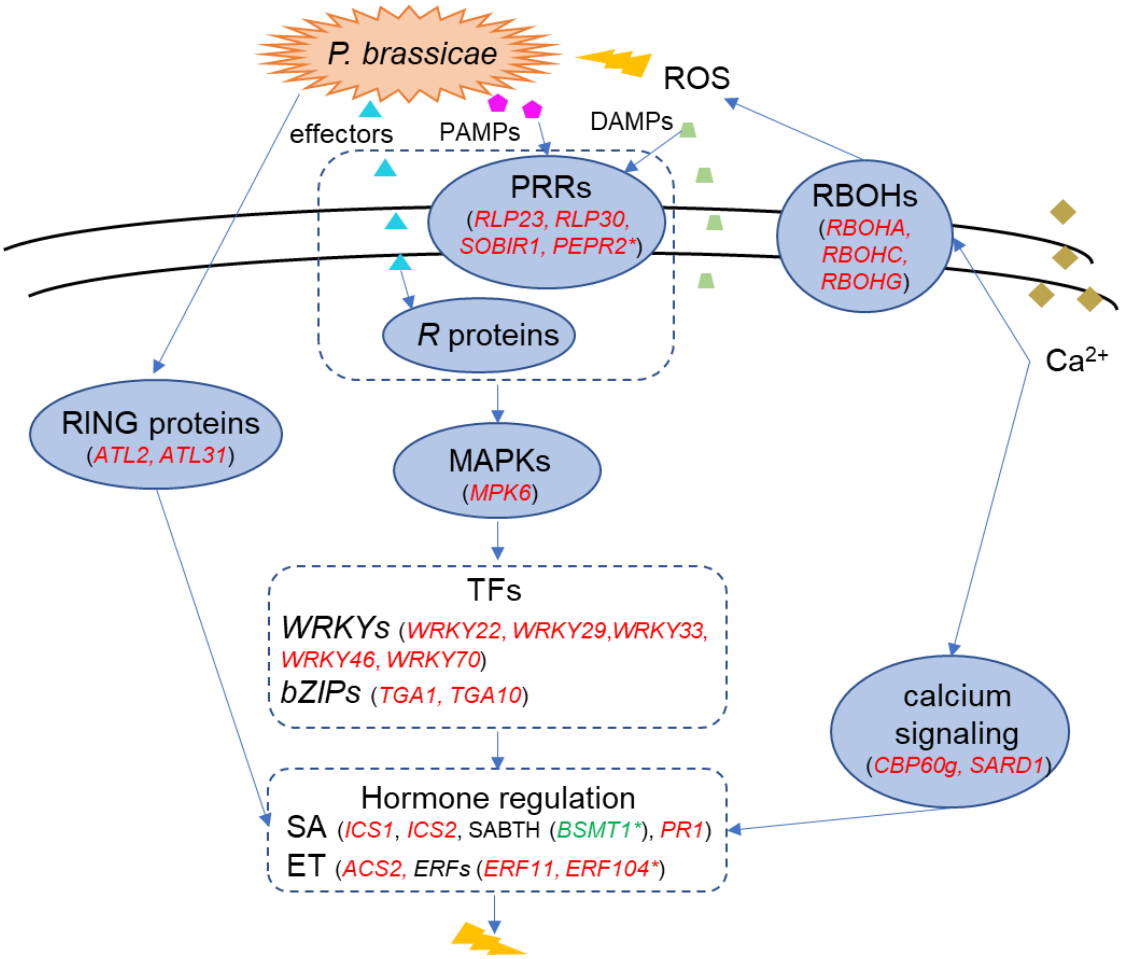

Figure 3.13 Model illustrating the major networks of the resistance response in the rutabaga 'Wilhemsburger' to *Plasmodiophora brassicae* pathotype 3A. Important genes in each functional category are indicated in parentheses. Genes in red are upregulated while those in green are downregulated in the resistant cultivar 'Wilhlemsburger'. Genes marked with an asterisk (*) were inversely regulated in the susceptible cultivar 'Laurentian'. Lightning bolt symbols indicate defense responses.

Chapter 4: Conclusions and future directions

4.1 Introduction

The planting of clubroot resistant (CR) canola (*Brassica napus* L.) is the most effective method to manage *Plasmodiophora brassicae* Woronin (Hwang et al., 2014). However, the widespread cultivation of CR canola in short rotations has exerted significant selection pressure on pathogen populations, resulting in the emergence of 'new' pathotypes of *P. brassicae* able to overcome the genetic resistance in most cultivars (Strelkov et al., 2016). Given that resistance represents the 'backbone' of clubroot management in western Canada (Peng et al., 2014), it is important to find novel ways to accelerate resistance breeding (Chapter 2).

Rutabaga (*B. napus* subsp. *napobrassica*) represents an excellent resistance source for the development of CR canola, as it possesses resistance to multiple pathotypes of *P. brassicae* and is closely related to canola. In a recent assessment, 79 of 124 rutabaga accessions tested were resistant to at least two *P. brassicae* pathotypes from Canada (Fredua-Agyeman et al., 2020). The rutabaga cultivars included in this thesis, 'Laurentian' and 'Wilhelmsburger', show resistance to many pathotypes (Strelkov et al., 2018). However, 'Laurentian' is susceptible to pathotype 3A, which is the predominant resistance-breaking pathotype in western Canada, while 'Wilhelmsburger' is resistant (Strelkov et al., 2018). An understanding of the differential responses of these cultivars may be informative for the development of canola cultivars with resistance to this important pathotype.

4.2 General conclusions

In Chapter 2, I discussed how omics and genome editing could open new opportunities to accelerate clubroot resistance breeding. Genomics and transcriptomics approaches, including single nucleotide polymorphism (SNP) arrays, genotype by sequencing (GBS), genome-wide association studies (GWAS), and bulked segregant RNA-seq (BSR-seq) analysis have been useful in identifying genome-wide SNP markers associated with clubroot resistance. Such information may be complemented by data from RNA-seq studies, which can provide an overview of the molecular responses of hosts to *P. brassicae*. In Chapter 3, I compared the transcriptional responses of 'Laurentian' and 'Wilhelmsburger' to *P. brassicae* pathotype 3A at multiple times after inoculation using RNA-seq technology. The results from this analysis suggested that the activation of receptor-like protein (*RPL*) genes, resistance (*R*) genes and genes involved in salicylic acid (SA) signaling are involved in defense against *P. brassicae* in both cultivars. In addition, genes related to calcium signalling, and genes encoding leucine-rich repeat (LRR) receptor kinases, the respiratory burst oxidase homolog protein, and transcription factors such as WRKYs, ethylene responsive factors and bZIPs, were upregulated in 'Wilhelmsburger', serving to restrict *P. brassicae* development. The comparison of the resistant and susceptible host responses allowed me to identify a set of candidate genes showing contrasting patterns of expression, which may be targeted in future studies aimed at further advancing knowledge and development of resistance.

4.3 Future studies

The results from my thesis may serve as the foundation for additional research into the *P. brassicae*/*B. napus* interaction. This could include not only studies to elucidate resistance/susceptibility mechanisms further, but also to incorporate these data in gene mapping studies, and to apply the CRISPR/Cas9 system to validate candidate genes involved in clubroot resistance. I discuss the details of these possible research directions below.

First, given the importance of this pathosystem, more research is needed to expand knowledge of the *B. napus*-*P. brassicae* interaction. For example, in Chapter 3 we found that SA-mediated responses are important in host defense against pathotype 3A, consistent with a recent report that these responses also are important in 'Laurentian' following inoculation with pathotype 5X (Galindo-González et al., 2020). We also uncovered, however, additional defense responses in 'Wilhelmsburger', indicating that there may be genotype-specific components to the resistance reaction. Therefore, the transcriptomic response of a larger suite of rutabaga (and other *B. napus*) genotypes should be considered. The application of proteomic and metabolomic techniques (Song et al., 2016; Yahaya et al., 2017) may also further advance understanding of the host reaction to clubroot, by complementing transcriptomics data with information at the post-transcriptional level. Moreover, while the focus of Chapter 3 was on the host transcriptome, the generated sequencing reads can also be aligned to the *P. brassicae* genome, enabling transcriptional profiling of the pathogen *in planta*. Ideally, such a study would identify *P. brassicae* genes that co-express with the host genes identified in Chapter 3, allowing inferences regarding molecular interactions.

Second, it is important to incorporate the results generated from this thesis with gene mapping studies. The combination of gene mapping approaches such as SNP arrays, GBS, GWAS and BSR-seq with RNA-seq represents a powerful approach for identifying loci associated with clubroot resistance. In particular, GWAS has been applied to identify resistance loci in rutabaga (Yu, 2019). Further analysis of the genes residing in these loci and genes showing significant changes in expression following *P. brassicae* inoculation could result in the identification of common genes that may be key regulators of clubroot resistance. Such research will also help to explain the resistance mechanisms mediated by certain CR genes/loci.

Last, the CRISPR/Cas9 system could be applied to confirm the roles of candidate genes identified in Chapter 3. In that chapter, I identified a set of candidate genes that showed contrasting patterns of expression between the resistant and susceptible rutabaga cultivars following *P. brassicae* inoculation. However, the functional roles of these genes in mediating clubroot resistance remain to be validated. The CRISPR/Cas9 system represents a powerful tool for this purpose, and it may be worthwhile to explore its application to the *B. napus/P. brassicae* interaction. Ultimately, a multi-disciplinary approach, which makes use of all available tools, will contribute to the sustainable and long-term management of clubroot of canola.

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