University of São Paulo
"Luiz de Queiroz" College of Agriculture

Comparative genomics and transcriptomics analyses reveal genetic complexity of soybean anthracnose caused by *Colletotrichum* species

Thaís Regina Boufleur

Thesis presented to obtain the degree of Doctor in Science.
Area: Plant Pathology

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I dedicate this work with love to

my parents Bernardo and Dirce, my brother Eduardo and
my godparents Laércio (in memorian) and Neiva,
which always supported my dreams.

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"It is not the strongest of the species that survives,
Not the most intelligent that survives.
It is the one that is the most adaptable to change"

- *Charles Darwin.*

CONTENTS

RESUMO

Análises de genômica comparativa e transcriptômica revelam a complexidade genética da antracnose da soja causada por espécies de *Colletotrichum*

O cultivo da soja (*Glycine max*) ocupa em torno de 6% da área plantada em escala mundial. Atualmente, mais de 90 doenças ameaçam a produção dessa cultura, que devido a sua importância global e múltiplos usos, como fonte de alimentação humana e animal, pode implicar na segurança alimentar. Dentre essas doenças está a antracnose, considerada uma doença de etiologia complexa pois pode ser causada por diversas espécies do gênero *Colletotrichum.* Com o advento das tecnologias de sequenciamento de nova geração, o sequenciamento de genomas e transcriptomas se tornou possível. Análises de genômica comparativa podem ser utilizadas para revelar repertórios de candidatos a efetores de importantes patógenos de plantas; por outro lado, o sequenciamento completo dos transcriptomas de plantas e patógenos durante sua interação permite a identificação de genes e vias bioquímicas e metabólicas envolvidas na defesa das plantas contra doenças. Para aprofundar o conhecimento e as informações disponíveis sobre a antracnose da soja e seus agentes causais, foi utilizada uma abordagem combinada entre experimentos biológicas e tecnologias de sequenciamento de nova geração. A revisão de todas as sequencias *ITS* de *Colletotrichum* associadas a soja disponíveis, revelou que mais de 37% delas está associada ao complexo de espécies errado. Ao menos 9 complexos de espécies associados com antracnose da soja foram encontrados, sendo os complexos *C. orchidearum* e *C. truncatum* os mais numerosos e distribuídos no mundo todo. O primeiro relato de *C. musicola*, como agente causal da antracnose da soja foi registrado. Os genomas de *C. sojae*, *C. plurivorum* e *C. musicola,* três espécies pertencentes ao complexo *C. orchidearum* foram sequenciados e comparados ao genoma de *C. truncatum* e oito espécies de *Colletotrichum* não patogênicas a soja. Análises de genômica comparativa sugerem que as espécies pertencentes ao complexo *C. orchidearum* e *C. truncatum* adquiriram a capacidade de infectar soja separadamente, devido à ausência de genes candidatos a efetores secretados compartilhados somente entre as quatro espécies. O sequenciamento do transcriptoma de quatro combinações entre soja/*C. truncatum* com diferentes níveis de resistência a antracnose revelou que a defesa da soja contra *C. truncatum* depende da ativação de centenas de genes após o reconhecimento do patógeno pela planta. Os resultados do revelaram que em interações com maior nível de resistência, a porcentagem do transcriptoma da planta modulado é maior quando comparado a interações mais suscetíveis, independentemente da cultivar de soja ou do genótipo de *C. truncatum*.

Palavras-chave: *Colletotrichum truncatum*, *Colletotrichum orchidearum, Glycine max*, Sequenciamento de RNA, Efetor, Defesa da planta

ABSTRACT

Comparative genomics and transcriptomics analyses reveal genetic complexity of soybean anthracnose caused by *Colletotrichum* species

Soybean (*Glycine max*) cultivation occupies around 6% of the world's arable land. Currently, there are more than 90 diseases that threaten soybean production, due to its global importance and multiple uses, as a source of human and animal feeds, may imply in food security. Among these diseases is soybean anthracnose, a disease of complex etiology which can be caused by several species of the genus *Colletotrichum*. The advent of next-generation sequencing technologies (NGS) allowed the high throughput sequencing of entire genomes and transcriptomes. Comparative genomic analyses are useful to reveal repertoires of effector candidates of important plant pathogens, potentially involved in plant infection; while the deep sequencing of transcriptomes of plant and pathogens during their interaction can allow the identification of important genes and pathways involved in plant defense. To gain insights into soybean anthracnose and its causal agents, we used a combined approach of biological experiments with NGS technologies. A revision of all public-available *Colletotrichum ITS* sequences associated with soybean revealed that more than 37% of those are assigned to the wrong species complex (s.c) level. We found at least 9 s.c. and one singleton species of *Colletotrichum* associated with soybean anthracnose worldwide, being the *C. orchidearum* s.c. and *C. truncatum* the most common and distributed. We reported for the first time that *C. musicola*, a member of the *C. orchidearum* s.c. was associated with soybean. Draft genomes of *C. sojae*, *C. plurivorum* and *C. musicola* were produced and compared with *C. truncatum* and eight additional *Colletotrichum* species not pathogenic to soybean. Comparative genomic analyses suggested that species belonging to the *C. orchidearum* s.c. and *C. truncatum* acquired the capability to infect soybean separately, due to the absence of secreted effector candidates (SECs) shared only among these four species. The transcriptomic sequencing of four different combinations of soybean/*C. truncatum* revealed that soybean defense against *C. truncatum* relies on the activation of several defense genes upon the recognition of the pathogen by the plant. Results revealed a higher modulation of the soybean transcriptome in more resistant interactions, when compared with more susceptible ones, independently of soybean cultivar and *C. truncatum* strain.

Keywords: *Colletotrichum truncatum*, *Colletotrichum orchidearum, Glycine max*, RNA sequencing, Effector, Plant defense

1. GENERAL INTRODUCTION

The improvement of crop production to attend to the increasing demand for food worldwide became one of the major problems to be solved in the XXI century (Tilman et al., 2011). Among those crops is soybean (*Glycine max*), considered one of the most cultivated crops (Shea et al., 2020), corresponding to around 6% of the world's arable land, being this area bigger than any other major crop (Hartman et al., 2011). World's soybean production reached up to 370 million tons in the 2019/20 season, being 80% of it produced in Brazil, the USA and Argentina (USDA, 2021).

Due to the global importance and multiple uses of soybean, as a rich source of oil and protein, soybean losses due to abiotic and biotic stresses may threaten food security (Hartman et al., 2011). There are currently more than 90 biotic diseases that threaten soybean production, among them soybean anthracnose (Hartman, 2015). This seed-borne disease can appear during all the stages of the culture in the field due to the possibility of long asymptomatic periods in infected plants (Yang and Hartman, 2015). In the early stages, characteristic symptoms are pre- and post-emergence damping-off. Symptoms appear in the aerial part of the plants after periods of high relative humidity, as petiole and veins necrosis as well as leaf rolling, which lead to premature defoliation. Lastly, during the early reproductive stages, dark, depressed and irregular spots can appear in pods, directly affecting seed quality and production (Yang and Hartman, 2015).

Over the years, several species of the hemibiotrophic fungus *Colletotrichum* were described as causal agents of soybean anthracnose, that became a disease of complex etiology (Dias et al., 2018). *Colletotrichum truncatum* is the most common associated species (Sharma et al., 2011), but *C. destructivum* (Manandhar et al., 1986), *C. coccodes* (Riccioni et al., 1998), *C. chlorophyti* (Yang et al., 2012, 2013), *C gloeosporioides* (Mahmodi et al., 2013), *C. incanum* (Yang et al., 2014), *C. plurivorum* (Barbieri et al., 2017), *C. sojae* (Damm et al., 2019) and *C. brevisporum* (Shi et al., 2020) were also described as causal agents of the disease.

Disease control rely on accurate species identification of the causal agent. *Colletotrichum* species differentiation based on morphology is unreliable (Cai et al., 2009; Jayawardena, 2016), and simple BLAST searches against the available databases can lead to wrong species identification, as it is estimated that around 10% of all deposited *internal transcribed space* (*ITS*) sequences are assigned to the wrong species (Nilsson et al., 2006). During the past years, with the advent of DNA-based characterizations, the genus *Colletotrichum* underwent many taxonomic revisions (Damm et al., 2009, 2012a, 2012b, 2014, 2019; Cannon et al., 2012; Weir et al., 2012; Liu et al., 2014; Jayawardena, 2016; Marin-Felix

et al., 2017), resulting in more than 200 accepted species classified into species complexes (s.c.) or singletons (Marin-Felix et al., 2017; Damm et al., 2019).

Advances in next-generation sequencing technologies (NGS) allowed the high throughput sequencing of entire genomes (Metzker, 2010) and transcriptomes (Ozsolak and Milos, 2011). The improvement of sequencing technologies, and pipelines for genome assembling and annotation, increased the number of high-quality genomes available (Gibriel et al., 2016). During recent years, at least 42 *Colletotrichum* genomes were published (http://www.colletotrichum.org/genomics/), and this number is increasing every year. The availability of genome sequences opens the possibility of innumerous studies, such as comparative genomic analyses that lead to the characterization of effector repertoires (Bhadauria et al., 2015; Gibriel et al., 2016; Baroncelli et al., 2017; de Queiroz et al., 2019), that may have roles in host-pathogen interactions and imply directly in management strategies of several diseases (Gibriel et al., 2016). On the other hand, RNA sequencing can provide an overview of the transcriptomic changes that occur during plant-pathogen interactions, allowing the identification of genes and pathways involved in different stages of plant defense (Vargas et al., 2012; Alkan et al., 2015; Bhadauria et al., 2017; Zhang et al., 2018).

Despite the growing importance of soybean anthracnose (Dias et al., 2018; Rogério et al., 2019) responsible for several losses worldwid (Wrather et al., 2010; Dias et al., 2016), advances in the research of this disease are just beginning to appear. So far, it is unclear if the association of new *Colletotrichum* species with the disease is related to emerging species or if it is due to the undergoing changes in the taxonomy of the genus. Multiple studies identified commercial soybean cultivars with different levels of resistance to anthracnose (Costa et al., 2009; Nagaraj et al., 2014; Dias et al., 2019), however, the genetic mechanisms that support soybean resistance, or *Colletotrichum* pathogenicity have not yet been investigated. To gain a better understanding of soybean anthracnose and its causal agents, this work aimed to:

- clarify which *Colletotrichum* species or complexes are associated with the disease and provide a comprehensive review for future studies on soybean anthracnose **(Chapter 2);**
- report for the first time that *Colletotrichum musicola* was found causing soybean anthracnose in Brazil **(Chapter 3);**
- produce draft genomes of three *Colletotrichum* species associated with soybean anthracnose **(Chapter 4)**;

- identify through a comparative computational approach, secreted effector candidates of four species of *Colletotrichum* related to soybean anthracnose **(Chapter 5)**;
- Identify genes and/or pathways involved in soybean defense against *C. truncatum* **(Chapter 6)**.

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2. SOYBEAN ANTHRACNOSE CAUSED BY *Colletotrichum* SPECIES: CURRENT STATUS AND FUTURE PROSPECTS

ABSTRACT

Soybean (*Glycine max*) is one of the most important cultivated plant worldwide as a source of protein-rich foods and animal feeds. Anthracnose, caused by different lineages of the hemibiotrophic fungus *Colletotrichum*, is one of the main limiting factors to soybean production. Losses due to anthracnose have been neglected, but their impact may threaten up to 50% of the grain production. While *C. truncatum* is considered the main species associated with soybean anthracnose, recently other species have been reported as pathogenic to this host. Until now, it has not been clear whether the association of new *Colletotrichum* species with the disease is related to emerging species or whether it is due to the undergoing changes in the taxonomy of the genus. Typical anthracnose symptoms are pre- and post-emergence damping-off; dark, depressed and irregular spots on cotyledons, stems, petioles and pods, and necrotic laminar veins on leaves that can result in premature defoliation. Symptoms may still evolve to pod rot, immature opening of pods, and premature germination of grains. As accurate species identification of the causal agent is decisive for disease control and prevention, in this work, we review the taxonomic designation of *Colletotrichum* isolated from soybean to understand which lineages are pathogenic to this host. We also present a comprehensive literature review of soybean anthracnose, focusing on distribution, symptomatology, epidemiology, disease management, identification and diagnosis. We also consider the knowledge emerging from population studies and comparative genomics of *Colletotrichum* spp. associated with soybean providing future perspectives in the identification of molecular factors involved in the pathogenicity process.

Keywords: *Glycine max*; *Glomerella*; Fungal pathogens, *Colletotrichum truncatum*, Emerging Diseases.

2.1 INTRODUCTION

The genus *Colletotrichum* constitutes a large monophyletic group of ascomycetes with more than 200 accepted species, classified into at least 14 species complexes (s.c.) and singletons (Marin-Felix *et al.*, 2017; Damm *et al.*, 2019). Considered one of the top 10 plant pathogenic fungi, *Colletotrichum* spp. is the causal agent of anthracnose in more than 3,000 plant species, leading to considerable yield reduction of economically important crops (Cannon *et al.*, 2012; Dean *et al.*, 2012; da Silva *et al.*, 2020). Due to its hemibiotrophic lifestyle and the facility of being manipulated in the laboratory, the genus is considered a model pathogen for biochemical, physiological and genetic studies (Perfect *et al.*, 1999; O'Connell *et al.,* 2012; Baroncelli *et al.*, 2017).

Soybean crop has great importance worldwide as a source of vegetable oil and proteins for human and animal feeds (Hartman *et al.*, 2011; Pagano and Miransari, 2016), contributing with 3.3% of the global human calorie intake (FAOSTAT, 2018) . In 2019/20, world soybean

production exceeded 330 million tons, of which approximately 86% were concentrated in Brazil, the United States and Argentina (USDA, 2020). Diseases are among the major factors that can affect soybean production, and anthracnose is becoming a major threat in producing areas around the world (Wrather *et al.*, 2010; Hartman *et al.*, 2015; Dias *et al.*, 2016; Subedi *et al.*, 2016; Nataraj *et al.*, 2020). This disease can reach up to 100% of incidence in the field (Hartman *et al.,* 1999), and incidence as low as 1% can cause yield losses of up to 90kg/ha (Dias *et al.,* 2016).

Soybean anthracnose is currently recognized as a disease of complex etiology (Dias *et al.*, 2018), with *C. truncatum* the most common associated species (Sharma *et al.*, 2011). During the past years, several other species have been reported as causal agents of the disease, such as *C. destructivum* (Manandhar *et al.*, 1986), *C. coccodes* (Riccioni *et al.*, 1998)*, C. chlorophyti* (Yang *et al.*, 2012, 2013)*, C. gloeosporioides* (Mahmodi *et al.*, 2013)*, C. incanum* (Yang *et al.*, 2014)*, C. plurivorum* (Barbieri *et al.*, 2017)*, C. sojae* (Damm *et al.*, 2019) and more recently, *C. musicola* (Boufleur *et al.*, 2020) and *C. brevisporum* (Shi *et al.,* 2020).

Colletotrichum species can affect soybean in all physiological stages (Sharma *et al*., 2011). Typical symptoms of anthracnose are pre and post-emergence damping-off, dark, depressed and irregular spots on stems, petioles and pods that can evolve and cause premature defoliation of the plants (Yang *et al.*, 2015). Such infections have the potential to cause severe losses that can reach up to 100% in soybean-producing areas under favorable conditions (Yang and Hartman, 2016).

An accurate species identification of a causal agent is decisive for disease control and prevention. The occurrence of multiple *Colletotrichum* species associated with soybean anthracnose may affect disease management since distinct species might respond differently to different control strategies. Different studies about the efficiency of fungicides in the control of soybean anthracnose showed contradictory results (Dias *et al.*, 2016; Chen *et al.*, 2018; Poti *et al.*, 2020), which could be due to different responses of *Colletotrichum* species to their active compounds.

Few discriminatory morphological characters are available, and the identification of *Colletotrichum* species based exclusively on these features is unreliable (Cai *et al.*, 2009; Jayawardena *et al.*, 2016). Currently, species identification of this genus is performed using a polyphasic approach that combines morphological and cultural characteristics with multilocus phylogenetic analyses of DNA sequences (Cai *et al.*, 2009; Liu *et al.*, 2016).

Several species within the *Colletotrichum* genus show a wide genetic variability however, the mechanisms responsible for such diversity are not yet fully understood (da Silva *et al.*, 2020).

Some population genetics studies of *Colletotrichum* species have offered tools for improving prevention and management strategies for plant diseases of important agricultural crops (Ureña-Padilla *et al.*, 2002; Ciampi-Guillardi *et al.*, 2014; Baroncelli *et al.*, 2015; Rogério *et al.*, 2019).

Many unresolved questions about soybean anthracnose remain. Until now, it is not clear whether the association of new *Colletotrichum* species with the disease is related to emerging species or whether it is due to the undergoing changes in the taxonomy of the genus. Most of the studies available for soybean anthracnose are focused on *C. truncatum*, with limited information about the other *Colletotrichum* species infecting this crop, which could reflect on obstacles during the management of the disease in the field. The aim of this work is to gain a better understanding of soybean anthracnose and its causal agents, clarify which *Colletotrichum* species or complexes are associated with the disease and provide a comprehensive review for future studies on soybean anthracnose.

2.2 *COlletotrichum* SPECIES ASSOCIATED WITH SOYBEAN, LIFESTYLE AND GEOGRAPHIC DISTRIBUTION

The lack of reliable morphological characteristics has transformed the identification of *Colletotrichum* spp. in a major challenge over the years (Cai *et al.*, 2009), leading to considerable taxonomic confusion. After the advent of DNA-based characterization, the taxonomy and nomenclature of *Colletotrichum* spp. underwent many revisions (Damm *et al.*, 2009; 2012a; 2012b; Cannon *et al.*, 2012; Weir *et al.*, 2012; Damm *et al.*, 2014; Liu *et al.*, 2014; Jayawardena, 2016; Marin-Felix *et al.*, 2017; Damm *et al.*, 2019). Currently, there are more than 200 recognized *Colletotrichum* species, either as singletons (ST) or as part of 14 s.c. (Marin-Felix *et al.*, 2017; Damm *et al.*, 2019). Classification of *Colletotrichum* into species complexes can be done using the *ITS-5.8S* rRNA region, a correct species identification requires a multilocus approach, with distinct complexes demanding different loci to be analyzed (Marin-Felix *et al.*, 2017; Damm et al., 2019).

Soybean anthracnose was first described in Korea in 1917, associated with *C. glycines* (Nakata and Takimoto, 1934). The same species was also reported as pathogenic to soybean in the USA in 1926 (Lehman and Wolf, 1926). Later, *C. glycines* (a synonym of *C. truncatum*) and *Glomerella glycines* were reported on soybean (Sinclair, 1989; Sharma *et al.*, 2011; Damm *et al.*, 2019). Until recently, studies about this disease have been mainly focused on *C. truncatum*. On the other hand, the *C. orchidearum* s.c. was described only recently (Damm *et al.*, 2019), and three species within this complex have already been reported as pathogenic to

soybean (Lehman and Wolf, 1926; Barbieri *et al.*, 2017; Boufleur *et al.*, 2020). The recent massive taxonomic revision of this genus has led to an increase of taxonomically wrongly assigned *ITS* sequences deposited in GenBank (Crouch *et al.*, 2009a; Damm *et al.*, 2009). To clarify the *Colletotrichum* s.c. associated with soybean anthracnose, and the worldwide distribution of this disease, all public available *ITS* sequences of *Colletotrichum* isolated from soybean and relative information were retrieved and compared with those of reference isolates through a phylogenetic analysis.

The *in-silico* screening for *Colletotrichum* species isolated from symptomatic and asymptomatic soybean plants whose nucleotide are deposited in GenBank yielded 499 ITS sequences originally assigned to eight *Colletotrichum* s.c. (*C. acutatum*, *C. boninense*, *C. dematium*, *C. gloeosporioides*, *C. magnum*, *C. orchidearum*, *C. spaethianum* and *C. truncatum*) and one ST species (*C. chlorophyti*) (Table S1). While it has been shown that around 10% of all deposited *ITS* sequences are assigned to the wrong species (Nilsson *et al.*, 2006), this number seems to be much higher in the case of *Colletotrichum.* In our dataset more than 37% of the sequences were wrongly assigned to the species-complex and therefore the proportion could be higher at the species level (Supplementary Table 1). Our analyses suggest that *Colletotrichum* strains isolated from soybean belong to nine complexes *C. acutatum*, *C. boninense*, *C. dematium*, *C. gloeosporioides, C. gigasporum*, *C. magnum*, *C. orchidearum, C. spaethianum* and *C. truncatum*) and one ST species (*C. chlorophyti*) (Figure 1). To our knowledge this is the first time that species belonging to the *C. gigasporum* s.c. have been associated with soybean (Table 1).

Although several species complexes have been associated with soybean (Figure 1), it was not possible to confirm if the strains belonging to the *C. acutatum*, *C. boninense* and *C. gigasporum* s.c. are truly pathogenic to soybean, as all the sequences retrieved that belong to those complexes came from asymptomatic plants or information related to pathogenicity were not available. It is known that *Colletotrichum* can go through a quiescent phase before the development of disease symptoms on the host (Prusky, 1996; Prusky *et al.*, 2013; de Silva *et al.*, 2017) and can live inside non-host plant tissues as endophytes (da Silva *et al.*, 2020). On the other hand, strains belonging to the *C. dematium*, *C. magnum, C. gloeosporioides*, *C. orchidearum, C. truncatum* s.c.; *C. chlorophyti* have been confirmed to be pathogenic (Table 1).

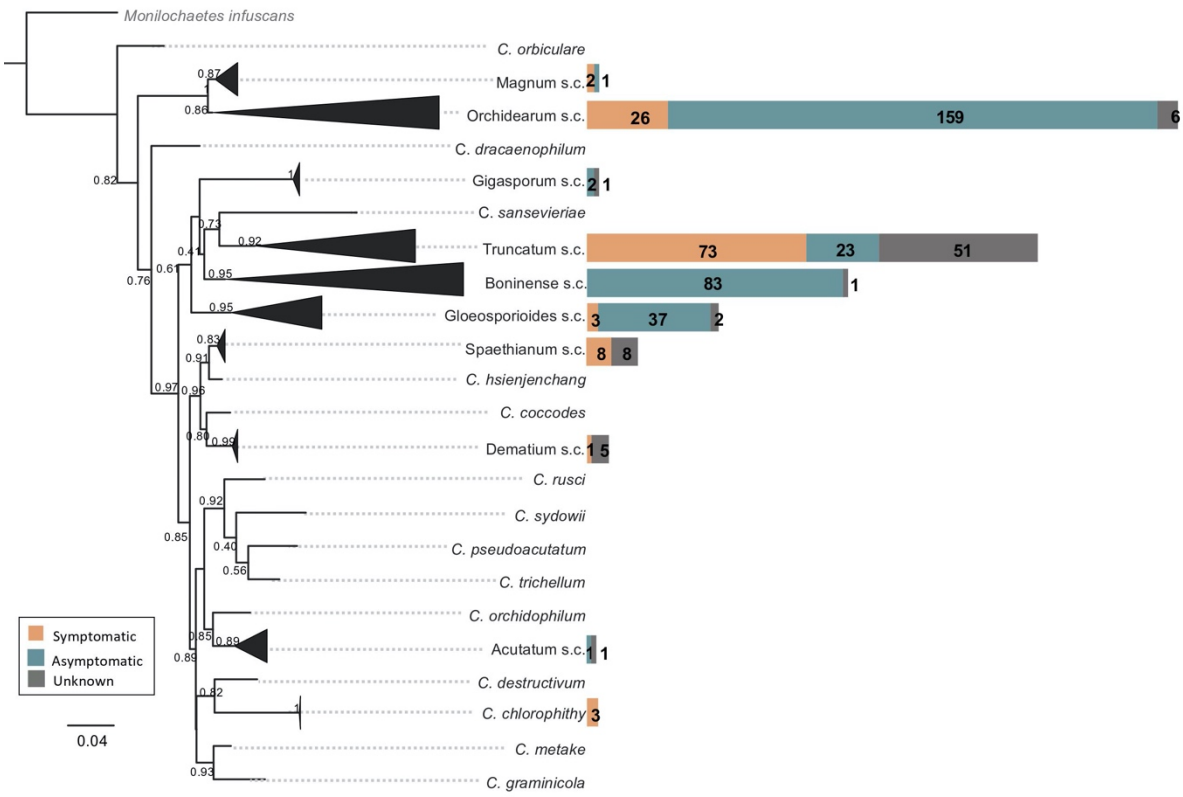

Figure 1: *Colletotrichum* species complexes (s.c.) associated with soybean worldwide identified based on a Bayesian phylogenetic analysis of ITS sequences. *Colletotrichum* s.c. associated with symptomatic, asymptomatic and unknown soybean plants are indicated by bars of distinct colors. All sequences were aligned using MAFFT 7.450 (Katoh, 2002; Katoh and Standley, 2013) and the multiple sequence alignment was exported to MEGA 10 (Stecher *et al.*, 2020), in which the best-fit substitution model was calculated for the sequence dataset. The concatenated alignment was performed with Geneious 2020.0.4 (https://www.geneious.com). A Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with Bayesian probabilities with MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001), based on the model of nucleotide substitution. The analyses were run from random trees for 5,000,000 numbers of generations and sampled every 1000 generations. The concatenated tree was compressed in FigTree 1.4.4 (Rambaut, 2014).

Colletotrichum coccodes and *C. destructivum* were also reported to be pathogenic to soybean (Manandhar *et al.*, 1986; Riccioni *et al.*, 1998), but no genetic information is available and therefore we could not confirm the taxonomic designation of the strains used in those studies. *Colletotrichum coccodes* is a singleton species isolated from soybean in the USA (Riccioni *et al.*, 1998) and *C. destructivum* was reported for the first time as pathogenic to soybean in the 1980s, associated with the sexual morph *G. glycines* (Manandhar *et al.*, 1986); thus this species was recently reclassified as *C. sojae* (Damm *et al.*, 2019). One of the most important soybean diseases in Argentina, the third major soybean producer of the world

(USDA, 2020), is the late season disease complex (LSDs) that includes soybean anthracnose caused by *C. truncatum* and *C. destructivum* (Ramos *et al.*, 2010, 2013). However, the real association of *C. destructivum* with the disease remains unclear, since those species were identified only based on morphological characteristics (Ramos *et al.*, 2013).

Sixteen countries or regions around the world reported the presence of at least one *Colletotrichum* lineage associated with soybean. Brazil, the USA and Taiwan have the largest diversity, followed by China, Colombia, Canada and Myanmar (Figure 2). The species complexes *C. orchidearum* and *C. truncatum* were the most widely distributed worldwide, present in nine countries, with the largest number of *ITS* sequences retrieved followed by the *C. gloeosporioides* s.c. present in four different countries (Figures 1-2). Whereas, the other species complexes seem to be restricted to one or two countries (Figure 2). Considering the number of records and the distribution of the *C. orchidearum* s.c. strains isolated since 2003 (Supplementary Table 1) and their presence in several soybean-producing countries, probably this complex is more important in the epidemiology of the disease than it is currently assumed, thus more studies need to be performed in order to understand its distribution, host specificity and impact in soybean fields around the world.

How many and which *Colletotrichum* species can be pathogenic or endophytic to soybean is still unclear. Historically, *C. truncatum* has been considered the prevalent species isolated from soybean or associated with soybean anthracnose (Sharma *et al.*, 2011). However, many questions remain open, and based on the genetic data available (Supplementary Table 1), we can hypothesize that the importance of *C. truncatum* has been overestimated. Precise knowledge about pathogen taxonomic designation and diversity are is crucial, having direct implications on disease management, either by cultural or chemical strategies as well as to disease resistance breeding programs (Chen *et al.*, 2018).

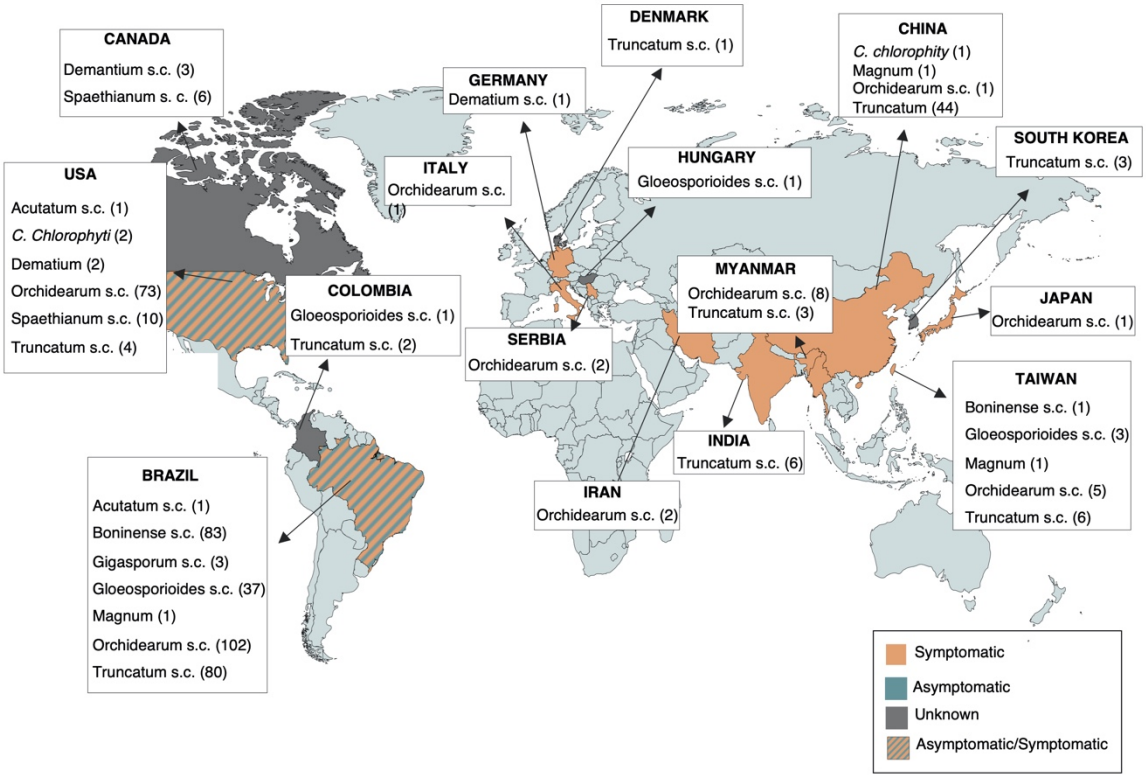

Figure 2: Global distribution of the Colletotrichum s.c. associated with soybean based on the information downloaded with sequences from GenBank. The number of isolates in each country or region are presented on the side of the names of the respective species complex. Countries or regions with the presence of symptomatic, asymptomatic and unknown soybean plants are indicated by different colors.

2.3. SYMPTOMATOLOGY AND EPIDEMIOLOGY

Favored by warm and humid conditions typical anthracnose symptoms can appear on all parts of soybean plants and in all physiological stages (Yang and Hartman, 2016). Systemic infections on seeds can cause pre and/or post-emergence damping-off and cotyledon lesions (Sharma *et al.*, 2011). Symptoms are commonly characterized by dark, depressed and irregular spots on stems, petioles and pods. Symptomatic leaves may be shrunken, rolled or wilted, and may have necrotic laminar veins, resulting in premature defoliation of the plants (Figure 3) (Yang *et al.*, 2015). This pattern of symptoms is reported for *C. truncatum*, *C. coccodes, C. gloeosporioides, C. plurivorum, C. musicola* and *C. incanum* (Riccioni *et al.,* 1998; Sharma *et al*., 2011; Mahmodi *et al*., 2013; Yang *et al.,* 2014; Dias *et al.,* 2018; Boufleur *et al.,* 2020), while *C. sojae* causes circular to irregularly grayish lesions with dark margins (Damm *et al.,* 2019), and *C. chlorophyti* cause necrotic lesions intra- and interveinal surrounded by slight chlorosis (Yang *et al.*, 2012).

Figure 3: Typical symptoms and signs of *C. truncatum* on infected soybean seeds (A-C). Dark, depressed and irregular spots on cotyledons, stem, petioles and pods (D-F).

Except for *C. truncatum,* almost no information is available on the life cycle of other *Colletotrichum* spp. associated with soybean anthracnose. *Colletotrichum truncatum* survives on seeds, crop residues, weeds, and can form soybean-infective microsclerotia (Hartman *et al.*, 1986; Khan and Sinclair, 1991; Yang and Hartman, 2016). Although the role of weeds and alternative hosts in the epidemiology of the disease is still unclear, probably the main source of primary inoculum of *C. truncatum* and *C. plurivorum* are infected seeds, which contribute to dispersion over long distances and the introduction of new fungal isolates in an area (Hartman *et al.,* 1999; Dias *et al.*, 2018).

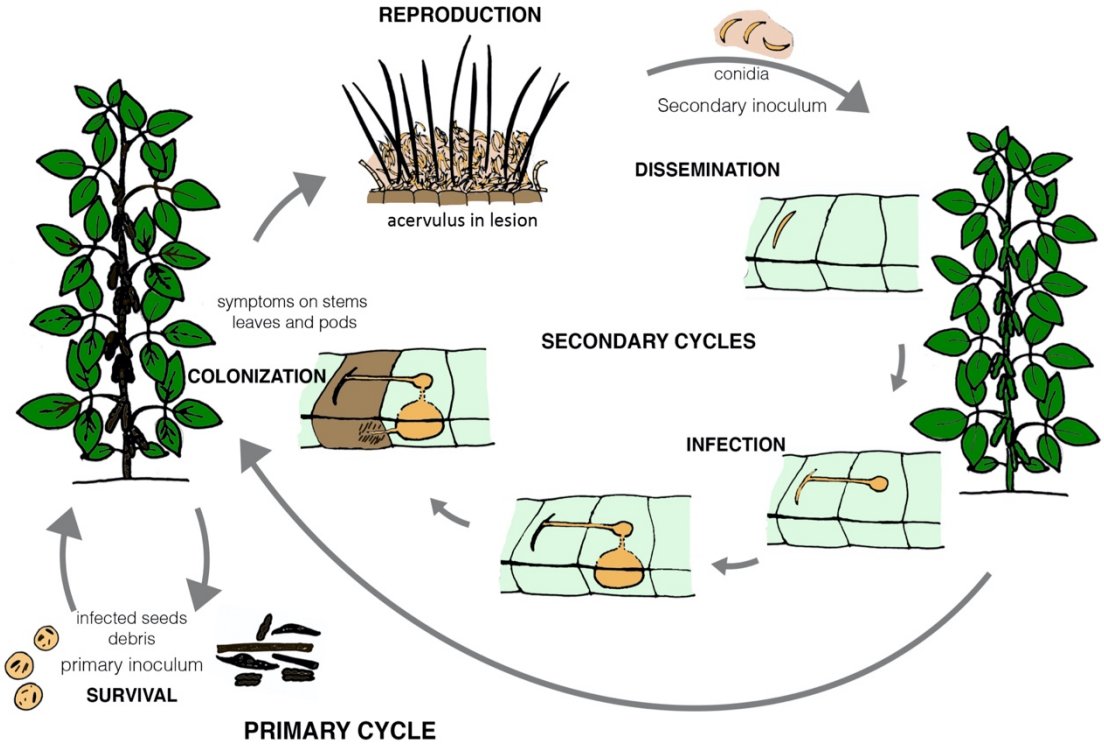

Figure 4: Disease cycle of soybean anthracnose caused by *Colletotrichum truncatum*.

Fungal penetration occurs directly after conidial germination and formation of an appressorium on the plant surface. *Colletotrichum truncatum* and *C. sojae* have the same patterns of infection and colonization of soybean leaves (Manandhar *et al.*, 1985). *Colletotrichum truncatum* have a hemibiotrophic lifestyle with a first stage on which the penetration peg develops into typical primary hyphae, that is a biotrophic vesicle between the cell wall and plasma membrane. The biotrophic phase is followed by a switch to the necrotrophic phase, with the production of secondary hyphae that colonize the tissue intra- and intercellularly causing cell death (Bhadauria *et al.*, 2013). The possibility of quiescent/endophytic infection is also reported (Bhadauria *et al.*, 2013), consequently, plant tissues can be infected without showing any symptoms (Chen *et al.*, 2006). Symptoms of anthracnose appear during the necrotrophic stage, on which occurs the formation of acervuli containing conidia. The conidia represent the secondary inoculum of the disease, disseminated by water splash that dissolve the mucilage in which they are covered, and aids short-range dispersal (Madden, 1997). The life cycle of *C. truncatum* is illustrated in Figure 4.

Understanding the relative importance of each species complex in the development of the disease in the field is fundamental to direct epidemiological studies, which are essential for its effective management. Some species associated with soybean anthracnose, such as *C. sojae*, *C. plurivorum*, and *C. musicola* have been reported to undergo the sexual state (Ramos *et al.*, 2013; Damm *et al.*, 2019; Boufleur *et al.*, 2020). *Glomerella glycines* (now *C. sojae*) was considered the sexual morph of at least three species of *Colletotrichum* in the past, creating a taxonomic confusion that was solved by Damm et al (2019), while others like *C. truncatum* and *C. destructivum* only occur in the asexual morph (Cannon *et al.*, 2012; Damm *et al.*, 2014). Can species with a sexual state have greater survivability on alternative hosts? Can genetic recombination play a role in survival or be an important source of variability in these species? Could sexual spores be spread differently from conidia? Do ascospores and conidia infect soybean tissues in the same way? These are some of the questions about the pathogen's epidemiology that still need answers as they directly impact the disease management.

2.4. DISEASE MANAGEMENT

The need for improvement of the disease management that motivated all efforts for a better understanding of soybean anthracnose proposed in this paper. The poor understanding of the life cycle and the epidemiological role of the *Colletotrichum* spp. associated with soybean may have led to the ineffective disease management, since it is not clear which species are responsible for the disease in the field.

Due to the potential for off-season survival of species of *Colletotrichum* that infect soybean, and the long-distance dissemination of the pathogen by seeds (Yang and Hartman, 2016) the management of soybean anthracnose should start with sowing disease-free seeds (Pellegrino *et al.*, 2010) and practicing crop-rotation. In most cases, seeds are symptomless and even low percentages of infection may lead to severe crop losses (Pellegrino *et al.*, 2010; Ciampi-Guillardi *et al.*, 2020). To prevent the disease, seeds can be treated with systemic fungicides such as carboxanilide; dimethyldithiocarbamate; benzimidazoles or triazoles (AGROFIT, 2020). Also, research on potential strategies of biological control with bio-priming have been performed. Soybean seeds inoculated with *Pseudomonas aeruginosa* and *Trichoderma harzianum* reduced *C. truncatum* field incidence up to 92%, offering the same efficiency as the fungicide benomyl (Begum *et al.*, 2010).

Currently, fungicides used as preventives are azoxystrobin, captan, mancozeb, carbendazim, thiophanate methyl and members of the sterol demethylation inhibitors (DMI),

such as triazoles (Dias *et al.*, 2016; Nataraj *et al.*, 2020; Poti *et al.*, 2020). However, in recent years several studies showed that fungicide efficiency is gradually reducing against soybean anthracnose (Dias *et al.*, 2016; Poti *et al.*, 2020).

In Brazil, when two seasons of soybean production were evaluated, the chemical control with the use of triazoles combined with strobilurins was efficient during the first season, but not during the second season under natural *Colletotrichum* spp. infection (Dias *et al.*, 2016). The resistance of *C. truncatum* isolates to multiple triazoles (flutriafol, fenbuconazole, tebuconazole and metconazole) and reduced sensitivity to difenoconazole and propiconazole have been reported, indicating an inherent resistance as a result of *CYP51A* and *CYP51B* gene variations (Chen *et al.*, 2018).

Carbendazim is a fungicide of the class of benzimidazole or methyl benzimidazole carbamate (MBC), which acts as a single-site inhibitor (Oliver and Hewitt, 2014). Different studies showed that carbendazim is the most effective fungicide reducing *C. truncatum* growth *in vitro* (Kale and Barhate, 2016; Ahamad *et al.*, 2018; Agam *et al.*, 2019). During two seasons of soybean production in Nepal, *in vivo* trials showed that carbendazim (12%) combined with mancozeb (63%) reduced the disease incidence and increased the yield of the treated plots when compared with the control; this was considered the best treatment among the tested fungicides (Subedi *et al.,* 2016). On the other hand, in a study with 52 *C. truncatum* isolates from different fields, 89% of them were considered highly resistant to carbendazim (EC_{50} > 1,000 µg/mL) and 86% showed a mutation at codon 198 of the *TUB2* gene, that prevents a hydrogen bond between carbendazim and *TUB2* and it is highly correlated with resistant fungi strains (Cai *et al.*, 2015; Poti *et al.*, 2020).

Despite that studies on the efficiency of fungicides against anthracnose in the field look promising, it remains unclear which *Colletotrichum* species are responsible for the disease. This may explain the contradictory results found in different field studies. The loss of efficacy has made producers intensify fungicide applications and doses, increasing their costs and inducing stronger selective pressure on the pathogen (Poti *et al.*, 2020). The correct identification of the causal agent of anthracnose is important to explain the real reason for the lack of efficiency of the active compounds in the field.

Besides the potential of losses due to infection by *Colletotrichum* species (Wrather *et al.*, 2010), there are no breeding programs for soybean cultivars resistant to anthracnose until now (Yang and Hartman, 2015). The implementation of resistant cultivars can generally reduce production costs and are a more ecofriendly solution when compared with chemical control (Talhinhas *et al.*, 2016).

Anthracnose resistance genes tend to be highly specific, and the emergence of additional *Colletotrichum* species associated with the disease in soybean-producing areas indicates the need to start focused programs (Dias *et al,* 2018). The inheritance of resistance to soybean anthracnose caused by *C. truncatum* was demonstrated to be governed by more than one gene (Nataraj *et al.*, 2020). In a test with 16 soybean accessions inoculated with a mixture of *C. truncatum* isolates, Dias *et al*. (2019) found that some soybean genetic materials with a high level of resistance in stems are highly susceptible to cotyledon infection. They hypothesized that the genetic resistance at cotyledons and stems might be under the control of genetically independent mechanisms. Sources of resistance of soybean to anthracnose caused by *C. truncatum* have been reported and studies in Brazil, India and the USA revealed that 22 commercial cultivars, nine genotypes and one soybean germplasm are highly resistant to *C. truncatum* (Costa *et al.*, 2009; Nagaraj *et al.*, 2014; Yang and Hartman, 2015; Dias *et al.*, 2019; Nataraj *et al.*, 2020). Research on sources of resistance to other *Colletotrichum* species associated with anthracnose have not yet been performed.

2.5. IDENTIFICATION AND MOLECULAR DIAGNOSTICS

Accurate identification of *Colletotrichum* strains to the species level is decisive in plant pathology with regard to fungal detection in propagative host material, quarantine measures, selection of biocontrol agents, screening varieties in plant breeding, population genetics, and genomics (Jayawardena, 2016). If cryptic species are to be confused with a single species, the integrity and understanding of the species will be compromised (Batista *et al.*, 2017), whose importance is fundamental in population genetics and genomic studies.

2.5.1 Morphological characters

Most of *Colletotrichum* lineages pathogenic to soybean can been divided into two major groups based on morphology, those that have curved conidia shape, including the *C. spaethianum*, *C. truncatum* and *C. demantium* s.c., plus *C. chlorophyti* (Damm *et al.,* 2009); and those with straight cylindrical conidia shape, including *C. gloeosporioides, C. gigasporum, C. magnum* and *C. orchidearum* s.c (Damm *et al.*, 2012a; 2012b; Weir *et al.*, 2012; Liu *et al.*, 2014; Damm *et al.*, 2019). The main character of the *C. acutatum* s.c. are cylindrical conidia with acute ends (Damm *et al.,* 2012a), while the *C. boninense* s.c have straight cylindrical to clavate conidia (Damm *et al.,* 2012b). Except for the *C. gigasporum* s.c., that have a distinctive morphological feature, with conidia up to 32 µm long and with av. length of 26 µm (Liu *et*

*al.,*2014), morphological characters overlap between species and s.c. associated with soybean, and were fully described before (Damm *et al.*, 2009; 2012a; 2012b; Weir *et al.*, 2012; Liu *et al.*, 2014; Damm *et al.*, 2019), therefore they should not be used for identification of *Colletotrichum*.

2.5.2 Molecular identification

Considering the importance of the *Colletotrichum* genus as a plant pathogen worldwide, rapid identification of a large collection of *Colletotrichum* isolates is often required. However, there is no consensus on the best molecular markers to discriminate species in each *Colletotrichum* s.c. (Vieira *et al.*, 2020). Currently, there is no minimum or optimal standard set of molecular markers able to discriminate all the *Colletotrichum* s.c. (Marin-Felix *et al.*, 2017; Vieira *et al.*, 2020). In general, five markers are amongst the most used to differentiate species among the distinct *Colletotrichum* s.c., especially those associated with soybean anthracnose: *ITS*, *GAPDH*, *TUB2*, *CHS-1*, and *ACT* (Damm *et al.*, 2009, 2019). Species within the *C. acutatum* s.c. can be effectively differentiated by both *TUB2* and *GAPDH* markers (Damm *et al.,* 2012a), while *GAPDH* alone can recognize all species within the *C. boninense* s.c. (Damm *et al.,* 2012b). Combined gene analysis of *ITS*, *GAPDH*, *CHS-1*, *ACT* and *TUB2* sequences can identify all the species within both the *C. dematium* and *C. gigasporum* s.c. (Liu *et al.*, 2014). For the *C. truncatum* s.c. *GAPDH* is the most informative marker, followed by *TUB2* and *ACT* (Vieira *et al.,* 2020); for *C. spaethianum* and *C. truncatum* s.c., the inclusion of the *HIS3* in the multilocus combination is needed for the precise discrimination of species, whereas this locus is not informative for other complexes (Jayawardena, 2016). The combination of *ITS*, *GAPDH*, *CHS-1*, *HIS3*, *ACT*, and *TUB2* can differentiate species within the *C. orchidearum* s.c. (Damm *et al.*, 2019). Species within the *C. gloeosporioides* s.c. can be distinguished by a combination of *ApMat* and *GS* sequences (Liu *et al.*, 2015).

Since it might be unrealistic for most researchers to sequence multiple loci across a large set of isolates, it is useful to recommend markers with more phylogenetic informativeness (Vieira *et al.,* 2020). In the majority of the 14 *Colletotrichum* species complexes *GAPDH*, *HIS3*, and *TUB2* were found as the most variable and informative markers for discriminating species.

The consensus is that species identification should not be based on BLAST searches of individual fungal sequences on NCBI/GenBank, but on robust phylogenetic analyses instead based on the concordance of multiple gene genealogies, including sequences from type species (Cannon *et al.,* 2012). One reason is that most molecular markers alone do not exhibit sufficient polymorphism to discriminate *Colletotrichum* species, mainly within complexes, so that

variation level among sequences is low. This is especially problematic in species with similar morphological characters that can be easily confused. Another issue is the problem of misidentification in the sequences deposited in NCBI as mentioned before, most likely as a consequence of the recent taxonomic reassessment of the genus that led to a massive increase in incorrectly assigned *ITS* sequences (Rogério *et al.*, 2017). The *ITS* region should not be used singly to describe new *Colletotrichum* taxa, because there is not enough signal for resolving the taxonomy.

Beyond phylogenetic trees, haplotype networks of concatenated sequences could be employed to infer geographical patterns of distribution or even host association among fungal lineages, below the species level. This approach was efficiently carried out in the identification of distinct clusters in *C. truncatum* strains causing soybean anthracnose in Brazil, by identifying groups of lineages associated with other Fabaceae hosts and weeds as well (Rogério *et al.*, 2017).

2.5.3 Molecular diagnosis

In general, molecular diagnostic tests are developed on the basis of molecular markers largely used in phylogenetic studies. Despite some caveats, the most widely used molecular marker to design specific primers to detect fungal pathogens is the nuclear ribosomal cluster (Mancini *et al.*, 2016; Pecchia *et al.*, 2019). Recent studies have pointed out the lack of variation in the *ITS* region needed to develop specific primers for most *Colletotrichum* species (Da Lio *et al.,* 2018). However, the *IGS* region can be an alternative to *ITS*, since it tends to contain more polymorphic sites and has proved to be an efficient marker for detecting *C. lupini* in lupins by polymerase chain reaction (PCR) and could, therefore, be considered as an alternative target for other *Colletotrichum* species (Pecchia *et al.*, 2019).

Molecular diagnosis techniques based on the detection of fungal DNA have been widely used for species-specific detection of *Colletotrichum* associated with soybean anthracnose. PCR is the method of choice in the field of molecular diagnosis of soybean pathogens, as it enables an exponential amplification of the target DNA sequence, making it a fast, efficient and attractive technique (Kumar *et al.*, 2020). Using these molecular techniques, tiny amounts of host samples are sufficient for the detection of *Colletotrichum* in soybean seeds or other plant tissues. Several PCR based strategies are available for these purposes, such as multiplex PCR, loop-mediated isothermal amplification (LAMP), real-time PCR or quantitative PCR (qPCR), droplet digital PCR (ddPCR), among others, using specific primer-pairs and sometimes

excluding the need for DNA extraction (Tian *et al.*, 2017; Wang *et al.*, 2017; Ciampi-Guillardi *et al.*, 2020).

Multiplex qPCR assays have been consistently used for pathogen diagnosis in plant material by allowing the simultaneous amplification of multiple DNA targets in a single reaction (Schena *et al.*, 2017). A highly sensitive multiplex Taqman qPCR assay targeting the *GAPDH* gene was developed to detect and quantify as little as 0.3 pg of *C. truncatum* DNA, along with two other pathogens in soybean seeds (Ciampi-Guillardi *et al.*, 2020). The method was able to access fungal DNA directly from seed soaking solution, amplifying only the target species and not any other fungi commonly associated with soybean seeds. The high specificity of the assay is provided by the internal TaqMan probes, which overcomes the risks of false positives and/or false negatives. For the diagnosis of *C. truncatum*, a multiplex qPCR assay targeting the *cox1* gene had also been proposed to distinguish four *Colletotrichum* species infecting soybean: *C. chlorophyti*, *C. sojae, C. incanum* and *C. truncatum* by using two duplex sets based on melting point temperatures. While successful detection was achieved with 0.1 pg of *C. truncatum* DNA, the assay may not be suitable for field diagnostics, because it was tested only on purified *Colletotrichum* DNA, not on host tissue samples (Yang *et al.*, 2015). Thus, the correct identification of *Colletotrichum* spp. in seeds is essential for diagnostic laboratories and producers, avoiding the introduction and dissemination of the pathogen in soybean fields (Ramiro *et al.*, 2019). To date, there are still no diagnostic tests for all *Colletotrichum* species associated with soybean.

New tools have been developed to quickly detect *Colletotrichum* DNA in host samples. LAMP is a new nucleic acid amplification technology that enables the synthesis of large amounts of DNA in a short period of time with high specificity (Notomi *et al.,* 2000; Fu *et al.,* 2011). It is assumed that it could be a potential alternative to PCR, since LAMP protocol does not require a thermocycler. Despite the great potential attributed to the technique, LAMP has not been widely used for detecting *Colletotrichum* species associated with soybean anthracnose so far. Rapid LAMP diagnostics assays were proposed to detect *C. truncatum*, targeting the large subunit of RNA polymerase II (*Rpb1*) coding gene (Tian *et al.*, 2017) and *C. gloeosporioides*, whose target was a glutamine synthetase (*GS*) gene (Wang *et al.*, 2017) in soybean samples. For *C. truncatum* the detection limit of the LAMP assay was 100 pg/µL of fungal DNA per reaction, a hundred times greater than the amount detected in the qPCR assay proposed by Tian *et al.,* (2017) and more than a thousand times less sensitive than the qPCR assay developed by Ciampi-Guillardi *et al.,* (2020).

An alternative approach would be to identify genomic regions specific to emerging *Colletotrichum* species or even to particular lineages using a computational approach based on whole genome comparison of distinct isolates or lineages. This approach has been successful used to develop specific markers for the detection of *C. lupini* and other plant pathogens (Pieck *et al.*, 2017; Pecchia *et al.*, 2019; Thierry *et al.*, 2020). Specific care must be taken in cases of recently diverged taxa, bearing in mind that it is unlikely that a single genomic region would perfectly meet all the requirements of a specific detection, especially in fungal lineages with very low divergence levels and recent genetic exchanges between them (Thierry *et al.*, 2020).

2.6. POPULATION GENETIC STUDIES

Genetic variability in the form of the presence of different alleles occurring at different frequencies in genes is crucial to provide greater endurance to environmental changes, and to increase species local adaptation over time (Hartl and Clark, 1997; Barrett and Schluter, 2008). Genetic investigation using a group of individuals provides a broader overview of species variability than a study with few individuals (McDonald, 1997) and can be used to make inferences about the predominant mode of pathogens reproduction, and their impact on genotypic diversity (McDonald and Linde, 2002). In recent years, plant pathologists have been interested in investigations of genetic variation in pathogen populations providing tremendous insights into the biology of fungal plant parasites (Giraud *et al.*, 2008).

The knowledge of the genetic structure, i.e., the amount and distribution of genetic variation within and among populations, allow us to investigate the evolutionary forces (gene flow, genetic drift, mutation, and natural selection) acting as modulators of genetic diversity in the populations (Giraud *et al.*, 2008). The evolutionary potential of pathogen populations is directly guided by their genetic diversity (Croll and Laine, 2016). Thus, access to the genetic structure gives information about the evolutionary processes that influenced the plant pathogen populations in the past and provides insights into their future evolutionary potential (McDonald and Linde, 2002). Such information could be useful to optimize the management of resistance genes and fungicides in agriculture, and therefore to control plant diseases more effectively (Zhan, 2009).

Several population genetic studies of *Colletotrichum* species have been published over the years, and these investigations have increased our knowledge of genetic variation of many important agricultural species (Ureña-Padilla *et al.*, 2002; Crouch *et al.,* 2009b; Ciampi-Guillardi *et al.*, 2014; Baroncelli *et al.*, 2015; Banniza *et al.*, 2018; Xavier *et al.*, 2018). Despite

the importance of soybean anthracnose, few studies are available on this pathosystem, and population genetic studies are even more scarce. Previous research mainly focused on genetic differences among *C. truncatum* isolates obtained from a range of hosts using distinct types of genetic markers, which revealed high genetic diversity and possible genetic recombination (Vasconcelos *et al.,* 1994; Ford *et al.,* 2004; Ranathunge *et al.,* 2009; Sant'anna *et al.,* 2010; Sharma 2009; Katoch *et al.,* 2017; Rogério *et al.,* 2017).

Considering that *C. truncatum* was the only fungal species associated with soybean anthracnose in Brazil up to 2007 (Rogério *et al.*, 2017), an investigation of the genetic structure of *C. truncatum* populations in the main soybean production areas was performed (Rogério *et al.*, 2019). High levels of genetic diversity within populations and no evidence of intraregional gene flow were revealed by microsatellite data. This study also suggested that Brazilian *C. truncatum* populations resulted from at least three founder events, which led to three genetic groups that spread throughout the country, conserving syntopy (Rogério *et al.*, 2019). Another study investigated populations of *C. truncatum* from Brazil and Argentina and identified intra-group similarity greater among the Argentinian isolates than the Brazilian group, with a strong correlation between geographical origin and genetic grouping (Dias *et al.*, 2019). Such a large difference in genetic diversity was associated with a greater geographic breadth of the sampling in Brazilian populations, in addition to a greater genetic variability of host cultivars, which could be reflected in the variability of the isolates among Brazilian regions.

Considering the increase of soybean anthracnose in South America, population studies suggest an association between the inherent variability of the pathogen and the climatic and cultural features, as well the genetic makeup of commercial soybean cultivars used (Dias *et al.*, 2019; Rogério *et al.*, 2019). These population studies highlight the intra-specific pathogen variability as a major feature in the genetic breeding for anthracnose resistance. Efforts into breeding programs aiming at anthracnose resistance should take into account the population structure and the genetic diversity levels of the pathogen, by using representative isolates of the genetic variability of the species for screening soybean resistant cultivars.

Although there have been advances in understanding the genetic variation in *C. truncatum* infecting soybean and its impact on disease management strategies, many gaps have not yet been filled. Expanding the discrimination of genetic groups recently detected and the estimation of recombination rates may provide a potent approach to elucidate the pathogen life history, and to address fundamental questions about the evolution and demographic history of this species (Stukenbrock, 2016; Rogério *et al.*, 2019).

2.7. GENOMICS AS A TOOL FOR UNDERSTANDING PATHOGENICITY FACTORS

Recent technological advances in next-generation sequencing and computational tools have made it possible to sequence and analyze whole genomes of many plant pathogens (Sant'Anna *et al.,* 2010). These technologies continue to advance rapidly, and costs have declined to the point that it is becoming affordable to sequence genomes of many individuals within a species (Raffaele and Kamoun, 2012; Grünwald *et al.*, 2016). This genomic revolution provides a major opportunity to connect the gaps between molecular biology, evolutionary genetics and epidemiology (Plissonneau *et al.*, 2017), playing a key role in plant disease management strategies (Klosterman *et al.*, 2016).

The availability of a large number of genetic markers distributed throughout the genome affords to refine molecular variation investigations. The employment of those markers provides fine-grained regarding processes of genetic divergence, recombination, demography, as well as evolutionary biology of pathogen populations, enabling more robust inferences compared to studies based on a limited number of genetic markers (Brumfield *et al.*, 2003; Luikart *et al.*, 2003; Helyar *et al.*, 2011). Population genomics analyses of a large number of loci offer an excellent opportunity to determine the genetic basis of many fungal phenotypes, including virulence (-Plissonneau *et al.*, 2017; Sarrocco *et al.*, 2020). Furthermore, techniques such as genome-wide association studies (GWAS), quantitative trait loci mapping (QTL), and genome scans for signatures of selection and selective sweeps are powerful tools to identify genes involved in host-specific interactions of fungal pathogens (Grünwald *et al.*, 2016; Plissonneau *et al.*, 2017).

To date, four *Colletotrichum* genomes isolated from symptomatic soybean plants have been sequenced: *C. truncatum* (IMI 507125), *C. plurivorum* (IMI 507127), *C. musicola* (IMI 507128), and *C. sojae* (IMI 507126) (Rogério *et al.*, 2020). Additionally, the genome of two other strains of *C. truncatum*, MTCC 3114 and TYU, isolated from *Capsicum annuum* and *Taxus cuspidata* respectively, and a strain of *C. chlorophyti* (NTL11) from tomato (*Lycopersicum esculentum*) are currently available (Gan *et al.*, 2017; Rao and Nandineni, 2017; Rogério *et al.*, 2020). A summary of the genome assembly statistics is available in Table 2. The pathogenicity of the four *Colletotrichum* spp. sequenced by Rogério *et al.,* (2020) to soybean fulfilled Koch's postulates on soybean. While the other sequenced isolates (MTCC 3114; TYU and NTL11) belong to species pathogenic to soybean,but it was not confirmed if they can infect soybean. These genome sequence data of *Colletotrichum* species pathogenic to soybean

currently available may greatly aid our understanding of host-pathogen interactions, besides offering a useful resource for further research in comparative genomics and evolutionary studies of *Colletotrichum*.

Scanning the *Colletotrichum* genomes for identification of full putative effector repertoires of the pathogen may be a useful tool in the hand of soybean breeders for the development of new cultivars with durable resistance against anthracnose (Oliver and Solomon, 2010; Lenman *et al.,* 2016; Barsoum *et al.,* 2019; Prasad *et al.,* 2019; Van de Wouw and Idnurm, 2019). Although there is no breeding program aimed at screening resistance to soybean anthracnose so far, genomic resources now available may support the development of future programs. Additionally, genome resources can be used to accelerate the development of diagnostic tools for plant pathogens (Klosterman *et al.,* 2016) impacting the application of more accurate management strategies.

Advances in comparative genomics and population genomic approaches open new perspectives to increase our understanding of the molecular mechanisms underpinning pathogenesis and adaptive processes of these pathogens (Stukenbrock and Bataillon, 2012; Klosterman *et al.,* 2016). Mechanisms regarding *C. truncatum* pathogenicity have been investigating in order to understand the factors involved in the pathogenesis of different hosts (Madden, 1997; Ranathunge *et al.*, 2009; Auyong *et al.,* 2012; Auyong, 2015). With the availability of genomic sequences for *Colletotrichum* species pathogenic to soybean, new advances into pathogenic processes at the molecular level are possible, contributing to improving our knowledge in the host-fungal interactions in the soybean-anthracnose pathosystem, and thus, developing effective and novel strategies to combat the pathogens.

2.8. CONCLUSIONS AND FUTURE PERSPECTIVES

Knowing precisely the diversity of the pathogen is crucial from the taxonomic, biological and ecological standpoints. Indeed, pathogen identity has direct implications for disease management, either by cultural or chemical strategies as well as for disease resistance breeding programs . Furthermore, an effective management of new *Colletotrichum* species requires tools to discriminate between emerging and established fungal populations associated with soybean, aiming to detect the pathogens at the earliest point to monitor and limit their spread.

Colletotrichum truncatum has been considered the most important causal agent of soybean anthracnose. However, our survey showed that at least twelve *Colletotrichum* lineages

are associated with soybean, with the *C. truncatum* and *C. orchidearum* s.c. having the greatest impact and the broadest worldwide distribution. Most of the information available on soybean anthracnose until now is limited to C. truncatum. Taking into account the numerous *Colletotrichum* species causing the disease, there is a gap in the knowledge of epidemiology, worldwide movement, distribution, identification, control measures, fungicide efficiency and genetic resistance for all of the species.

In agreement with Vieira et al., (2020), more robust genomic sampling is required to improve our understanding of relationships among taxa in the genus *Colletotrichum*, and also our ability to distinguish species within complexes. Genome data is now available for several *Colletotrichum* species associated with anthracnose in soybean, such as *C. truncatum*, *C. musicola*, *C. plurivorum*, *C. sojae*, *C. chlorophyti* (Rao and Nandineni, 2017; Gan et al., 2017; Rogério et al., 2020), yet a comprehensive phylogenomic study of the genus is still needed. A population genomics approach and comparative genomics investigations can be used for identifying candidate genes involved in pathogenicity, virulence (or aggressiveness), host specialization, fungicide resistance, and adaptation to different environments, with higher precision, contributing to a better understanding of *Colletotrichum* species dynamic in the agroecosystems.

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SUPPORTING INFORMATION

Table S1: Strains of *Colletotrichum* spp. studied, with collection details and GenBank accession numbers

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. lupini	*C. acutatum*	*C. acutatum*	JQ936134	Brazil	2010
C. boninense	*C. boninense*	*C. gloeosporioides*	JX258676	Brazil	2010
C. boninense	*C. boninense*	*C. gloeosporioides*	JX258682	Brazil	2010
C. boninense	*C. boninense*	*C. gloeosporioides*	JX258690	Brazil	2010
C. boninense	*C. boninense*	*C. gloeosporioides*	JX258692	Brazil	2010
C. boninense	*C. boninense*	*C. gloeosporioides*	JX258698	Brazil	2010
C. boninense	*C. boninense*	*C. gloeosporioides*	JX258705	Brazil	2010
C. boninense	*C. boninense*	*C. gloeosporioides*	JX258717	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258773	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	EF503672	Taiwan	-
C. boninense	*C. boninense*	*C. boninense*	JQ936106	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936107	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936108	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936109	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936110	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936169	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936170	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936171	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936172	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936173	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936174	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936175	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936208	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936209	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936307	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JQ936308	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258675	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258694	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258700	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258706	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258707	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258708	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258709	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258710	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258713	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258719	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258728	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258729	Brazil	2010

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. boninense	*C. boninense*	*C. boninense*	JX258740	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258741	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258744	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258745	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258746	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258751	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258752	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258753	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258755	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258756	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258762	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258768	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258769	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258772	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258774	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258775	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258776	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258778	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258779	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258781	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258782	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258783	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258784	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258786	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258788	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258789	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258790	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258791	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258792	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258796	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258799	Brazil	2010
C. boninense	*C. boninense*	*C. truncatum*	JX258714	Brazil	2010
C. boninense	*C. boninense*	*C. truncatum*	JX258747	Brazil	2010
C. boninense	*C. boninense*	*C. orchidearum*	JX258687	Brazil	2010
C. boninense	*C. boninense*	*C. orchidearum*	JX258701	Brazil	2011
C. boninense	*C. boninense*	*C. orchidearum*	JX258703	Brazil	2012
C. boninense	*C. boninense*	*C. orchidearum*	JX258711	Brazil	2013
C. boninense	*C. boninense*	*C. orchidearum*	JX258720	Brazil	2014
C. boninense	*C. boninense*	*C. orchidearum*	JX258724	Brazil	2015

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. boninense	*C. boninense*	*C. orchidearum*	JX258736	Brazil	2016
C. boninense	*C. boninense*	*C. orchidearum*	JX258738	Brazil	2017
C. boninense	*C. boninense*	*C. boninense*	JX258763	Brazil	2010
C. boninense	*C. boninense*	*C. boninense*	JX258766	Brazil	2010
C. dematium	*C. dematium*	*C. dematium*	MT472099	Germany	2019
C. dematium	*C. dematium*	*C. spaethianum*	MT557117	USA	2020
C. dematium	*C. dematium*	*C. spaethianum*	MT557170	USA	2020
C. dematium	*C. dematium*	*C. spaethianum*	MT557302	USA	2020
C. dematium	*C. dematium*	*C. spaethianum*	MT557363	USA	2020
C. dematium	*C. dematium*	*C. spaethianum*	MT557374	USA	2020
C. dematium	*C. dematium*	*C. spaethianum*	MT557460	USA	2020
C. dematium	*C. dematium*	*C. dematium*	MH299930	Canada	2017
C. dematium	*C. dematium*	*C. dematium*	MH299931	Canada	2017
C. dematium	*C. dematium*	*C. dematium*	MH299932	Canada	2017
C. dematium	*C. dematium*	*C. spaethianum*	MK447736	Canada	-
C. dematium	*C. dematium*	*C. spaethianum*	MK447742	Canada	-
C. fragariae	*C. gloeosporioides*	*C. gloeosporioides*	JQ936111	Brazil	2010
C. fragariae	*C. gloeosporioides*	*C. gloeosporioides*	JX258785	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	MT557525	USA	2020
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	MT557555	USA	2020
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	DQ286198	Hungary	2003
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	FJ172223	Taiwan	2003
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	FJ172232	Taiwan	2003
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	FJ172233	Taiwan	2003
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936116	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936118	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936121	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936124	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936125	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936129	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936130	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936214	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936218	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936225	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936229	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JQ936237	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258683	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258684	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258685	Brazil	2010

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258693	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258695	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258696	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258732	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258733	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258764	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258765	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258770	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258771	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258787	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258798	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	JX258803	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gloeosporioides*	MN298754	Colombia	
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258674	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258679	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258681	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258686	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258689	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258691	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258697	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258699	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258702	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258704	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258712	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258716	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258718	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258723	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258727	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258735	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258737	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258739	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. boninense*	JX258743	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. truncatum*	JX258767	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	FJ172224	Taiwan	2003
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	FJ172234	Taiwan	2003
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	FJ172235	Taiwan	2005
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	FJ172236	Taiwan	2005
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	FJ172237	Taiwan	2003
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	FJ185790	Iran	2005

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936112	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936113	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936114	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936115	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936117	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936119	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936120	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936122	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936123	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936126	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936127	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936131	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936132	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936133	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936176	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936177	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936213	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936215	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936216	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936217	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936219	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936220	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936221	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936222	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936223	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936224	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936226	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936227	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936230	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936231	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936232	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936233	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936234	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936235	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936236	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936238	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936239	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936240	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936241	Brazil	2010

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936309	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936310	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936311	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936312	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936313	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936314	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936315	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936316	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936317	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936318	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936319	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936320	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936321	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936322	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936323	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936324	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JQ936325	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258677	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258680	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258688	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258721	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258722	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258725	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258726	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258730	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258731	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258734	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258742	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258757	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258758	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258759	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258760	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258761	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258777	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258780	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258793	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258794	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258795	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258797	Brazil	2010

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258800	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258801	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258802	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. orchidearum*	JX258804	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. magnum*	FJ172225	Taiwan	2005
C. gloeosporioides	*C. gloeosporioides*	*C. magnum*	JX258678	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gigasporum*	JQ936128	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gigasporum*	JQ936228	Brazil	2010
C. gloeosporioides	*C. gloeosporioides*	*C. gigasporum*	KM246279	Brazil	2013
C. brevisporum	*C. magnum*	*C. magnum*	MT361074	China	2020
C. cliviae	*C. orchidearum*	*C. orchidearum*	MG182676	USA	2015
C. musicola	*C. orchidearum*	*C. orchidearum*	Unsequenced	Brazil	2016
C. musicola	*C. orchidearum*	*C. orchidearum*	Unsequenced	Brazil	2017
C. plurivorum	*C. orchidearum*	*C. orchidearum*	LC383779	Myanmar	2017
C. plurivorum	*C. orchidearum*	*C. orchidearum*	LC383780	Myanmar	2017
C. plurivorum	*C. orchidearum*	*C. orchidearum*	LC383781	Myanmar	2017
C. plurivorum	*C. orchidearum*	*C. orchidearum*	KT696336	Brazil	2012
C. plurivorum	*C. orchidearum*	*C. orchidearum*	KT696337	Brazil	2013
C. plurivorum	*C. orchidearum*	*C. orchidearum*	KT696338	Brazil	2013
C. plurivorum	*C. orchidearum*	*C. orchidearum*	KT696339	Brazil	2014
C. plurivorum	*C. orchidearum*	*C. orchidearum*	KT696340	Brazil	2014
C. plurivorum	*C. orchidearum*	*C. orchidearum*	KT696341	Brazil	2009
C. plurivorum	*C. orchidearum*	*C. orchidearum*	LC383782	Myanmar	2017
C. plurivorum	*C. orchidearum*	*C. orchidearum*	LC383783	Myanmar	2017
C. plurivorum	*C. orchidearum*	*C. orchidearum*	MG600725	Japan	2017
C. plurivorum	*C. orchidearum*	*C. orchidearum*	MK142672	Brazil	2013
C. plurivorum	*C. orchidearum*	*C. orchidearum*	MK142673	Brazil	2013
C. sojae	*C. orchidearum*	*C. orchidearum*	KT696354	Brazil	2013
C. sojae	*C. orchidearum*	*C. orchidearum*	MG600749	USA	2017
C. sojae	*C. orchidearum*	*C. orchidearum*	MG600752	Italy	2017
C. sojae	*C. orchidearum*	*C. orchidearum*	MG600753	Serbia	2017
C. sojae	*C. orchidearum*	*C. orchidearum*	MG600754	Serbia	2017
C. sojae	*C. orchidearum*	*C. orchidearum*	MG600755	Iran	2017
Glomerella glycines	*C. orchidearum*	*C. orchidearum*	KC110792	USA	2010
Glomerella glycines	*C. orchidearum*	*C. orchidearum*	KC110793	USA	2010
C. incanum	*C. spaethianum*	*C. spaethianum*	KC110787	USA	2010
C. incanum	*C. spaethianum*	*C. spaethianum*	KC110788	USA	2010
C. spaethianum	*C. spaethianum*	*C. spaethianum*	MH299924	Canada	2017
C. spaethianum	*C. spaethianum*	*C. spaethianum*	MH299925	Canada	2017
C. spaethianum	*C. spaethianum*	*C. spaethianum*	MH299926	Canada	2017

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. spaethianum	*C. spaethianum*	*C. spaethianum*	MH299946	Canada	2017
C. capsici	*C. truncatum*	*C. truncatum*	EF458670	China	-
C. capsici	*C. truncatum*	*C. truncatum*	EF458671	China	-
C. capsici	*C. truncatum*	*C. truncatum*	EF458672	China	-
C. capsici	*C. truncatum*	*C. truncatum*	EF458673	China	-
C. capsici	*C. truncatum*	*C. truncatum*	EF458674	China	-
C. capsici	*C. truncatum*	*C. truncatum*	EF458675	China	-
C. capsici	*C. truncatum*	*C. truncatum*	EF458676	China	-
C. capsici	*C. truncatum*	*C. truncatum*	JQ936210	Brazil	2010
C. capsici	*C. truncatum*	*C. truncatum*	JQ936211	Brazil	2010
C. capsici	*C. truncatum*	*C. truncatum*	JQ936212	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	LC360104	Myanmar	2017
C. truncatum	*C. truncatum*	*C. truncatum*	LC360108	Myanmar	2017
C. truncatum	*C. truncatum*	*C. truncatum*	LC360105	Myanmar	2017
C. truncatum	*C. truncatum*	*C. dematium*	MT557422	USA	2020
C. truncatum	*C. truncatum*	*C. dematium*	MT557438	USA	2020
C. truncatum	*C. truncatum*	*C. boninense*	JX258750	Brazil	2010
C. truncatum	*C. truncatum*	*C. boninense*	JX258754	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	FJ172226	Taiwan	2004
C. truncatum	*C. truncatum*	*C. truncatum*	FJ172227	Taiwan	2003
C. truncatum	*C. truncatum*	*C. truncatum*	FJ172228	Taiwan	2004
C. truncatum	*C. truncatum*	*C. truncatum*	FJ172229	Taiwan	2005
C. truncatum	*C. truncatum*	*C. truncatum*	FJ172230	Taiwan	2005
C. truncatum	*C. truncatum*	*C. truncatum*	FJ172231	Taiwan	2005
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936135	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936136	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936137	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936138	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936139	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936140	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936242	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936243	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936244	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936245	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936246	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936247	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936248	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936249	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JQ936250	Brazil	2010

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. truncatum	*C. truncatum*	*C. truncatum*	JX258748	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	JX258749	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	KC110790	USA	2010
C. truncatum	*C. truncatum*	*C. truncatum*	KC110791	USA	2010
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614291	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614292	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614293	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614294	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614295	Brazil	2005
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614296	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614297	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614298	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614299	Brazil	2007
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614300	Brazil	2007
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614301	Brazil	2007
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614302	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614303	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614304	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614305	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614306	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614307	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614308	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614309	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614310	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614311	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614312	Brazil	2005
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614313	Brazil	2005
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614314	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614315	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614316	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614317	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614318	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614319	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614320	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614321	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614322	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614323	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614324	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614325	Brazil	2004

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614326	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614327	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614328	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614329	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614330	Brazil	
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614331	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614332	Brazil	2004
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614333	Brazil	2002
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614334	Brazil	2006
C. truncatum	*C. truncatum*	*C. truncatum*	KJ614335	Brazil	1992
C. truncatum	*C. truncatum*	*C. truncatum*	KT696342	Brazil	2012
C. truncatum	*C. truncatum*	*C. truncatum*	KT696343	Brazil	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KT696344	Brazil	2013
C. truncatum	*C. truncatum*	*C. truncatum*	KT696345	Brazil	2013
C. truncatum	*C. truncatum*	*C. truncatum*	KT696346	Brazil	2012
C. truncatum	*C. truncatum*	*C. truncatum*	KT696347	Brazil	2012
C. truncatum	*C. truncatum*	*C. truncatum*	KT696348	Brazil	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KT696349	Brazil	2012
C. truncatum	*C. truncatum*	*C. truncatum*	KT696350	Brazil	2013
C. truncatum	*C. truncatum*	*C. truncatum*	KT696351	Brazil	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KT696352	Brazil	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KT696353	Brazil	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KY287672	India	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KY287673	India	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KY287674	India	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KY287675	India	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KY287676	India	2014
C. truncatum	*C. truncatum*	*C. truncatum*	KY287677	India	2014
C. truncatum	*C. truncatum*	*C. truncatum*	MK123447	South Korea	-
C. truncatum	*C. truncatum*	*C. truncatum*	MK123448	South Korea	-
C. truncatum	*C. truncatum*	*C. truncatum*	MK123449	South Korea	-
C. truncatum	*C. truncatum*	*C. truncatum*	MN298752	Colombia	-
C. truncatum	*C. truncatum*	*C. truncatum*	MN298753	Colombia	-
C. truncatum	*C. truncatum*	*C. orchidearum*	JX258715	Brazil	2010
C. truncatum	*C. truncatum*	*C. truncatum*	GU227865	USA	2009
C. truncatum	*C. truncatum*	*C. truncatum*	GU227866	USA	2009
C. truncatum	*C. truncatum*	*C. truncatum*	GU227867	Denmark	2009
C. chlorophyti	None	None	KU594267	China	2016
C. chlorophyti	None	None	JX126475	USA	2009

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
C. chlorophyti	None	None	KC110786	USA	2010
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409134	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409135	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409136	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409137	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409138	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409139	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409140	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409141	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409142	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409143	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409144	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409145	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409146	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409147	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409148	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409149	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409150	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409151	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409152	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409153	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409154	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409155	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409156	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409157	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409158	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409159	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409160	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409161	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409162	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409163	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409164	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409165	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409166	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409167	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409168	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409169	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409170	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409171	USA	2015

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409172	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409173	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409174	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409175	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409176	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409177	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409178	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409179	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409180	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409181	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409182	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409183	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409184	USA	2015
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409185	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409186	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409187	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409188	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409189	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409190	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409191	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409192	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409193	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409194	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409195	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409196	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409197	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409198	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409199	USA	2016
Colletotrichum sp.	Unknown	*C. orchidearum*	MK409200	USA	2016
Colletotrichum sp.	Unknown	*C. gloeosporioides*	MK933713	Brazil	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871589	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871590	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871591	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871592	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871593	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871594	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871595	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871596	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871597	China	2020

Table S1 (Continued)

Submitted as species	Submited as complex	Reclassification	Accession (ITS)	Country	Date
Colletotrichum sp.	Unknown	*C. truncatum*	MN871598	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871599	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871600	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871601	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871602	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871603	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871604	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871605	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871606	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871607	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN871608	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883902	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883903	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883904	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883905	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883906	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883907	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883908	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883909	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883910	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883911	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883912	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883913	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883914	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883915	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883917	China	2020
Colletotrichum sp.	Unknown	*C. truncatum*	MN883918	China	2020
Colletotrichum sp.	Unknown	*C. orchidearum*	MN883916	China	2020
Colletotrichum sp.	Unknown	*C. spaethianum*	MT557198	USA	2020
Colletotrichum sp.	Unknown	*C. spaethianum*	MT557254	USA	2020
Colletotrichum sp.	Unknown	*C. acutatum*	MT557274	USA	2020

3. FIRST REPORT OF *Colletotrichum musicola* CAUSING SOYBEAN ANTHRACNOSE IN BRAZIL

Keywords: *Glycine max*, *Glomerella*, Legumes, Fungal Disease.

Soybean (*Glycine max* L.) is one of the most important crops worldwide as a source of protein-rich foods and animal feeds. Anthracnose, one of the major limiting factors to soybean production (Dias et al., 2016), is caused by species such as *Colletotrichum truncatum, C. sojae,* and *C. plurivorum* (Damm et al., 2009, 2019). In December 2016 and 2017, soybean plants of cultivars Monsoy 8768 and Pioneer y-70 with typical symptoms of anthracnose (necrotic and irregular brown lesions on stems, leaves and pods) were collected in Mato Grosso state, Brazil. Commercial fields sampled showed 10 – 15 % of incidence of anthracnose in each sampled area. In total, 10 different geographic locations were sampled.

Colletotrichum strains were isolated and cultured on potato dextrose agar at 25 ºC with a 12-h light photoperiod from surface-disinfested (70 % alcohol and 0.5 % sodium hypochlorite) plant tissues. Among others, three single-spore isolates (LFN0048 from Sinop, LFN0074 and LFN0090 from Lucas do Rio Verde) showed different morphology; isolates LFN0048 and LFN0074 were selected for further characterization. Total genomic DNA was extracted and partial *GAPDH* (*Glyceraldehyde 3-phosphate dehydrogenase), HIS3* (*histone H3*), and *TUB2* (*beta-tubulin*) genes were amplified and sequenced. The sequences were deposited in GenBank (accession numbers MN604249 and MK163893 for HIS3, MN604248 and MK142674 for *GAPDH*, and MN604250 and MK142675 for *TUB*) and were compared with most similar reference sequences of *Colletotrichum* (Damm et al., 2019). Both isolates clustered with *C. musicola* epitype (CBS 132885), showing 100% and 98.5% similarity in GAPDH, 99.5% and 98.9% in HIS3 and 99.2% in TUB2.

On PDA, colonies showed dark-grey aerial mycelium with entire margins, reverse violaceous-black. Conidia and ascospores size and shape match the previously described by Damm et al. (2019): 12.12 – 15.86 x 4.93 – 6.95 µm and 15.5 – 19.34 x 5 – 7.84 µm, respectively (n = 100). Appressoria (n=50) were single or in loose-groups, violaceous-black with predominant obovoid, truncated and cylindrical shapes, with smooth, undulate or lobate margin, with 9.25 – 29.79 x 7.22 – 21.06 µm in size. Perithecia, paraphyses; and unitunicate 8-spored asci were also observed. Asci were cylindrical to clavate, smooth-walled and 48.12 – 68.78 x 9.59 – 14.47 µm in size (n = 50) (Figure S1).

Soybean anthracnose is seed-borne (Rogério et al., 2017; Dias et al., 2018), therefore pathogenicity tests were carried out in pre-germinated seeds. Five seeds of Brasmax 8579 cultivar were inoculated with 10 µL drop of a conidial suspension (10^6 conidia/mL) placed in the emerging radicle while five mock-inoculated seeds were used as control. Seeds were incubated at 25 ºC with a 12-h photoperiod. After seven days, inoculated plants showed necrotic lesions on the cotyledons, leaflets, and hypocotyl, whereas control plants remained asymptomatic. The experiment was repeated three times. *C. musicola* was re-isolated from all the symptomatic tissues and the identity was confirmed by morphology and multilocus phylogeny (Figure S2).

Until now, *C. musicola* has been reported to be associated with *Musa* sp. (Damm et al., 2019) and *Colocasia esculenta* (Vásquez-López et al., 2019) in Mexico, and with *Phaseolous lunatus* in Brazil (Cavalcante et al., 2019). To our knowledge, this is the first report of *C. musicola* causing anthracnose in soybean producing regions around the world.

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SUPPORTING INFORMATION

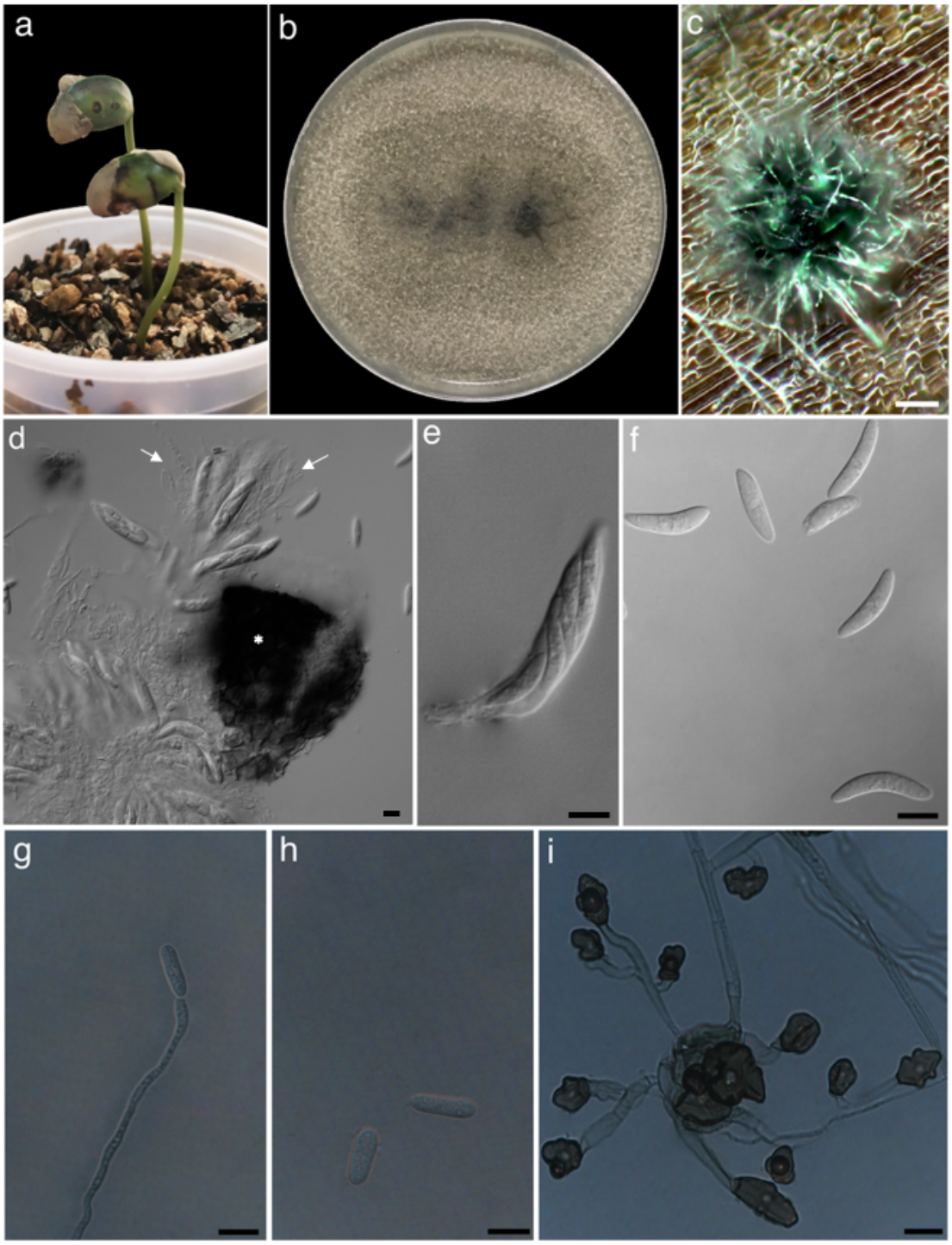

Figure S1: (a) Necrotic lesions on soybean cotyledons seven days after inoculation with *Colletotrichum musicola* conidial suspension. (b) Fourteen-day- old *C. musicola* colony on PDA. (c) Ascomata. (d) Perithecia (*), paraphyses (arrows), asci and ascospores (e) Ascus. (f) Ascospores. (g) Conidia produced in a conidiogenus cell. (h) Conidia. (i) Appressoria. Scale bars: (c) 100 m. (d-i) 10 m

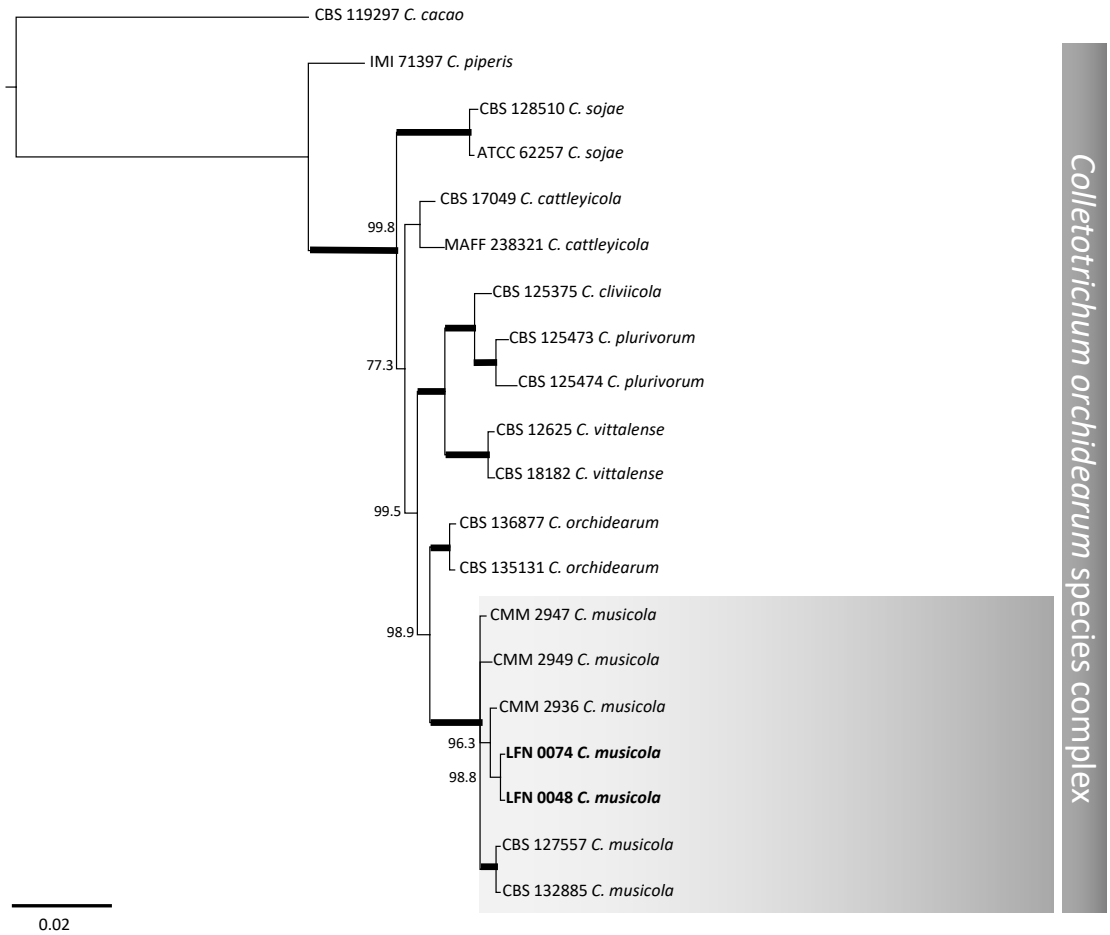

Figure S2: Bayesian inference phylogenetic tree reconstructed from the combined GAPDH, HIS3, and TUB2 sequence alignment of 19 isolates of the *Colletotrichum orchidearum* species complex. *C. cacao* CBS 119297 was used as outgroup. Thickened nodes represent Bayesian posterior probability (BPP) > 0.9; BPP ≤ 0.9 are shown at the nodes. *Colletotrichum musicola* strains isolated from soybean are emphasized in bold. The scale bar represents the number of expected changes per site.

4. GENOME SEQUENCES RESOURCES OF *Colletotrichum plurivorum*, *C. musicola* AND *C. sojae*: THREE SPECIES OF THE *C. orchidearum* SPECIES COMPLEX PATHOGENIC TO SOYBEAN

ABSTRACT

Colletotrichum is a large genus of plant pathogenic fungi comprising more than 200 species. In this work we present the genome sequences of three *Colletotrichum* species pathogenic to soybean: *C. plurivorum*, *C. musicola* and *C. sojae.* These three species belong to the *C. orchidearum* species complex and have been described and associated with soybean only recently. The genome sequences will provide insights into factors that contribute to pathogenicity towards soybean and will be useful for further research into the evolution of *Colletotrichum.*

Keywords: Anthracnose, Comparative Genomics, *Glomerella*, Illumina, Single Molecule Real-Time Cells, Sequencing.

Colletotrichum is a large genus of plant pathogenic fungi, currently considered one of the top 10 most important fungal pathogens by plant pathologists (Dean et al., 2012; Crouch et al., 2014). Several species of *Colletotrichum* have been associated with soybean (*Glycine max*) anthracnose worldwide, with *C. truncatum* globally considered the most prominent (Rogério et al., 2017). In recent years emerging species such as *C. plurivorum*, *C. sojae* (Barbieri et al., 2017; Damm et al., 2019) and *C. musicola* (Boufleur et al., 2020) were associated with the disease for the first time. Such species belong to the *C. orchidearum* species complex (s.c.) (Damm et al. 2019), a monophyletic clade of species closely related to the *C. orbiculare* s.c. Here, we report the reference genome sequences of three species pathogenic to soybean: *C. sojae*, *C. musicola*, *C. pluvivorum* from the *C. orchidearum* species complex.

All isolates used in this study were cultivated in potato dextrose broth medium and genomic DNA was extracted from fresh mycelial tissue. Genomic DNA was extracted using a Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA). Short reads genomic DNA libraries of all three strains were constructed using an NEBNext® Ultra^{TM} Prep Kit (paired-end 2x150 bp, with a 500 bp insert size) and sequenced on an Illumina HiSeq2000 sequencer (Illumina Inc., San Diego, California, USA). Illumina reads belonging to *C. musicola, C. plurivorum* and *C. sojae* were trimmed with Trim Galore v.0.4.5 (Krueger et al., 2012) and forward and reverse reads were merged using Flash v.1.2.7 (Magoc and Salzberg, 2011). *De novo* assemblies of combined and uncombined reads were performed with SPAdes v3.13.1 (Bankevich et al., 2012). All genomic regions with deep read coverage (such as those

belonging to rRNA clusters and the mtDNA) and low coverage scaffolds were identified and masked. Assembly completeness was estimated with the program BUSCO (v3.0.2) (Simão et al., 2015), based on the sordariomyceta_odb9 lineage dataset. Gene annotation was performed with MAKER3 v3.01.02 (Holt and Yandell, 2011) as described by Baroncelli et al., (2016). Secreted proteins were identified using SignalP (v.5.0b) (Nielsen, 2017).

All three newly sequenced species had similar genome sizes, GC contents and number of genes (Table 1) as other *Colletotrichum* species (O'Connell et al., 2012; Baroncelli et al., 2014; Alkan et al., 2015; Rogério et al., 2020). To the best of our knowledge the genome sequences of *C. musicola, C. plurivorum* and *C. sojae* represent new resources. The data reported here may provide insights into pathogenicity factors towards soybean and will be a useful resource for further research into comparative genomics and evolutionary studies in *Colletotrichum.*

Table 1. Summary statistics of *Colletotrichum truncatum*, *C. plurivorum*, *C. musicola* and *C. sojae* genomes

	Statistics		
Variables	**C. *plurivorum***	**C. *sojae***	**C. *musicola***
Isolate	LFN00145	LFN0009	LFN0074
Culture Collection	IMI 507127	IMI 507126	IMI 507128
Tissue sampled	Stem	Leaf	Stem
Average coverage	280X	285X	315X
Number of scaffolds	984	1140	2463
Total assembly length (Mb)	49.70	49.35	52.73
Largest contig length (bp)	850,834	726,643	344,569
Scaffold N50 (bp)	130,438	104,192	46,381
Scaffold L50	104	125	232
N's per 100 kbp	35.82	21.33	59.80
GC (%)	55.86	55.92	54.97
BUSCO completeness (%)	98.8	98.6	99.0
Number of predicted genes	16,153	16,124	16,826
Secreted proteins	1,989	1,931	1,988
Genome accession	WIGO00000000	WIGN00000000	WIGM00000000

DATA DEPOSITION

The genome assemblies and annotations have been deposited at DDBJ/ENA/GenBank: *Colletotrichum plurivorum* (BioProject: PRJNA577396, Biosample: SAMN13025176,

Genome: WIGO00000000, Version: WIGO01000000); *Colletotrichum sojae* (BioProject: PRJNA577398, Biosample: SAMN13025178, Genome: WIGN00000000, Version: WIGN01000000); *Colletotrichum musicolq* (BioProject: PRJNA577394, Biosample: SAMN13025166, Genome: WIGM00000000, Version: WIGM01000000).

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5. COMPARATIVE GENOMIC ANALYSIS REVEAL SECRETED EFFECTOR CANDIDATES OF FOUR SPECIES OF *Colletotrichum* PATHOGENIC TO SOYBEAN

ABSTRACT

Colletotrichum is considered one of the most important genera of fungi by plant pathologists due to its great potential for destruction. *Colletotrichum* spp. have a hemibiotrophic lifestyle and can infect a wide range of hosts, causing losses in essential crops worldwide, such as soybean (*Glycine max*). This crop is one of the most important agricultural commodities. Large areas of soybean cultivation under monoculture affects the intensity of diseases, amongst them, anthracnose. In the past, soybean anthracnose was manly associated with *C. truncatum*, but during the last decade, other species have emerged from commercial soybean fields, including three species belonging to the *C. orchidearum* species complex (s.c.) (*C. musicola*, *C. plurivorum* and *C. sojae*), becoming one limiting factor to soybean production in several regions. To better understand the pathogenicity factors of species of *Colletotrichum* that infect soybean we analyzed the repertoire of secreted effector candidates (SECs) of *C. truncatum*, *C. plurivorum*, *C. sojae* and *C. musicola*, and compared them with 8 additional *Colletotrichum* species not pathogenic to soybean. Through an *in-silico* approach, we tested the hypothesis that all the *Colletotrichum* species that infect soybean have a common ancestor, and therefore share the repertoire of SECs. The repertoire of *C. orchidearum* specific and species-specific SECs were revealed, however, no host-specific SECs were spotted, indicating that the two major species complexes that infect soybean acquired the pathogenicity genes separately. This is the first characterization of effector repertoires of *Colletotrichum* species related to soybean anthracnose. Our results provided a set of candidate genes to be further investigated during functional characterization and could help to understand the role of effector proteins host-pathogen interactions of these species.

Keywords: *Colletotrichum truncatum*, *Colletotrichum musicola, Colletotrichum sojae, Colletotrichum plurivorum, Glycine max*, Comparative Genomics.

5.1 INTRODUCTION

With more than 200 accepted species classified into species complexes (s.c.) or singletons (Marin-Felix et al., 2017; Damm et al., 2019), the hemibiotrophic fungus *Colletotrichum* is considered among the ten most destructive genera of phytopathogenic fungi (Dean et al., 2012), responsible for several losses in important crops (Vargas et al., 2012; Amil-Ruiz et al., 2016; Dias et al., 2016; Banniza et al., 2018; de Silva et al., 2019). Several species of *Colletotrichum* have been reported as pathogenic to soybean, being the *C. orchidearum* and *C. truncatum* s.c. found as the most distributed complexes associated with symptomatic soybean plants worldwide (Boufleur et al., 2021). While *C. truncatum* is associated with soybean since 1917 (Nakata and Takimoto, 1934), *C. musicola, C. plurivorum* and *C. sojae*, members of the *C. orchidearum* s.c. were only described in the past 5 years (Barbieri et al.,

2017; Damm et al., 2019; Boufleur et al., 2020), however this species complex is misidentified at least since 2003 (Boufleur et al., 2021).

A dispute for survival and adaptation marks the evolutionary battle between plants and pathogens throughout history (Jones and Dangl, 2006; Dong et al., 2014, 2015). This arms race is partially described by the "zig-zag" model (Jones and Dangl, 2006). In this model, the first layer of defense of plants recognizes molecular patterns associated with pathogens (PAMPs) or damage-associated molecular patterns (DAMPs) and active a pattern triggered immune response (PTI) (Jones and Dangl, 2006; Boller and Felix, 2009; Couto and Zipfel, 2016). On the other hand, pathogens can overcome this layer of defense releasing effectors, that are surface-exposed or secreted proteins that cause alterations in structure or processes of the host cell, suppressing the defense responses or enhancing access to nutrients, promoting the colonization of the host by the pathogen (Win et al., 2012). The recognition of effectors or effector targets by resistance (*R*) genes of the host will then trigger the second layer of defense, called effector-triggered immunity (ETI), being a stronger response than PTI that can lead to a hypersensitive reaction (HR) (Win et al., 2012; Dong et al., 2014).

Understading the pathogenicity mechanisms of *Colletotrichum* and the way that they adapt to their hosts can be a powerful tool in developing sustainable control strategies (Dussert et al., 2019). The evolution through adaptation of pathogens to different hosts can involve sets of effectors, that can specialize and infect a specific host (Ma et al., 2010; Poppe et al., 2015; Hartmann et al., 2017; Petre et al., 2020). Over the past few years, the genomes of at least 42 species of *Colletotrichum* have been sequenced (http://www.colletotrichum.org/genomics/), including *C. truncatum, C. sojae, C. plurivorum* and *C. musicola* (Rogério et al., 2020). The availability of genome sequences from multiple species of *Colletotrichum* enables unprecedented insights into genome composition (Lo Presti et al., 2015), including the prediction of effector candidates. Indentification of fungal genes involved plant infection is becoming a useful tool to improve disease management (Mousavi-Derazmahalleh et al., 2019). The evolutionary trajectory of host-pathogen interactions can help clarify the mechanisms underlying the threat of pathogens to crops (Sánchez-Vallet et al., 2018).

Despite some studies of the sets of effectors in different species of *Colletotrichum* (Bhadauria et al., 2015; Baroncelli et al., 2016; de Queiroz et al., 2019; Lelwala et al., 2019), until now, the total number of effectors of *Colletotrichum* species that infect soybean and how many are unique to each species is unknown. The repertoire of candidate effectors from those species may reveal genes involved in host specificity during the *Colletotrichum*-soybean interaction. In this work, we predicted the repertoire of secreted effector candidates (SECs) in

the proteomes of four species of *Colletotrichum,* pathogenic to soybean and compared these with eight closely related species of *Colletotrichum* non-pathogenic to this host, providing a useful platform for future works regarding soybean anthracnose.

5.2 METHODS

5.2.1 Isolate and genome sampling

To gain insights into the repertoire of SECs of *Colletotrichum* species pathogenic to soybean, we selected 12 *Colletotrichum* proteomes and correspondent strains (Table 1). Four of them are soybean pathogenic, including *C. musicola* (*CMUS*)*, C. plurivorum* (*CPLU*) and *C. sojae* (*CSOJ*), members of the *C. orchidearum* s.c. and *C. truncatum* (*CTRU*). Eight additional proteomes, isolated from multiple hosts, were included in the analysis: *C. orbiculare* (*CORB*), *C. gloeosporioides sensu lato* (*CGLO*), *C. higginsianum* (*CHIG*), *C. tofieldiae* (*CTOF*), *C. graminicola* (*CGRA*)*, C. orchidophilum* (*CORC*), *C. fioriniae* (*CFIO*) and *C. nymphaeae* (*CNYM*) (Table 1).

Table 1: *Colletotrichum* strains used in the pathogenicity test and comparative genomics analysis

Strain	Species	Species complex	Host	Origin	Reference
MAFF 240422	*C. orbiculare*	orbiculare	Cucumis sativus	Japan	(Gan et al., 2019)
LFN0074	*C. musicola*	orchidearum	Glycine max	Brazil	(Rogério et al., 2020)
LFN00145	*C. plurivorum*	orchidearum	Glycine max	Brazil	(Rogério et al., 2020)
LFN0009	*C. sojae*	orchidearum	Glycine max	Brazil	(Rogério et al., 2020)
1059	*C. truncatum*	truncatum	Glycine max	Brazil	(Rogério et al., 2020)
Cg-14	*C. gloeosporioides s.s.*	gloeosporioides	Persea americana	Israel	(Alkan et al., 2015)
IMI 349063	*C. higginsianum*	destructivum	Brassica rapa	Trinidad & Tobago	(Zampounis et al., 2016)
CBS 168.49	*C. tofieldiae*	spaethianum	Lupinus polyphyllus	Germany	(Hacquard et al., 2016)
M1.001	*C. graminicola*	graminicola	Zea mays	USA	(O'Connell et al., 2012)
IMI 309357	*C. orchidophilum*	none	Phalaenopsis sp.	United Kingdom	(Baroncelli et al., 2018)
IMI 504882	*C. fioriniae*	acutatum	Fragaria x ananassa	New Zealand	(Baroncelli et al., 2014)
IMI 504889	*C. nymphaeae*	acutatum	Fragaria x ananassa	Denmark	(Baroncelli et al., 2016)

5.2.2 Pathogenicity assays

Pathogenicity assays were performed to confirm the capability (or not) of the selected strains to cause soybean anthracnose. Except for *CGRA* and *CORB*, all the strains were retrieved from culture collections to perform the tests (Table 1). Seeds of the soybean cultivar IPRO7739, from Monsoy company, were superficially disinfected with NaClO (1%) for 1 min, then rinsed three times in sterile distilled water (SDW). Disinfected seeds were placed in Petri dishes containing 100 g of sterile sand, soaked with 10 mL of SDW. Each Petri dish contained 5 seeds and was incubated at 25°C for 32 h until germination.

Colletotrichum strains were grown on potato dextrose agar (PDA) culture medium and incubated at 25ºC for 15 days. Conidia suspensions were prepared by washing and filtering the cultures and were adjusted to a final concentration of 1×10^6 conidia/mL. Each pre-germinated seed was inoculated with 5 µL of conidia suspension of each *Colletotrichum* strain as described by Dubrulle et al., (2020). Water was used as a negative control. Inoculated seedlings were initially incubated in the dark at 25ºC for 4 h and then transferred to 100 mL pots filled with sterilized vermiculite and randomly distributed in a green-house for 7 days when the severity of anthracnose was evaluated using a diagrammatic scale that range from 0 to 5, adapted from Yang and Hartman (2015). Severity data were analyzed with the post-hoc Tuckey method at 0.05 significance level, using the ExpDes R package (v.1.2.0).

5.2.3 Identification of specific effector protein candidates (SECs) of soybean pathogenic *Colletotrichum* species

The proteomes of four species of *Colletotrichum* pathogenic to soybean, and eight additional non-pathogenic *Colletotrichum* species were included in the analysis (Table 1). A phylogeny of the genus *Colletotrichum* was constructed based on publicly available DNA sequences of three nuclear loci belonging to the 12 selected species: *actin* (*ACT*), *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and *chitin synthase* (*CHS*). The analyses were run from random trees for 5,000,000 generations and sampled every 1000 generations. The predicted proteomes of the 12 *Colletotrichum* spp. were clustered based on similarity with OrthoFinder (v. 2.3.5) (Emms and Kelly, 2015) and the clusters of proteins were analyzed with the R package UpsetR (v. 1.4.0) (Conway et al., 2017) to identify unique and shared orthogroups between the species and species complexes.

Specific secreted effector candidates (SSECs) proteins are defined as proteins with no (detectable) homology to any other available proteins inside or outside the genus *Colletotrichum*. To check if the four *Colletotrichum* species that are related to soybean anthracnose evolved the capability to infect soybean from a common ancestor, we looked to the SSECs proteins through a computational biology pipeline (Figure 2). The prediction of SECs of soybean pathogenic species of *Colletotrichum* was made using the proteomes predicted by Rogério et al., (2020). The initial secretome was predicted with SignalP (v.5.0) (Almagro Armenteros et al., 2019), then sequences containing transmembrane (TM) domains and glycophosphatidylinositol (GPI)-anchors were identified using THMMM (v.2.0) (Krogh et al., 2001) and PredGPI (Pierleoni et al., 2008) respectively, and those proteins that are predicted

to have a signal peptide cleavage site, no transmembrane domains and no GPI-anchors were considered as the initial set of SECs for each species of *Colletotrichum.*

The set of SECs of each *Colletotrichum* species were submitted individually to a sequence of BLAST searches using an *e*-value threshold of 1E-5 and classified into shared (proteins with homology to proteins from other members of the genus *Colletotrichum*), species-complex specific (those that had homology only within other species from the same s. c.), host-specific (shared only between the four species that infect soybean) and species-specific (those that had no homology to any other protein either within or outside of the same genus). The final set of predicted SECs was submitted to a BLAST against the non-redundant database of NCBI and PHI-base to check similarity with known genes of other microorganism species; being those proteins with similarity outside the genus *Colletotrichum* considered conserved among microorganisms.

Species-specific and species complex SSECs were characterized. For the prediction of subcellular localization within the plant cell, mature protein sequences were submitted to LOCALIZER (Sperschneider et al., 2017), and to the prediction of apoplastic SECs the proteins were submitted to ApoplastP (Sperschneider et al., 2018). The percentage of cysteines was identified in Geneious (v. 2020.10.4) and repeat-containing proteins were predicted using T-REKs (Jorda and Kajava, 2009).

5.3 RESULTS

5.3.1 Among the selected *Colletotrichum* species, only *C. truncatum* and members of the *C. orchidearum* s.c. are pathogenic to soybean

Pathogenicity assays confirmed that only *CTRU* and the three species belonging to the *C. orchidearum* s.c. (*CPLU, CMUS* and *CSOJ*) are able to cause anthracnose in soybean, being *CTRU* more aggressive to the tested soybean cultivar than *CPLU*, *CMUS* and *CSOJ*; while *CGLO*, *CHIG*, *CTOF*, *CORC, CFIO* and *CNYM* are not pathogenic (Figure 1). Strains of *CGRAM* and *CORB* species were not included in the assay.

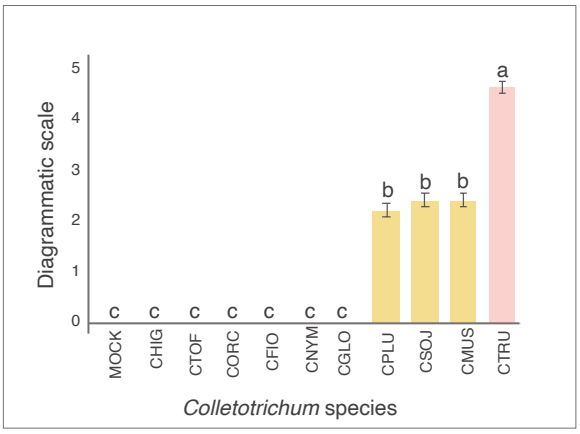

Figure 1: Level of aggressiveness of *Colletotrichum* species to soybean. Tuckey test applied on transformed data ((X+1)^0.5). Equal letters do not differ in average of aggressiveness among *Colletotrichum* strains in Tuckey test with *p*-value = 0.05%. Species belonging to the *C. orchidearum* species complex are represented by yellow bars, while *C. truncatum* is represented by the pink bar. *CHIG*: *C. higginsianum*; *CTOF*: *C. tofieldiae*; *CORC*: *C. orchidophilum*; *CFIO*: *C. fiorinae*; *CNYM*: *C. nymphaeae*; *CGLO*: *C. gloeosporioides*; *CPLU*: *C. plurivorum*; *CSOJ*: *C. sojae*; *CMUS*: *C. musicola*; and CTRU: *C. truncatum*.

5.3.2 The majority of candidate effectors of *Colletotrichum* species show conservation microorganisms

To better understand the evolution of the two main *Colletotrichum* s. c. that infect soybean worldwide (*C. truncatum* s. c. and *C. orchidearum* s. c.) (Boufleur et al., 2021) we conducted *in-silico* analyses (Figure 2) to check if the representative species of those complexes (*CTRU*, *CMUS*, *CSOJ* and *CPLU*) evolved the capability to infect soybean from a common ancestor, and therefore share the set of SSECs.

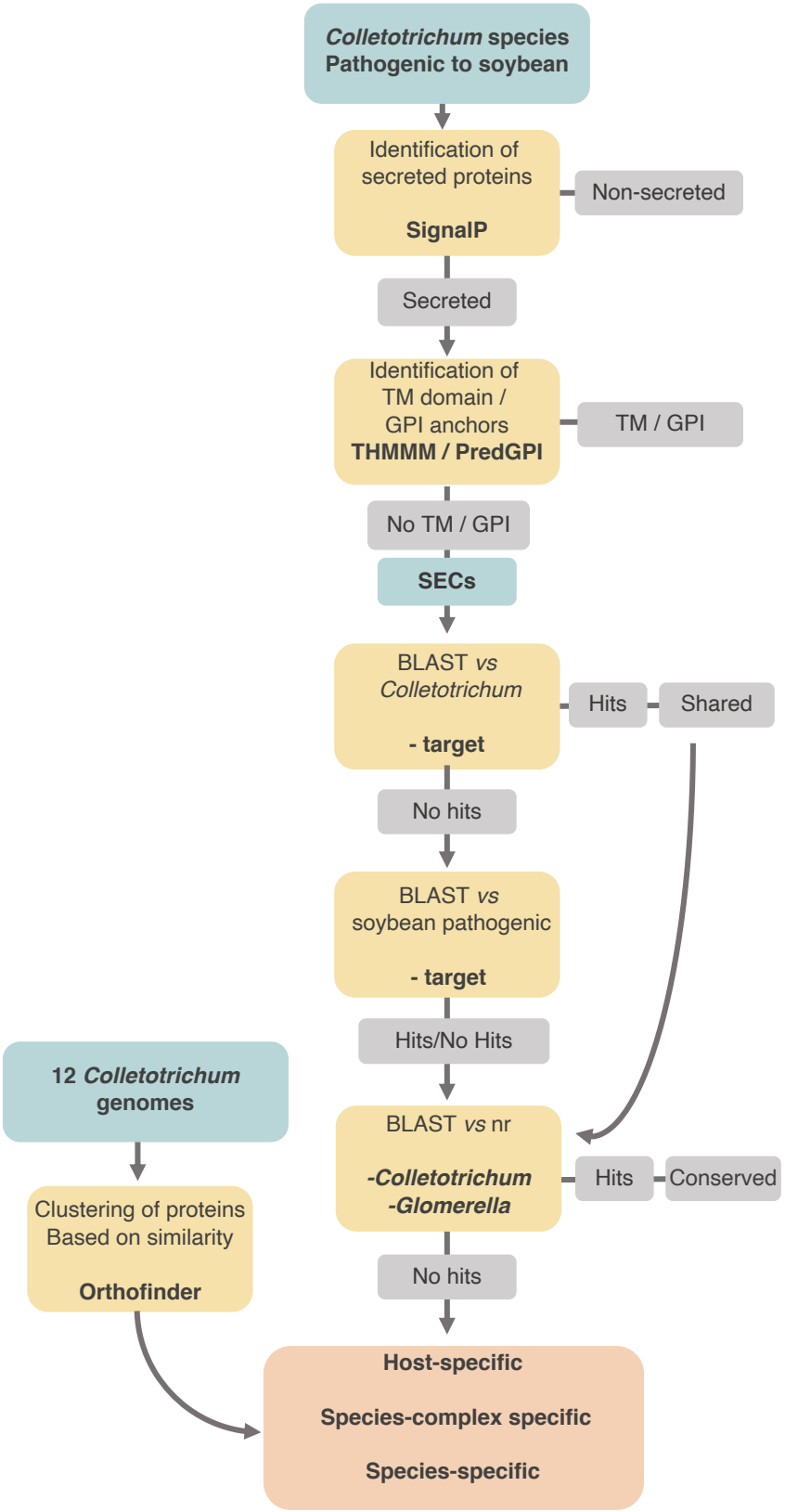

Figure 2: In silico analysis workflow for the prediction of effector candidates in *Colletotrichum truncatum*, *C. musicola*, *C. plurivorum* and *C. sojae.*

The proteomes of the 12 *Colletotrichum* species compared in this work were assigned to 32,018 orthogroups, among those 7,428 are shared among all the proteomes analyzed (Figure 3B). Orthology analysis identified 66 orthogroups comprising 338 *Colletotrichum* spp. genes, common only to the four species infecting soybean; and 764 orthogroups shared only between the species belonging to the *C. orchidearum* s.c. While 1,214 (1,695 genes); 1,103 (1,126 genes); 943 (952 genes) and 760 (771 genes) orthogroups were specific to *C. truncatum*, *C. musicola*, *C. sojae* and *C. plurivorum*, respectively (Figure 23C).

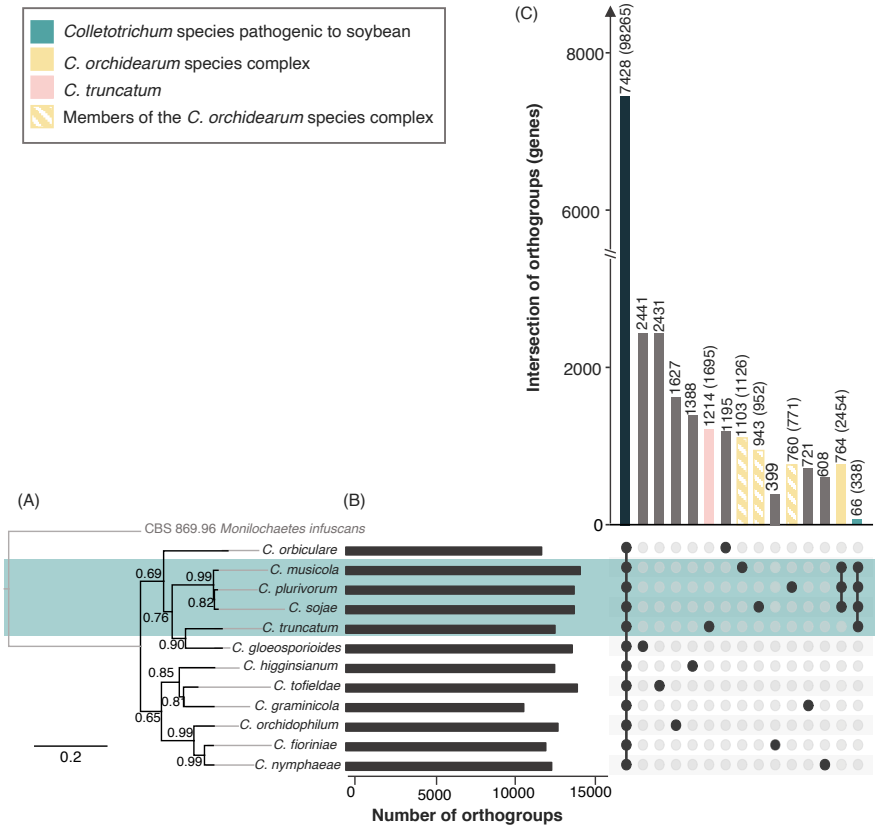

Figure 3: Protein clustering analysis of 12 *Colletotrichum* species. Species highlight in light blue represent the four species pathogenic to soybean; the *C. orchidearum* species complex (s.c) is represented in yellow, and striped yellow bars correspond to each species belonging to the *C. orchidearum* s.c. (*C. musicola, C. plurivorum* and *C. sojae*); while *C. truncatum* is represented in pink. (A) Species phylogenetic tree reconstructed from concatenated nucleotide alignments of the *ACT*, *CHS*, and *GAPDH* genes. Posterior probabilities are reported next to the nodes. (B) The bars on the right side of the tree represent the total number of orthogroups in each proteome. (C) Bars in the upper side represent the number of orthogroups shared by the species highlighted by the black dots reported in the bottom side. Number of genes correspondent to the orthogroups are in parentheses.

Proteomes of the four species of *Colletotrichum* were scanned for the presence of signal peptides. Those proteins with predicted signal peptide, but presence of TM domains and/or GPI-anchors were eliminated from the analysis. The secretomes of the four species vary between 9-10%, being the initial defined set of SECs 1,638; 1,495; 1,447; and 1,485 proteins for *CTRU*, *CPLU*, *CSOJ* and *CMUS* respectively (Table 2). SECs of each species went through serial BLAST filtrations (Figure 2). Our results revealed that most SECs of the four *Colletotrichum* species are conserved amongst microorganisms, corresponding to 80% of *CTRU*, 83% of *CPLU*, 85% of *CSOJ* and 84% of *CMUS*. Among these, 6; 9 and 8 SECs are *C. orchidearum* specific, corresponding to *CPLU, CSOJ* and *CMUS* respectively; 19; 3 and 1 species-specific SECs were identified for *CTRU*, *CSOJ* and *CMUS*, while one SEC identified in *CTRU*, is also found in *CPLU, CSOJ* and *CMUS* proteomes, being those host-SSECs (Table S1-S4).

Table 2: Secretome size of the four species of *Colletotrichum* that infect soybean, compared in this study.

Species	Proteome	Signal peptide	Absence of TM/GPI anchor	% of secreted proteins
CTRU	15,901	2,116	1,638	10
CPLU	15,153	1,989	1,495	10
CSOJ	16,124	1,931	1,447	9
CMUS	16,826	1,871	1,485	9

Around 17% of *CTRU*, 13% of *CSOJ*, 14% of *CMUS* and 15% of *CPLU* are shared only between the genus *Colletotrichum.* A comparison of all SECs, with no similarity inside or outside the genus highlighted 13; 18 and 13 *C. orchidearum* SSECs in *CPLU*, *CSOJ* and *CMUS*. We also identified 41 *CTRU*-specific, 8 *CPLU*-specific, 8 *CSOJ*-specific and 15 *CMUS*-SSECs. Host SSECs shared only between the four *Colletotrichum* species that infect soybean were not identified (Figure 4). All the sets of s.c. specific, species-specific and host-SSECs aforementioned were assigned to their corresponding orthogroups based on the similarity analysis of the proteins (Tables S1-S4).

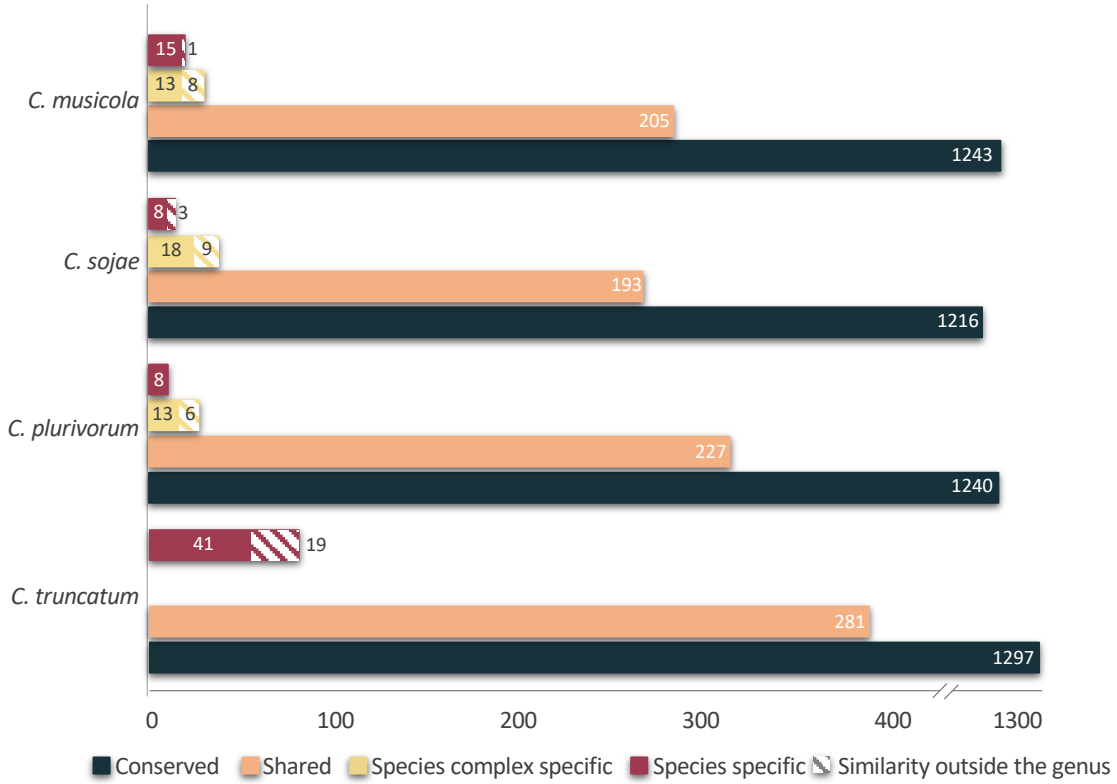

Figure 2: Putative secreted effector candidates (SECs) of *Colletotrichum* species pathogenic to soybean.

Several characteristics are commonly observed in effector proteins, such as high percentage of cysteines (cysteine-rich), with >2% of cysteines in its amino acid sequences (Lu and Edwards, 2016), repeat containing proteins (Mesarich et al., 2015) and the translocation to different subcellular compartments, such as the chloroplast or mitochondria when they have a transit peptide, or to the plant cell nucleus, when the possess nucleus localization signals (NLS) (Sperschneider et al., 2017), or can be delivered to the plant apoplast (Sperschneider et al., 2018). We scanned the *Colletotrichum orchidearum* s.c. specific with or without similarity outside the *Colletotrichum* genus for these characteristics. All *C. orchidearum* SSECs, have at least one of the aforementioned characteristics, from those, 8; 9 and 11 SECs were predicted as effectors by EffectorP 2.0 tool for *CPLU, CSOJ* and *CMUS*. Among the species SSECs without similarity outside the genus, 7; 7 and 11 of *CPLU*, *CSOJ* and *CMUS* have at least one of these characteristics, among these 5; 2 and 3 were predicted to be effectors by EffectorP 2.0 tool; while all the species-SSECs with similarity outside the genus are predicted to be effectors and have at least one characteristic. Among the *CTRU*-SSECs, 35 without similarity outside the genus and all the 20 with similarity outside the genus were predicted to have at least one of those characteristics, being 18 and 13 of them predicted by EffectorP 2.0 respectively (Table 3).

Table 3: Predicted *C. orchidearum* species complex and species SSECs of the four species of *Colletotrichum* pathogenic to soybean, containing characteristics commonly associated to effector proteins in fungi, and the total number of protein sequences predicted as effectors by EffectorP 2.0 tool.

***C. orchidearum* s.c. SSECs**								
Species	**SSECs (NR)**	**SSECs**	**RCP**	**SL (NLS)**	**SL (other)**	**Apoplast**	**CR**	**EffectorP**
CPLU	6	13	9	2	2	13	14	8
CSOJ	9	18	6	4	5	13	17	9
CMUS	8	13	6	0	2	15	14	11
Species SSECs								
CTRU	19	41	13	7	5	32	39	31
CPLU	0	8	0	0	4	1	4	2
CSOJ	3	8	2	0	1	3	8	5
CMUS	1	15	2	0	2	5	9	6

SSECs: specific secreted effector candidates; NR: similarity with the non-redundant database (NCBI); RCP: repeat containing proteins; SL: subcellular localization; NLS: nuclear localization signal; CR: cysteine-rich proteins (>2%); NA: not applicable.

5.4 DISCUSSION

The availability of four *Colletotrichum* representative genomes of the *C. truncatum* s.c. and *C. orchidearum* s.c. (Rogério et al., 2020), reported as the most distributed s.c. associated with soybean worldwide (Boufleur et al., 2021), along with the genomes of several *Colletotrichum* species associated with other hosts (http://www.colletotrichum.org/genomics/), allowed us to investigate if the four species pathogenic to soybean evolved the capability of infecting this host from a common ancestor, by looking to the set of SECs of each species and compare it with the proteomes of 8 additional *Colletotrichum* species non-pathogenic to soybean. Effectors proteins produced by plant pathogens are surface-exposed or secreted proteins, many of those translocated to the apoplast or cytoplasm of the host, where they inhibit the host defense responses to allow colonization by the pathogen (Hogenhout et al., 2009; Win et al., 2012).

Sets of effector candidates of different *Colletotrichum* species were revealed. (Baroncelli et al., 2016; de Queiroz et al., 2019). The initial interaction between a pathogen and its host occurs in the apoplast located between the plant cell wall and the plasma membrane (Sperschneider et al., 2018; Wang and Wang, 2018). Effector proteins secreted to the apoplast have several functions, such as enzyme inhibitors, suppression of the recognition by the host and undermining plant PTI response (Lo Presti et al., 2015; Wang and Wang, 2018). Our results revealed that most of the *C. orchidearum* specific and species SSECs are secreted to the plant

apoplast while only a few genes are secreted to the plant cell nucleus or other subcellular compartments (Table 3), suggesting that the initial contact is determinant for the capability of *Colletotrichum* species to infect soybean. These results are in accordance with the results of Kleemann et al., (2012) and Irieda et al., (2014) who showed that representants of *C. higginsianum* and *C. orbiculare* effector candidates, that were already shown to be delivered to the plant apoplast during the early biotrophic stage of the fungus.

By definition, orthologues trace back to a gene that was present in a common ancestor (Emms and Kelly, 2019), and therefore, searching for orthology is considered the most accurate way to compare the composition of genomes of different species (Gabaldón and Koonin, 2013). Protein clustering analysis revealed a set of host-specific orthogroups, however, no host-SSECs without similarity outside the *Colletotrichum* genus were revealed among those. On the other hand, SSECs were found among the sets of *C. orchidearum* s.c. species-specific orthogroups, supporting the results.

The evolution of effector proteins rely on the arms-race between plants and pathogens, with the aim of escape detection and evolve the capability of cause disease in different hosts (Lo Presti et al., 2015), therefore the pathogenicity to specific hosts and/or cultivars can be a result of the evolution of effector proteins from a common ancestor (Stergiopoulos et al., 2010, 2012), as shown for the hemibiotrophic pathogen *Phythophthora infestans* (Dong et al., 2014) and for *Venturia* spp. (Prokchorchik et al., 2020). The absence of host SSECs, allied to the evolutionary distance between the two species complexes from the last common ancestor (Fig. 3A) suggests that *C. truncatum* and *C. orchidearum* s.c. evolved separately and acquire the pathogenicity genes to infect soybean later.

The identification of sets of SSECs of the *C. orchidearum* s.c. and *CTRU* open the field to perform evaluations of the functional role of these genes in soybean infection. Besides cultural and chemical control strategies have already been described for soybean anthracnose, recent outbreaks of the disease have been reported by researchers (Rogério et al., 2017, 2019; Dias et al., 2018) and producers (personal communication), suggesting that the control strategies used are not always effective. This may be a consequence of different *Colletotrichum* species present in soybean fields, that allied to the separate evolution of these species, may imply directly in disease management strategies, as the correct identification of the causal agent is crucial to an efficient control (Cai et al., 2009; Jayawardena, 2016).

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SUPPLEMENTARY INFORMATION

Table S1: *Colletotrichum musicola* secreted effector candidates (SECs)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CMUS01_03451	Host-Specific with similarity outside the genus	-	-	432	49,3	3,9	-	Yes	OG0013514
CMUS01_16415	*C. orchidearum* specific	Yes	-	83	8,5	12	-	Yes	OG0015052
CMUS01_13947	*C. orchidearum* specific	Yes	-	85	8,9	7,1	-	Yes	OG0014941
CMUS01_13948	*C. orchidearum* specific	Yes	-	86	8,8	7	-	Yes	OG0014942
CMUS01_14213	*C. orchidearum* specific	Yes	-	58	5,9	6,9	-	Yes	OG0014958
CMUS01_05086	*C. orchidearum* specific	Yes	-	117	12,1	6,8	-	Yes	OG0014526
CMUS01_06861	*C. orchidearum* specific	Yes	-	108	11,2	5,6	-	Yes	OG0014603
CMUS01_06820	*C. orchidearum* specific	Yes	-	179	19,7	3,4	-	Yes	OG0014599
CMUS01_04008	*C. orchidearum* specific	-	-	123	13,3	2,4	-	-	OG0014481
CMUS01_09629	*C. orchidearum* specific	-	Yes	448	48,5	0,2	-	-	OG0014733
CMUS01_05796	*C. orchidearum* specific	-	Yes	232	23,9	0	-	-	OG0014562
CMUS01_06435	*C. orchidearum* specific	-	Yes	185	20,1	0	-	-	OG0014587
CMUS01_11399	*C. orchidearum* specific	-	-	156	15,3	0	-	Yes	OG0014819
CMUS01_14944	*C. orchidearum* specific	Yes	-	75	7,7	0	-	Yes	OG0014984
CMUS01_12127	*C. orchidearum* specific with similarity (nr db)	Yes	-	77	7,8	10,4	-	Yes	OG0014848
CMUS01_02809	*C. orchidearum* specific with similarity (nr db)	-	-	77	7,7	7,8	-	Yes	OG0014439
CMUS01_15349	*C. orchidearum* specific with similarity (nr db)	-	-	39	3,8	7,7	-	Yes	OG0015004
CMUS01_15354	*C. orchidearum* specific with similarity (nr db)	Yes	-	154	16,9	3,9	-	Yes	OG0015005
CMUS01_11539	*C. orchidearum* specific with similarity (nr db)	-	Yes	129	12,1	3,1	-	Yes	OG0014824
CMUS01_11011	*C. orchidearum* specific with similarity (nr db)	-	-	154	16,3	2,6	Chloroplast	-	OG0014808
CMUS01_11524	*C. orchidearum* specific with similarity (nr db)	Yes	Yes	96	9,5	1	-	Yes	OG0014823

Table S1: (continued)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CMUS01_11534	*C. orchidearum* specific with similarity (nr db)	-	Yes	323	34,6	0	Chloroplast	-	OG0010939
CMUS01_05231	Species-specific	Yes	-	68	7,0	11,8	-	Yes	OG0022631
CMUS01_06715	Species-specific	-	-	70	6,9	4,3	-	Yes	OG0022730
CMUS01_01303	Species-specific	-	-	283	30,2	4,2	Chloroplast	-	-
CMUS01_11365	Species-specific	Yes	-	122	14,1	4,1	-	-	OG0023016
CMUS01_03684	Species-specific	-	-	81	8,7	3,7	-	-	OG0022524
CMUS01_03859	Species-specific	Yes	-	108	11,4	3,7	-	-	OG0022534
CMUS01_04271	Species-specific	-	-	159	17,1	3,1	Chloroplast	-	OG0022565
CMUS01_07042	Species-specific	Yes	-	234	26,0	2	-	-	OG0022750
CMUS01_09430	Species-specific	-	Yes	110	11,5	1,8	-	-	OG0022867
CMUS01_09803	Species-specific	Yes	-	131	14,3	1,5	-	Yes	OG0022901
CMUS01_14349	Species-specific	-	-	131	14,3	1,5	-	-	OG0023199
CMUS01_04275	Species-specific	-	-	90	9,1	1,1	-	-	OG0022568
CMUS01_14394	Species-specific	-	-	143	15,1	0,7	-	-	OG0023201
CMUS01_13216	Species-specific	-	Yes	84	8,9	0	-	Yes	OG0023127
CMUS01_15804	Species-specific	-	-	39	4,1	0	-	-	OG0023301
CMUS01_10910	Species-specific with similarity (nr db)	Yes	-	101	10,4	7,9	-	Yes	OG0022982

* nr db: non-reduntant database.

Table S2: *Colletotrichum plurivorum* secreted effector candidates (SECs)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CPLU01_14551	Host-specific with similarity outside the genus	-	-	432	46,1	4,2	-	Yes	OG0013466
CPLU01_04300	*C. orchidearum* specific	-	Yes	226	23,3	0	-	-	OG0014562
CPLU01_06231	*C. orchidearum* specific	-	-	576	62,9	0,2	Nucleus	-	OG0014733
CPLU01_08815	*C. orchidearum* specific	Yes	-	85	8,9	7,1	-	Yes	OG0014941
CPLU01_08816	*C. orchidearum* specific	Yes	-	86	8,9	7	-	Yes	OG0014942
CPLU01_09347	*C. orchidearum* specific	-	Yes	126	12,1	3,2	-	Yes	OG0014824
CPLU01_09355	*C. orchidearum* specific	-	Yes	320	34,3	0	Chloroplast	-	OG0010939
CPLU01_10047	*C. orchidearum* specific	Yes	-	71	7,3	0	-	Yes	OG0014984
CPLU01_11912	*C. orchidearum* specific	Yes	Yes	117	12,3	6,8	-	Yes	OG0014526
CPLU01_12757	*C. orchidearum* specific	-	Yes	194	21,0	0	-	-	OG0014587
CPLU01_12854	*C. orchidearum* specific	Yes	-	141	15,1	4,3	-	-	OG0014599
CPLU01_13748	*C. orchidearum* specific	-	Yes	198	19,4	0	-	Yes	OG0014819
CPLU01_14053	*C. orchidearum* specific	-	Yes	80	7,7	0	-	Yes	OG0014823
CPLU01_14227	*C. orchidearum* specific	-	-	77	7,7	7,8	-	Yes	OG0014439
CPLU01_04841	*C. orchidearum* specific with similarity (nr db)	Yes	-	238	25,3	3,8	-	-	OG0014958
CPLU01_09762	*C. orchidearum* specific with similarity (nr db)	Yes	-	70	7,2	11,4	-	Yes	OG0014848
CPLU01_11683	*C. orchidearum* specific with similarity (nr db)	-	Yes	72	6,9	5,6	Nucleus	-	OG0015004
CPLU01_12148	*C. orchidearum* specific with similarity (nr db)	-	-	242	26,2	4,1	-	-	OG0015005
CPLU01_12611	*C. orchidearum* specific with similarity (nr db)	Yes	-	108	11,2	5,6	-	Yes	OG0014603
CPLU01_15546	*C. orchidearum* specific with similarity (nr db)	-	-	154	16,3	2,6	Chloroplast	-	OG0014808

Table S2: (continued)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CPLU01_02254	Species-specific	-	-	190	19,9	2,1	Chloroplast	-	OG0026898
CPLU01_03023	Species-specific	Yes	-	140	15,2	2,1	-	-	OG0026942
CPLU01_05972	Species-specific	-	-	235	25,9	0,9	Chloroplast	-	OG0027064
CPLU01_09167	Species-specific	-	-	79	8,2	1,3	-	-	OG0027222
CPLU01_10072	Species-specific	-	-	68	7,2	0	-	-	OG0027269
CPLU01_12177	Species-specific	-	-	165	17,8	0,6	Chloroplast	-	OG0027338
CPLU01_12778	Species-specific	Yes	-	148	15,9	2	Chloroplast	-	OG0027383
CPLU01_15430	Species-specific	-	-	67	6,6	4,5	-	Yes	OG0027496

* nr db: non-reduntant database.

Table S3: *Colletotrichum sojae* secreted effector candidates (SECs)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CSOJ01_15762	Host-specific with similarity outside the genus	-	-	432	46,0	3,9	-	Yes	OG0013466
CSOJ01_01718	*C. orchidearum* specific	Yes	-	97	9,9	10,3	-	Yes	OG0013588
CSOJ01_02292	*C. orchidearum* specific	-	Yes	240	24,9	0	-	-	OG0014562
CSOJ01_03941	*C. orchidearum* specific	-	-	120	12,9	2,5	Nucleus	-	OG0014472
CSOJ01_05277	*C. orchidearum* specific	-	Yes	155	16,3	0,6	-	-	OG0014748
CSOJ01_05688	*C. orchidearum* specific	Yes	-	137	14,8	4,4	-	-	OG0014599
CSOJ01_06508	*C. orchidearum* specific	-	-	263	28,7	1,1	Nucleus	-	OG0014480
CSOJ01_07932	*C. orchidearum* specific	-	-	243	26,9	3,3	-	-	OG0015056
CSOJ01_08820	*C. orchidearum* specific	-	-	436	47,7	1,8	Chloroplast/mitochondria	-	OG0014948
CSOJ01_09256	*C. orchidearum* specific	-	Yes	274	27,5	1,5	Chloroplast	Yes	-
CSOJ01_09501	*C. orchidearum* specific	-	-	123	11,8	3,3	-	Yes	OG0014824
CSOJ01_09508	*C. orchidearum* specific	-	Yes	323	34,6	0	Chloroplast	-	OG0010939
CSOJ01_10279	*C. orchidearum* specific	Yes	-	113	11,6	6,2	-	Yes	OG0014526
CSOJ01_11132	*C. orchidearum* specific	-	-	168	18,2	0	-	-	OG0014587
CSOJ01_11943	*C. orchidearum* specific	-	-	71	7,3	0	-	Yes	OG0014984
CSOJ01_12003	*C. orchidearum* specific	Yes	-	91	9,2	8,8	-	Yes	OG0014848
CSOJ01_13106	*C. orchidearum* specific	-	Yes	95	9,4	1,1	-	-	OG0014823
CSOJ01_14785	*C. orchidearum* specific	-	-	532	57,9	0,2	Nucleus	-	OG0014733
CSOJ01_15809	*C. orchidearum* specific	-	-	77	7,7	7,8	-	Yes	OG0014439
CSOJ01_04556	*C. orchidearum* specific with similarity (nr db)	Yes	-	85	9,0	11,8	-	Yes	OG0013491
CSOJ01_09129	*C. orchidearum* specific with similarity (nr db)	Yes	-	86	8,9	7	-	Yes	OG0014942
CSOJ01_09130	*C. orchidearum* specific with similarity (nr db)	Yes	-	85	8,9	7,1	-	Yes	OG0014941
CSOJ01_09799	*C. orchidearum* specific with similarity (nr db)	-	-	252	26,7	4,8	Chloroplast	-	OG0014958
CSOJ01_11173	*C. orchidearum* specific with similarity (nr db)	Yes	-	83	8,5	12	-	Yes	OG0015052

Table S3: (continued)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CSOJ01_11318	*C. orchidearum* specific with similarity (nr db)	Yes	-	118	12,2	5,1	-	Yes	OG0014603
CSOJ01_11751	*C. orchidearum* specific with similarity (nr db)	-	-	242	26,1	4,1	-	-	OG0015005
CSOJ01_13856	*C. orchidearum* specific with similarity (nr db)	-	-	154	16,3	2,6	Chloroplast	-	OG0014808
CSOJ01_14303	*C. orchidearum* specific with similarity (nr db)	-	Yes	72	6,9	5,6	Nucleus	Yes	OG0015004
CSOJ01_02886	Species-specific	-	-	294	32,6	1,7	Mitochondria/nucleus	-	OG0027709
CSOJ01_03097	Species-specific	-	Yes	100	10,4	2	-	-	OG0027727
CSOJ01_04860	Species-specific	Yes	-	74	7,9	2,7	-	-	OG0027822
CSOJ01_07468	Species-specific	Yes	-	82	8,5	12,2	-	Yes	OG0027980
CSOJ01_07714	Species-specific	-	-	88	9,5	2,3	-	-	OG0027995
CSOJ01_08358	Species-specific	-	-	96	10,7	0	-	-	OG0028038
CSOJ01_10965	Species-specific	Yes	-	96	10,3	5,2	-	-	OG0028170
CSOJ01_13469	Species-specific	-	Yes	108	11,9	0	-	Yes	OG0028327
CSOJ01_00179	Species-specific with similarity (nr db)	-	-	72	7,6	2,8	-	-	OG0027559
CSOJ01_14688	Species-specific with similarity (nr db)	Yes	-	147	16,0	4,8	-	-	OG0028402
CSOJ01_15961	Species-specific with similarity (nr db)	Yes	-	93	9,8	6,5	-	Yes	OG0028482

* nr db: non-reduntant database.

Table S4: *Colletotrichum truncatum* secreted effector candidates (SECs)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CTRU02_14336	Host-specific with similarity (nr db)	-	-	435	46,1	4,1	-	Yes	OG0013466
CTRU02_00830	Species-specific	Yes	-	131	14,304	2,3	-	-	OG0030959
CTRU02_00831	Species-specific	-	Yes	549	59,415	1,6	Mit-Chlo	-	OG0030960
CTRU02_00942	Species-specific	-	-	258	28,068	2,3	Nucleus	-	OG0030967
CTRU02_02134	Species-specific	Yes	-	87	9,287	8	-	Yes	OG0031056
CTRU02_02414	Species-specific	-	-	85	9,306	0	-	-	OG0031076
CTRU02_02635	Species-specific	Yes	-	79	7,991	7,6	-	Yes	OG0031095
CTRU02_04831	Species-specific	Yes	-	52	5,747	5,8	-	Yes	OG0031248
CTRU02_04889	Species-specific	-	-	48	5,234	2,1	-	-	OG0031252
CTRU02_05045	Species-specific	-	Yes	70	7,095	4,3	-	Yes	OG0031265
CTRU02_05072	Species-specific	-	-	71	8,002	1,4	-	-	OG0031268
CTRU02_05528	Species-specific	-	-	99	10,855	2	-	-	OG0031319
CTRU02_05538	Species-specific	-	-	53	5,58	1,9	-	Yes	OG0031320
CTRU02_05671	Species-specific	-	Yes	161	17,465	7,5	Chlo-mit	-	OG0031344
CTRU02_05841	Species-specific	-	-	119	13,202	0	-	-	OG0031355
CTRU02_06815	Species-specific	-	-	120	12,315	1,7	-	-	OG0031429
CTRU02_07028	Species-specific	-	-	108	11,908	0,9	-	-	OG0031438
CTRU02_07468	Species-specific	Yes	-	90	9,561	8,9	-	Yes	OG0031469
CTRU02_08110	Species-specific	Yes	-	164	18,212	6,1	-	-	OG0031506
CTRU02_08233	Species-specific	Yes	-	78	8,632	1,3	-	Yes	-
CTRU02_08935	Species-specific	-	-	146	16,718	4,1	Chloroplast	-	OG0031544
CTRU02_09022	Species-specific	-	-	87	9,037	2,3	-	-	OG0031552
CTRU02_09174	Species-specific	Yes	-	69	7,416	1,4	-	-	OG0031556
CTRU02_09190	Species-specific	Yes	-	80	8,944	7,5	-	Yes	OG0031557

Table S4: (continued)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CTRU02_09640	Species-specific	-	-	48	4,786	4,2	-	Yes	OG0031584
CTRU02_10342	Species-specific	Yes	-	77	8,507	6,5	-	-	OG0031637
CTRU02_10581	Species-specific	Yes	-	170	18,701	3,5	-	-	OG0031657
CTRU02_11186	Species-specific	-	-	85	8,868	1,2	-	Yes	OG0031694
CTRU02_11196	Species-specific	-	-	312	34,3	1,3	Nucleus	Yes	OG0031695
CTRU02_11952	Species-specific	Yes	-	147	15,897	5,4	-	Yes	OG0031756
CTRU02_11978	Species-specific	-	Yes	308	32,681	0,6	-	Yes	OG0031757
CTRU02_12587	Species-specific	-	Yes	196	19,922	0	-	Yes	-
CTRU02_13132	Species-specific	Yes	-	120	13,218	7,5	-	-	OG0031827
CTRU02_13374	Species-specific	-	-	77	8,08	0	-	Yes	OG0031846
CTRU02_13651	Species-specific	Yes	-	134	15,173	4,5	-	-	OG0031862
CTRU02_13804	Species-specific	-	-	38	4,396	0	-	-	OG0031876
CTRU02_13901	Species-specific	-	Yes	151	15,386	0	-	-	OG0031879
CTRU02_14087	Species-specific	Yes	-	75	7,695	12	-	Yes	OG0031886
CTRU02_14662	Species-specific	-	Yes	173	18,514	0,6	Nucleus	-	-
CTRU02_14861	Species-specific	Yes	-	90	9,749	2,2	-	-	OG0031943
CTRU02_15496	Species-specific	-	Yes	173	18,514	0,6	Nucleus	-	-
CTRU02_15750	Species-specific	-	-	219	23,553	0,9	Nucleus	-	OG0032015
CTRU02_00515	Species-specific with similarity (nr db)	-	-	595	65,663	4,5	-	-	OG0030931
CTRU02_02969	Species-specific with similarity (nr db)	-	-	120	12,214	5	-	Yes	OG0031118
CTRU02_07605	Species-specific with similarity (nr db)	-	-	119	12,255	1,7	-	Yes	OG0031479
CTRU02_08303	Species-specific with similarity (nr db)	Yes	-	109	11,617	7,3	-	Yes	OG0031510
CTRU02_08569	Species-specific with similarity (nr db)	Yes	-	121	13,308	5	-	Yes	OG0031530
CTRU02_09092	Species-specific with similarity (nr db)	Yes	-	161	17,207	1,2	-	Yes	OG0031553

Table S4: (continued)

GeneID	BLAST	EffectorP 2.0	Tandem repeats	Lenght (a.a.)	Mol. weight (kdA)	% of cisteynes	Subcellular localization	Apoplast	Orthogroup
CTRU02_12840	Species-specific with similarity (nr db)	-	Yes	261	28,784	3,4	-	-	OG0031814
CTRU02_12970	Species-specific with similarity (nr db)	-	-	325	36,078	0	-	Yes	OG0031821
CTRU02_13195	Species-specific with similarity (nr db)	Yes	-	218	23,551	5,5	-	Yes	OG0031832
CTRU02_13647	Species-specific with similarity (nr db)	Yes	-	146	15,496	2,7	-	Yes	OG0031861
CTRU02_13981	Species-specific with similarity (nr db)	Yes	-	213	23,007	6,6	-	Yes	OG0031883
CTRU02_14089	Species-specific with similarity (nr db)	Yes	-	146	15,943	4,1	-	Yes	OG0031887
CTRU02_14439	Species-specific with similarity (nr db)	Yes	-	201	20,923	4,5	Nucleus	Yes	OG0017420
CTRU02_15174	Species-specific with similarity (nr db)	-	Yes	157	16,661	0	-	-	OG0031975
CTRU02_15290	Species-specific with similarity (nr db)	Yes	-	106	11,413	5,7	-	Yes	OG0031984

* nr db: non-reduntant database.

6. TRANSCRIPTOME ANALYSES OF DIFFERENT SOYBEAN CULTIVARS PROVIDES NOVEL INSIGHTS INTO THE SOYBEAN RESPONSE TO *Colletotrichum truncatum* INFECTION

ABSTRACT

Soybean (*Glycine max*) is one of the major crops worldwide as a source of protein-rich foods and animal feeds. Soybean anthracnose, mainly associated with the hemibiotrophic fungus *Colletotrichum truncatum*, is one of the limiting factors to soybean production. In order to gain a better understanding of the genetic basis of soybean defense against *C. truncatum*, we used a combined approach of pathogenicity assays and deep RNA sequencing. We examined the transcriptional pattern of two soybean commercial cultivars (*Gm1* and *Gm2*) during the development *C. truncatum* infection by two different strains (γ and β). Pathogenicity assays showed that the same soybean cultivar can have contrasting levels of resistance to different strains of *C. truncatum*, and this behavior is confirmed by the sequenced data. We divided the four treatments into two conditions accordingly to the level of resistance to anthracnose. Based on our results, we suggest that upon recognition of the fungus *C. truncatum,* treatments with a higher level of resistance modulate several genes related to plant immune responses, including signaling pathways and extensively transcriptional reprogramming that led to a hypersensitive response related to oxidative burst and activation of genes encoding compounds that enhanced plant resistance to pathogens.

Keywords: Time-course, RNA sequencing, Gene Expression, *Glycine max*, Anthracnose.

6.1 INTRODUCTION

Soybean (*Glycine max*) is one of the most produced cropss worldwide, which can be extensively used for human and animal feeds due to its valuable nutritional composition (Hartman et al., 2011; Shea et al., 2020). Its production is constantly threatened by multiple diseases, including anthracnose, caused by several species of the hemibiotrophic fungus *Colletotrichum* (Riccioni et al., 1998; Sharma et al., 2011; Yang et al., 2012, 2014; Barbieri et al., 2017; Damm et al., 2019; Boufleur et al., 2020; Shi et al., 2020), being *C. truncatum* one of the most distributed species worldwide (Boufleur et al., 2021). Losses due to this disease can reach up to 100% under favorable climate conditions (Wrather et al., 2010), where 90kg/ha can be lost for each 1% of increment of the disease in the field (Dias et al., 2016).

The plant immune system has multiple layers (Jones and Dangl, 2006a; Poland et al., 2009; Cook et al., 2015; Jones et al., 2016). The resistance of plants against pathogens can be either qualitative, when a plant has a high level or is completely resistant, and is well represented by the zig-zag model, which describes pamp-triggered immunity (PTI) and effector-triggered immunity (ETI) (Jones and Dangl, 2006a) or quantitative, that is referred to incomplete or partial levels of resistance phenotypes, and can be explained by the invasion

model, which describes plant immunity as a surveillance system that is constantly evolving to trigger plant defense (Cook et al., 2015). However, there is no clear separation among qualitative and QDR genetic mechanisms, as QDR may or may not be a result of variation in components of PTI and ETI (Poland et al., 2009; Corwin and Kliebenstein, 2017).

Even if it is considered the predominant resistance found in nature, genes and mechanisms involved in QDR are poorly understood (Bartoli and Roux, 2017; Delplace et al., 2020), and resistance involving the QDR is more difficult to select due the involvement of several genes (Corwin and Kliebenstein, 2017). Because of the reduced selective pressure on the pathogen and the possibility of providing broad-spectrum resistance for more than one species and/or race of a pathogen, QDR has great potential to be more durable in the field (Krattinger et al., 2009; Corwin and Kliebenstein, 2017; Pilet-Nayel et al., 2017; Nelson et al., 2018).

The coordination of early defense responses to pathogen attack in plants may be required by QDR (Delplace et al., 2020) and New Generation Sequencing (NGS) strategies, such as RNAseq can be a useful tool to understand some components of the complex defense mechanisms involved in QDR, shedding light on genes and pathways involved in plant resistance against pests and pathogens, allowing the design of more resistant genotypes that can be used in agriculture (Nelson et al., 2018; Delplace et al., 2020). Multiple hosts have QDR against *Colletotrichum* species, among them strawberry (*Fragaria x ananassa*) resistance to *C. acutatum* and *C. gloeosporioides* (Amil-Ruiz et al., 2016; Anciro et al., 2018), bean (*Phaseolus vulgaris*) resistance to *C. lindemunthianum* (Geffroy et al., 2000; Mungalu et al., 2020), and potato (*Solanum tuberosum*) resistance against *C. coccodes* (Massana-Codina et al., 2020).

Although chemical control is an efficient method for disease management and allowed farmers to increase production due to the reduction of losses caused by pests and diseases; genetic resistance is a more eco-friendly solution for efficient production systems (Oerke and Dehne, 2004; Nelson et al., 2018), and soybean breeding should be employed as part of a strategy to control soybean anthracnose. Multiple studies aiming the identification of soybean resistant sources were performed around the world (Costa et al., 2009; Nagaraj et al., 2014; Yang and Hartman, 2015; Dias et al., 2019), however, genetic mechanisms that underpin soybean resistance against anthracnose have not yet been investigated, and until now, research progress on this field has been slow. With the advance of biotechnology, mechanisms involved in several pathways underlying plant defense against *Colletotrichum* species have been reported as a result of the investigation of transcriptomic data (Vargas et al., 2012; Amil-Ruiz et al.,

2016; Padder et al., 2016; Bhadauria et al., 2017; Diniz et al., 2017, 2017; Miranda et al., 2017; Mehmood et al., 2021).

In this study, we provide a wide-range transcriptomic analysis of the soybean-*C. truncatum* interaction during the development of anthracnose disease. We sequenced the transcriptome of two soybean cultivars that alters their level of resistance upon inoculation with two different strains of *C. truncatum*. The combined approach between pathogenicity assays and transcriptomic analyses revealed a strong correlation, that will pave the way towards a better understanding of the complex molecular mechanisms underlying the resistance of soybean to *C. truncatum*.

6.2 MATERIALS AND METHODS

6.2.1 Pathogenicity assays for assessment of soybean susceptibility to anthracnose

A preliminary screening of the interaction between 7 commercial soybean cultivars and 15 strains of *C. truncatum* was carried out in controlled conditions in a screenhouse (data not shown). As results, we spotted four combinations to access the differences in the genetic response during the development of *C. truncatum* infection. These combinations consisted of two soybean cultivars, IPRO7739 (*Gm1*) and IPRO8372 (*Gm2*) from Monsoy company and two *C. truncatum* strains, CMES1059 (β) and CMES1080 (γ).

Soybean seeds were superficially disinfected with NaClO 1% for 1 minute, then rinsed three times with sterile distilled water (SDW). Seeds were placed in plates filled with 100 g of sterile sand, soaked with 10 mL of SDW and incubated at ± 25°C for 32 h until germination. Conidia of *C. truncatum* were cultured in PDA medium for 15 days prior to collection for inoculations. Conidia suspensions were prepared by washing the cultures with SDW. For the inoculation, suspensions were adjusted to a final concentration of 1×10^6 conidia/mL, and the 3 pre-germinated seeds were inoculated with 5µL of conidia suspension as described by Dubrulle et al., (2020) in each treatment; seedlings inoculated with SDW were used as negative control. Inoculated seedlings were initially incubated in the dark at 25ºC for 4 h and then transferred to 100 mL pots filled with sterilized vermiculite and randomly distributed and incubated in a Conviron plant growth chamber at 25°C and watered with 25 mL of SDW every day. In total, three biological repetitions were performed. *Colletotrichum truncatum* was reisolated from all interactions with soybean at 120 hpi. To double check the presence of the pathogen in inoculated plants, polymerase chain reaction (PCR) was performed using *C. truncatum* specific

glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene primers (ColTF6/R5) as described by Ciampi-Guillardi et al., (2020) at 48 and 120 hpi.

The severity of anthracnose for each of the four interactions (γ -*Gm1*; γ -*Gm2*; β-*Gm1* and β-*Gm2*) was estimated at 120 hpi. A diagrammatic scale ranging from 0 to 5 adapted from Yang and Hartman (2015) was used for the evaluations; were 0 = no visible symptoms; 1 = 1 to 10% of the plant with symptoms (initial affected cotyledons); 2 = 11 to 35% of the plant affected (initial symptoms on cotyledons and on hypocotyls); 3 = 36 to 65% of the plant with symptoms (severe necrosis of cotyledons and symptoms on hypocotyl); 4 = 66 to 90% of the plant affected (necrosis in cotyledons, hypocotyl and leaflets); 5 = 91 to 100% of the plant affected (severe necrosis in all tissues and plant death). A double factorial scheme analysis was performed on the three biological replicates, using the ExpDes R package (v.1.2.0) with the post-hoc Tuckey method at 0.05 significance level. The treatments were grouped in two conditions (A and B) based on the similarity of the defense response.

6.2.2 Preparation of RNA samples and extraction

For total RNA extractions, five soybean plants were infected per treatment per time point as described in 6.2.1. Hypocotyls fragments of 0.5 cm of five randomly selected plants were collected and pooled together at 0; 12; 48 and 120 hpi. Harvested plant tissue was flash-frozen in liquid N_2 and stored at -80ºC until RNA extraction. Tree biological replicates of the experiment were performed. The collected tissue was ground using mortar and pestle and total RNA was purified using the PureLink RNA Mini Kit (Invitrogen, USA) following the manufacturer's instructions. Total RNA was treated with RNAse-free DNAse (Life Technologies) to remove DNA contamination. The quantity of total RNA was estimated using Qubit 2.0 fluorometer (Life Technologies) and RNA integrity was checked using Agilent TapeStation 4200 (Agilent Technologies).

6.2.3 Library construction and Illumina RNA sequencing (RNA-seq)

Total extracted RNA was sent to Genewiz (South Plainfield, USA) for Illumina sequencing. In total, 48 libraries derived from all the combinations (Figure 2) were prepared using the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB) using manufacturer's instructions. Sequencing libraries were validated on the Agilent TapeStation (Agilent technologies) and quantified in Qubit 2.0 fluorometer (Invitrogen) and by quantitative PCR (KAPA Biosystems). Libraries were sequenced using Illumina HiSeq4000 (2x150bp).

6.2.4 Quantification of transcript abundance and time-course differential expression analysis

The quality of reads was accessed using FastQC (v.0.11.7) and clean reads were obtained by removing reads containing adapters with CutAdaptors (v.1.9.1). Paired-end clean reads were mapped against the 88,646 transcripts of the soybean reference genome Wiliams 82 (*G. max* Wm.82.a2.v1) downloaded from Phytozome (v.13) (https://phytozome-next.jgi.doe.gov/) (Schmutz et al., 2010) using HISAT (v.2.1.0). Alignments from each library were processed with StringTIE (v.1.3.5) to quantify expression values of transcripts.

The biological variability of DESeq2's median of ratios normalized samples within each time point was accessed with a principal component analysis (PCA) using the plotPCA function of DESeq2 (v.1.28.1). A hierarchical *k*-means time-course clustering to access the expression profiles of soybean transcripts in condition A and condition B was performed using the TCseq package (v.1.12.1). To detect DEGs in a time-course approach, transcripts which had a minimum of 10 counts per million in at least three repetitions were kept, and statistical analysis among libraries were performed with DESeq2 R (v.1.28.1) package. Only those transcripts with a false-discovery rate (FDR) of ≤ 0.05 and log2-fold change (L2FC) ≥ 2 or L2FC ≤ -2 were considered DE. The used approach it shown in Figure 2.

6.2.7 Functional annotation of selected genes

Venn diagrams of DEGs were generated using the Venn Diagram module of Intervene online tools (Khan and Mathelier, 2017) for the selection of transcripts related to condition A and condition B in each time-point. Selected transcripts were grouped into functional categories accordingly to annotations of the soybean reference genome and manually checked using InterProScan, PFAM terms and the Kyoto Encyclopedia of Genes and Genomes (KEGG).

BinGO app in Cytoskape (Maere et al., 2005) was used to predict overrepresented biological processes (BP) and molecular functions (MF) among common up and downregulated transcripts in each condition. Enriched GO terms were detected using the hypergeometric exact tests with an FDR ≤ 0.05.

6.3 RESULTS

6.3.2 Inversion of behavior of soybean cultivars response upon inoculation with different strains of *C. truncatum*

Two *C. truncatum* strains (γ and β) with inverse behavior in two different soybean cultivars (*Gm1* and *Gm2*) were selected for further analysis. Preliminary studies showed that *C. truncatum* conidia of γ and β strains germinate and form appressoria within 6 hpi when inoculated on *Gm1* and *Gm2* soybean pre-germinated seeds (data not shown). First anthracnose symptoms in *Gm1* and *Gm2* soybean cultivars started to appear 72 hpi and evolved to severe necrosis by 120 hpi in γ-*Gm*2 and β-*Gm1* (Figure 1A), while in γ-*Gm*1 and β-*Gm2* symptoms appeared to be milder. Plants used as negative controls remained asymptomatic.

At 120 hpi symptoms on soybean seedlings were evaluated based on a diagrammatic scale ranging from 1 to 5 (Yang and Hartman, 2015) and the results were submitted to a Tukey test ($p$value ≤ 0.05). For further analyses, the combinations were grouped into two biological conditions based on the isolate level response inside each cultivar. Treatments γ-*Gm*1 and β-*Gm2* belong to condition A and have a higher level of resistance; while treatments γ-Gm2 and β-*Gm1* belong to condition B, which presented a higher level of susceptibility (Figure 1B).

Colletotrichum truncatum specific PCR of the *GAPDH* gene confirmed the presence of the fungus on inoculated soybean plants during the asymptomatic and symptomatic phase of the disease (Figure 1C). Both, γ and β strains were successfully reisolated from all the inoculated combinations.

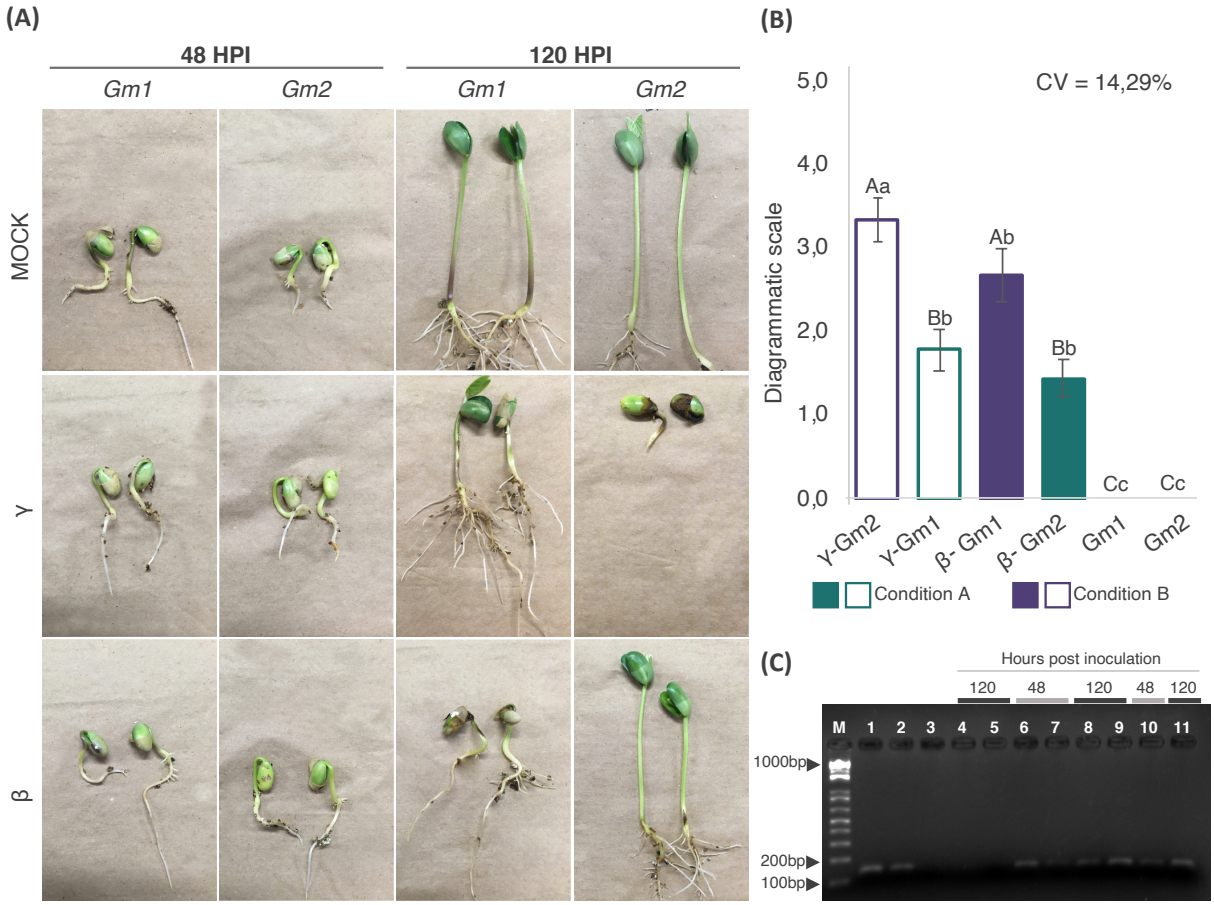

Figure 1: Response of two soybean cultivars (*Gm1* and *Gm2*) to inoculation with two different strains of *C. truncatum* (γ and β). (**A**) Symptoms observed in soybean 48 and 120 hours pos-inoculation (hpi). (**B**) Tuckey test applied on transformed data ((X+1)ˆ0.5). Upper case equal letters do not differ in average of isolate level inside each cultivar; lower case equal letters do not differ in average of cultivar level inside each isolate in Tuckey test with *p*-value = 0.05%. (**C**) *C. truncatum* specific polymerase chain reaction (PCR) results of GAPDH gene (ColF6/R5) visualized in a 3% agarose gel. M: 1 Kb leader; 1: γ positive control; β positive control; 3: negative reaction control; 3: *Gm1* non-inoculated; 4: *Gm2*: non-inoculated; 6,8: γ-*Gm1*; 7,9: β-*Gm2*; 10-11: γ-*Gm2*. Condition A: more resistant combinations; Condition B: more susceptible combinations.

6.3.3 Transcriptomic profiles confirm the biological behavior of soybean response to *C. truncatum* in all the treatments

To reveal the transcriptomic reprogramming of soybean during the development of *C. truncatum* infection, samples were collected for RNAseq at 0; 12; 48 and 120 hpi, and 48 cDNA libraries were sequenced. A total of 1,202,534,286 raw reads were generated in the Illumina HiSeq 4000 sequencing. Overall from 83.1 to 92.3% of paired-end reads were mapped to the *G. max* genome with 78.5 to 87.5% being uniquely mapped; only one library of γ-*Gm2* have 60% of mapped reads with 55.3% uniquely mapped (Figure S2A).

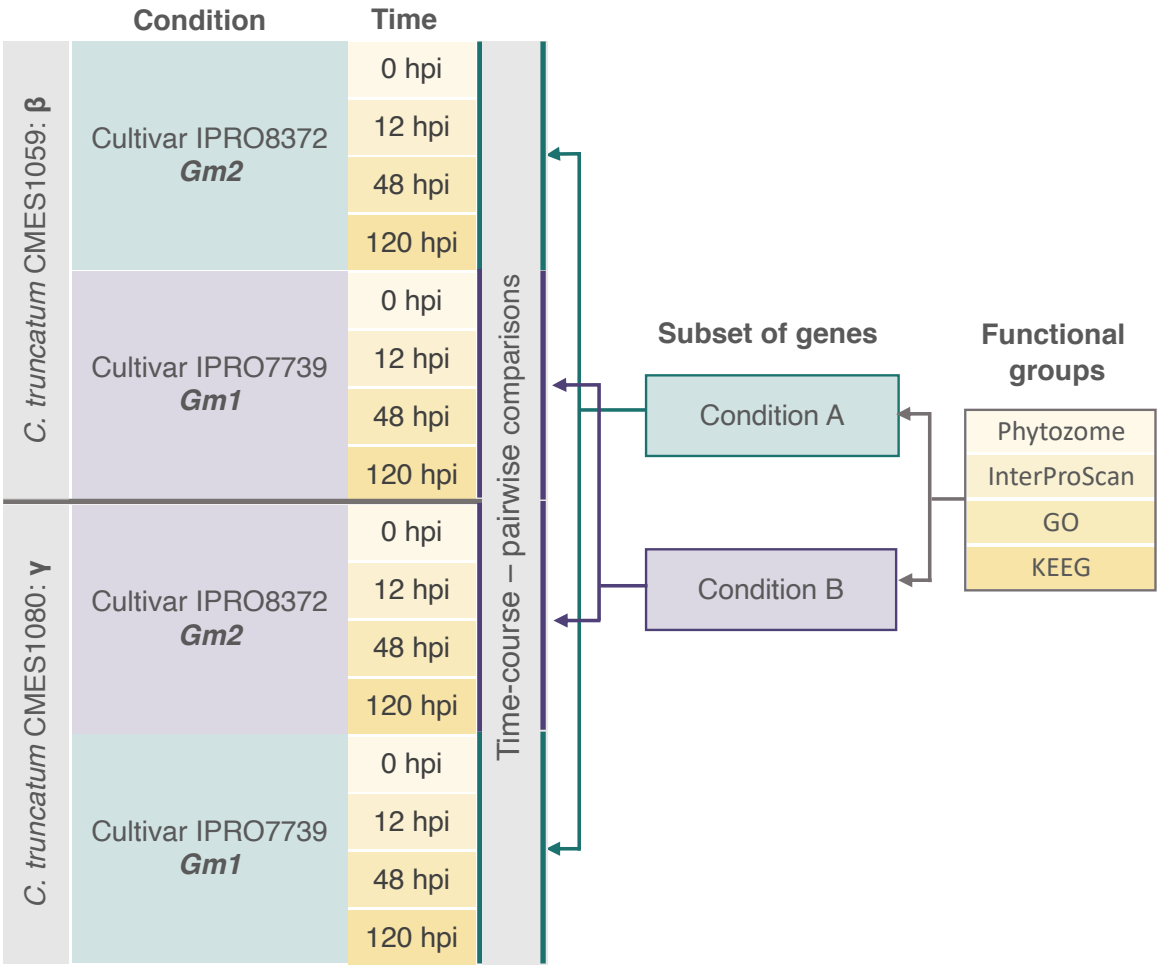

Figure 2: Approach used for the identification, subset and functional annotation of differentially expressed genes of two *Glycine max* cultivars during the evolution of infection by two *C. truncatum* strains.

A PCA of normalized samples within each time-point showed that the three biological independently generated libraries for each treatment are highly correlated (Figure S2B). At 0 and 12 hpi PCA analysis clustered combinations based on the soybean cultivar; while at 48 hpi combinations with a higher level of resistance clustered with β-*Gm1,* and at 120 hpi clusters were based on the *C. truncatum* strain (Figure S1B).

To preview the gene expression pattern in both conditions, raw transcript counts were clustered based on the *k*-means method. More resistant combinations have 10,522 genes in clusters, while the more susceptible ones have 3,004 genes in clusters. Clusters that represent ~75% of the transcripts of both conditions are shown in Figure S2C.

6.3.4 Combinations with a higher level of resistance to anthracnose have a more coordinated change in the soybean transcriptome when compared to the susceptible ones

To understand the development of *C. truncatum* in interaction with different cultivars of soybean, a time-course approach was used to identify DE genes (Figure 2). A total of 13,478 DEGs were identified (Figure 3), among these 5,495 were DE only more resistant combinations; 2,979 were DE only in more susceptible combinations and 4,904 were DE in both. The percentage of changes in the soybean transcriptome and the number of DEG in the time course comparison observed in each combination are shown in Figure 3. More resistant combinations show the highest percentage of changes in the soybean transcriptome when compared to more susceptible ones. In both resistant combinations, the total number of DEGs increases proportionally with the time, and the number of genes shared among both treatments in each timing are 154; 976 and 1675, respectively; while in more susceptible combinations, each have a different response, and the number of shared genes in each timing are 111; 4 and 10, respectively. In combination γ-Gm*2* the number of DEGs decreases in time; while in β-*Gm1* the number of DEGs decreases between 12 and 48 hpi and increases between 48 and 120 hpi.

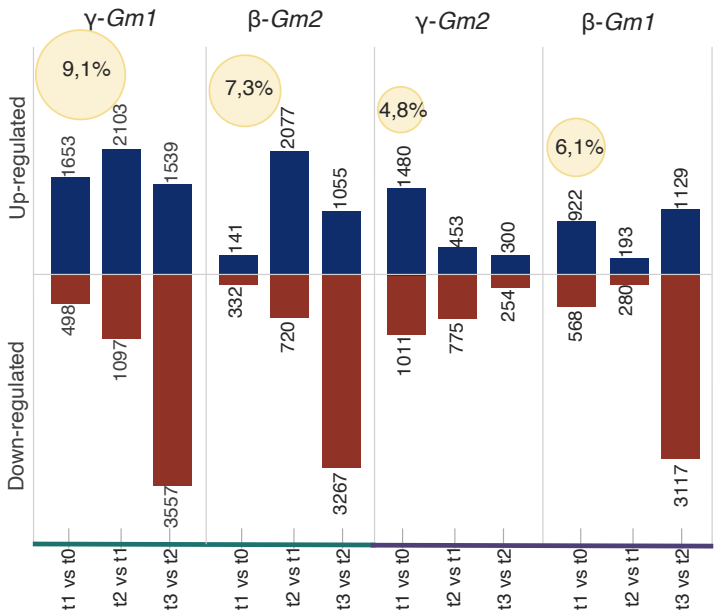

Figure 3: Changes in soybean transcriptome upon inoculation with different strains of *C. truncatum*. Total percentage of changes in each combination are represented inside yellow circles. The number of differentially expressed transcripts in each time pairwise comparison is represented by blue (upregulated) and red (downregulated) bars. The line in the bottom of the graph represent combinations that belong to condition A (green – more resisstant) and condition B (purple – more susceptible). T1: 0 hours pos-inoculation (hpi); T2: 12 hpi; T3: 48 hpi; and T4: 120 hpi.

The relationship among all combinations in each time point was visualized to identify genes associated with soybean resistance against *C. truncatum* and undergoing dynamic changes at the transcriptome level during the development of the disease, (Figure 4A). More resistant combinations shared the highest number of DEGs at all times when compared to more susceptible combinations, being the highest number of up (850) and downregulated (88) genes at 48 *vs* 12 hpi among the more resistant ones. The subset of 1,512 transcripts that are shared uniquely among both resistant and/or susceptible combinations on at least one timing was selected for further analysis. Among those, 1,401 have a coordinated expression only in the resistant combinations, 431 of them not modulated in the susceptible ones; 31 had a coordinated expression only in susceptible combinations, among these 12 are not modulated in the more resistant ones; and 79 were coordinated both conditions. For those DE on more resistant combinations, 72% had the pattern of expression showed in *k*-means cluster 2, while for the more susceptible ones the majority of 25% are represented by *k*-means cluster 1 (Figure 4B).

Functional annotations were assigned to the 1,512 genes based on the gene annotations in the soybean reference genome (*G. max* Wm.82.a2.v1). InterProScan, PFAM and KEGG terms were manually checked, and the expression patterns of the annotated genes, grouped in different functional categories are shown in Figure 4C.

From those 1,512 genes, around 27.5% (418) are involved in other plant functions, 34% (517) are involved in pathogen recognition and signaling, such as receptors, proteins related with kinase cascades, G-proteins, calcium signaling, hormones, transcription factors and epigenetics. General defenses such as pathogenesis-related (PR) proteins, cell wall related proteins, enzymes, reactive oxygen species (ROS), and other known defense proteins represent 28% (426 genes) of the annotated genes and around 8% (126) of the transcripts have no known domains or annotations or domains of unknown function.

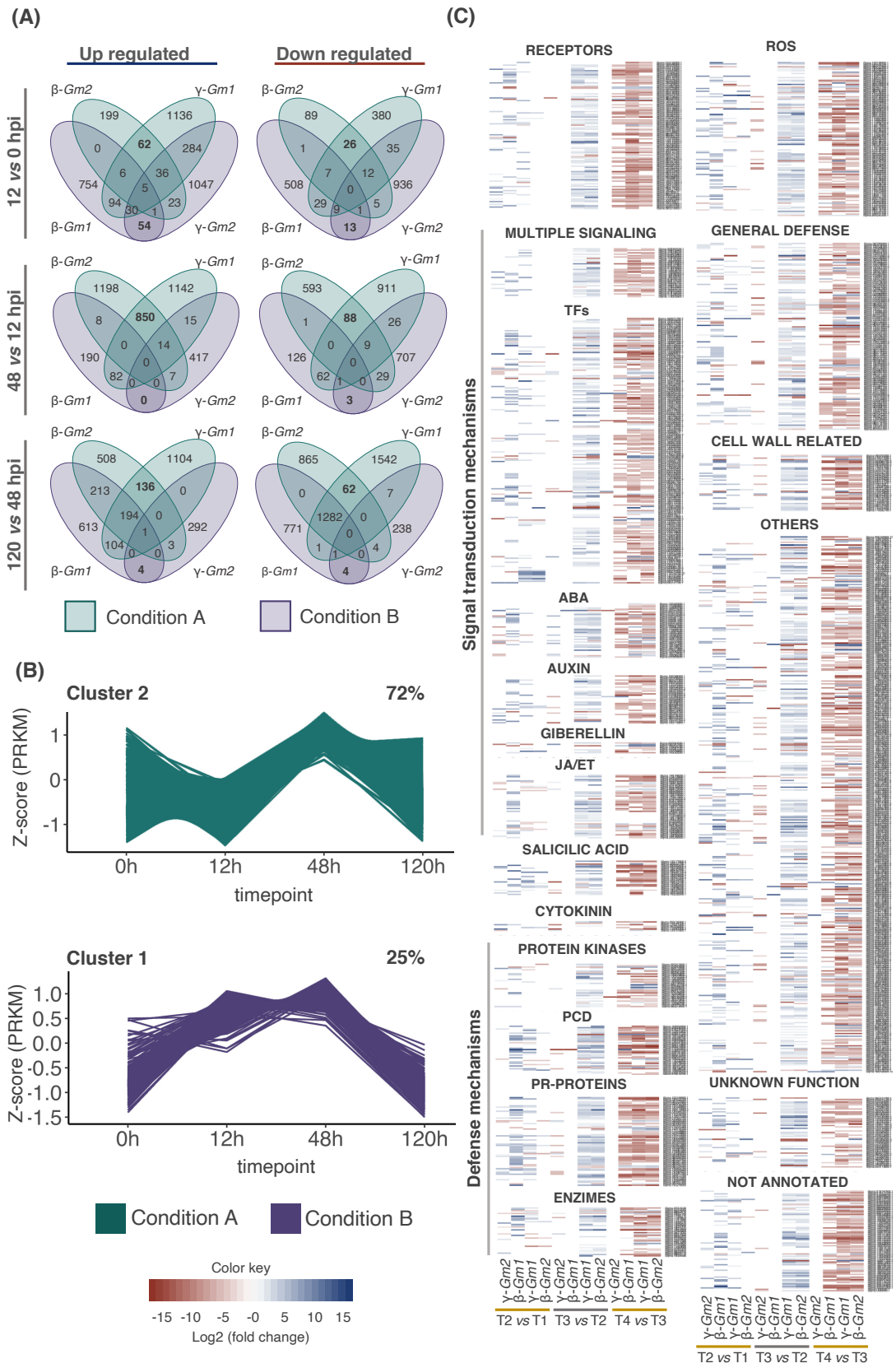

Figure 4: Visualization of differentially expressed (DE) transcripts shared among combinations of condition A (more resistant) (γ-*Gm1* and β-*Gm2*) and condition B (more susceptible) ((γ-*Gm2* and β-*Gm1*). (**A**) Venn diagrams displaying the overlaps among combinations of condition A and condition B at different time pairwise comparisons. (**B**) Representative *k*-means clusters corresponding to the majority of DE transcripts identified as common at condition A or B and (**A**). (**C**) Heatmaps showing the pattern of expression of 1,512 transcripts common to A and/or B in at least one time point. DE transcripts were grouped into different functional categories.

6.3.5 GO enrichment analysis revealed genes enriched in defense-related pathways in the more resistant condition

The subset of genes obtained in 6.3.4 was used to perform Gene Ontology (GO) enrichment analysis. Up and downregulated genes from each pairwise comparison in both conditions were scan separately, to identify overrepresented molecular functions (MF) and biological processes (BP) activated by soybean during the development of infection of *C. truncatum*.

For more susceptible combinations and for the first timing (12 *vs* 0 hpi) in more resistant combinations, no enriched GO terms were revealed. In more resistant combinations, hypergeometric test (p-value ≤ 0.05) detected 40 MF and 42 BP upregulated at 48 *vs* 12 hpi, while at 120 *vs* 48 hpi the analysis revealed 12 and 46 up and downregulated MF and 2 and 39 up and downregulated BP (Figures S2-S3). Of those, 32 MF and BP that are upregulated between 12 and 48 hpi are downregulated between 48 and 120 hpi. Even with different numbers of DE transcripts, this indicates that most of the enriched processes are triggered between 12 and 48 hpi and are deactivated between 48 and 120 hpi. Among those, several MF and BP involved in plant defense against pathogens are enriched, such as MFs "transcription regulation activity", "peroxidase activity", "antioxidant activity", "chitinase activity", "chitin binding" and "pattern binding". The BPs are "response to stress", "response to oxidative stress", "defense response", "response to biotic stimulus", and "response to wounding" (Figure S4). On the other hand, "defense response to fungus" and "response to stimulus" BPs are upregulated at 48 *vs* 12 hpi and "kinase activity" and "protein kinase activity" are downregulated only at 120 *vs* 48 hpi, meaning that the last two probably have a basal activity in soybean.

6.3.6 Identification of genes involved in different stages of soybean defense against *C. truncatum*

Genes DE uniquely at each timing and shared among both combinations of more resistant or more susceptible conditions were subset. In total, 235 genes involved in several defense responses were DE in different timings and selected for further analysis. Among those, 229 are specific to more resistant conditions; while 6 genes are specific to more susceptible conditions (Figure 5). In more resistant conditions, twenty-five genes are upregulated between 12 and 48 hpi and subsequentially downregulated at 120 hpi; all the others are associated with a unique timing. The pattern of expression of the genes grouped in different functional categories is presented in Figure 6. The subset of genes with respective L2FC values are shown in Table S1-S2.

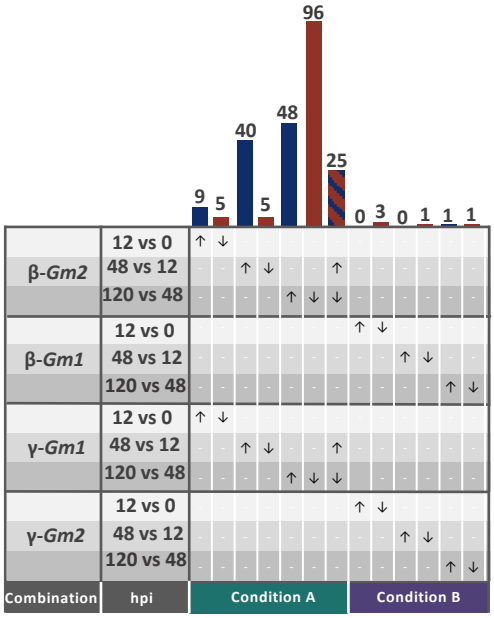

Figure 5: UpsetR plot showing differentially expressed genes, specific to each time pairwise comparisons for condition A (more resistant) and B (more susceptible). The number of up regulated (arrows pointing up) and downregulated (arrows pointing down) genes is represented in the upper side of each intersection bar. Genes commonly up and downregulated are colored with blue and red, respectively. Bar colored with diagonal stripes represent genes that are up regulated at 48 hpi (hours pos inoculation) and down regulated at 120 hpi.

6.3.7 Protein kinases and *C. truncatum* recognition are involved in soybean defense

Protein kinases (PKs) are involved in essential cellular functions and plant defense against biotic and abiotic stresses (Lehti-Shiu and Shiu, 2012). In our study, 99 genes (6.5%) out of the subset of 1,512 encoded PKs (Figure 4), among these, 38 are only modulated in more resistant conditions, 21 of those associated only with specific timings; while only two genes with kinase domains are specific to more susceptible conditions (Figure 6).

In more susceptible conditions, a *dual-specificity protein-kinase* (*YAK1*) (*Glyma.08G266300.2*) is highly downregulated (>-11 L2FC) between 48 and 120 hpi; and one lectin receptor like-kinase (Lectin-RLK) (*Glyma.10G228900.1*) is downregulated between 0 and 12 hpi, being the downregulation 3 times stronger in combination β-*Gm1* when compared with γ-*Gm2* (Figure 6).

A gene encoding a cyclin dependent protein kinase inhibitor (SMR) is downregulated at 120 hpi in more resistant conditions, while 3 genes with PK domains (*Glyma.06G127700.4*; *Glyma.07G088000.1*; *Glyma.08G318300.1*) are upregulated only during the symptomatic phase of anthracnose disease (120 hpi) (Figure 6).

Receptors like-kinases (RLKs) and receptors-like proteins (RLPs) are surface located in the plasma membrane of the plant cell and are part of the innate immune system of plants, responsible by the recognition of elicitors of the pathogen and early activation of downstream signaling events and plant defense (Jones and Dangl, 2006b; Cook et al., 2015; Boutrot and Zipfel, 2017; Kanyuka and Rudd, 2019). From the 40 genes with domains related to surface receptors modulated only in more resistant conditions, 16 genes with RLK domains and one gene with an RLP domain are putatively involved in *C. truncatum* recognition at specific timing. From those, 3 Lectin-RLKs are upregulated between 12 and 48 hpi, among these one (*Glyma.07G080700.1*) did not have alterations on the pattern of expression at 120 hpi and two (*Glyma.07G154100.1, Glyma.12G144500.1*) are downregulated at 120 hpi, being *Glyma.12G144500.1* upregulated in >9 L2FC in both combinations. Other 2 Lectin-RLKs (*Glyma.07G184000.1*; *Glyma.18G185400.1*), one WAK (*Glyma.07G094100.1)* and 3 RLK-GNK2 (*Glyma.11G206700.3*, *Glyma.20G137500.1*, *Glyma.20G139300.1*) are downregulated at 120 hpi (Figure 6). One leucine-rich-repeat (LRR)-Kinase (*Glyma.11G141400.2*) is upregulated between 12 and 0 hpi. Five LRR-RLKs (*Glyma.02G302600.1*, *Glyma.03G165800.1*, *Glyma.03G166300.1*, *Glyma.07G013700.1*, *Glyma.18G199000.2*) and one LRR-RLP (*Glyma.16G169500.1*) were also upregulated at 48 and downregulated at 120 hpi. Except for *Glyma.07G154100.1*, *Glyma.03G165800.1* and *Glyma.03G166300.1*, L2FC values are similar among both combinations (Figure 6). It is important to highlight that a Lectin-RLK receptor was commonly modulated only in more susceptible conditions, within the first 12 hpi (Figure 6).

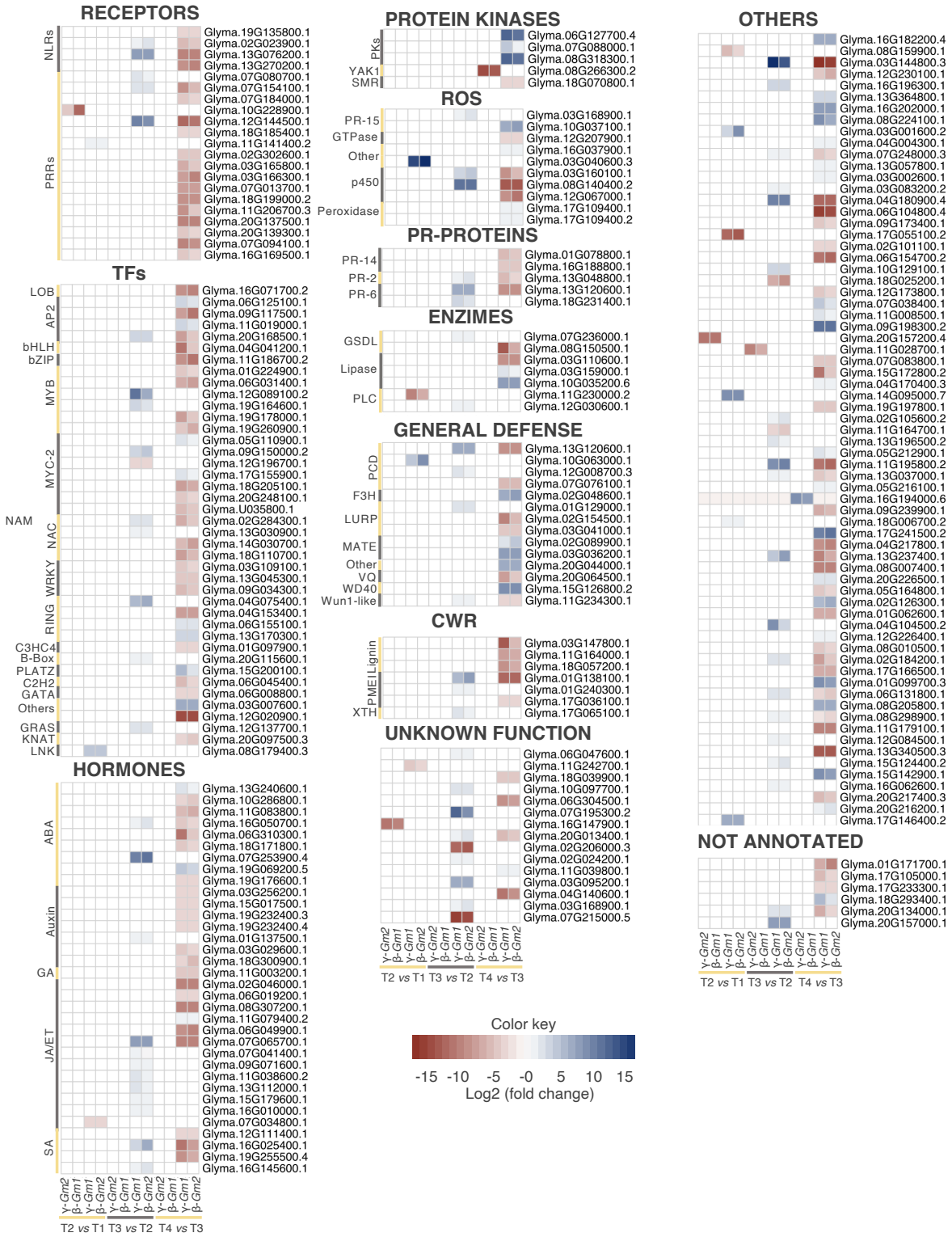

Figure 6: Heatmaps of differentially expressed (DE) transcripts related to specific time-points after soybean infection with *C. truncatum*. The annotated genes are grouped into different functional categories. Transcripts involved in recognition of the pathogen by the plant (receptors); transcripts involved into signal transduction (signaling, transcription factors (TFs), and hormones); transcripts involved into defense response of plants against pathogens (protein kinases, reactive oxygen species (ROS), enzymes, pathogenesis related (PR)-proteins and

general defense responses); other genes modulated upon soybean infection (cell wall related (CWR), others); genes with domains of unknown function and genes with no annotations (not annotated). T1: 0 hours pos-inoculation (hpi); T2: 12 hpi; T3: 48 hpi; and T4: 120 hpi.

Part of the *R* genes family, another class of receptors called nucleotide binding domain leucine-rich repeat containing (NLRs) are located in the cytoplasm of the plant cell (Kanyuka and Rudd, 2019; Shao et al., 2019). They are characterized to have an NB (nucleotide binding) and an LRR domain that can require specific sensors such as toll/interleukin-1 receptor (TIR) or coiled-col (CC) for downstream signaling (Collier et al., 2011; Cook et al., 2015; Jones et al., 2016). In the soybean genome, a total of 319 NLRs were putative identified (Kang et al., 2012). In this study, 22 genes with annotated domains associated with NLRs have its expression changed during soybean interaction with *C. truncatum,* among these 10 are activated only during more resistant conditions (Figure 4). Three of those have their expression changed only at specific timing. A CC-NBS-LRR (*Glyma.19G135800.1*) is upregulated at 48 and downregulated at 120 hpi. Two TIR-NBS-LRR genes (*Glyma.02G023900.1*, *Glyma.13G076200.1*) are upregulated earlier (between 12 and 48 hpi) and downregulated at 120 hpi (Figure 6).

6.3.8 Signaling transduction mechanisms involved in soybean defense against *C. truncatum*

After the recognition of the pathogen by cell surface or intracellular receptors, multiple signaling mechanisms can be activated, such as MAPKs and calcium fluctuations (Cook et al., 2015; Andersen et al., 2018). Out of the subset of 1,512 genes, 39 that are part of this two signaling mechanisms were activated during soybean interaction with *C. truncatum*. None of those have a time coordinated expression in more susceptible combinations, while 13 are only DE in more resistant combinations, and shared by both in at least one timing. Including 10 genes involved in calcium fluctuations: 4 of them activated between 12 and 48 hpi while the other 6 are downregulated at 120 hpi; and 3 genes involved in MAPK cascade, including a homologous to the MKK9, and a homologous of the *mitogen-activated protein kinase phosphatase 1* (*MKP1*), activated between 12 and 48 hpi, and a histidine kinase (HK), upregulated only at 120 hpi (Figure 4; 6).

Many forms of plant hormones act downstream of pathogen recognition into signal transduction for plant defense activation (Couto and Zipfel, 2016; Andersen et al., 2018). In the current work, 171 (11%) genes encoding molecules involved in the biosynthesis of 7 classes of

hormones were annotated. Among those, the most representative hormones were jasmonic acid (JA) and ethylene (ET) (36%), followed by abscisic acid ABA (23%), auxin (20%), salicylic acid (15%), gibberellin (GA) (4.5%) and cytokinin (CK) (4%) (Figure 4; 6)

Fifty-three genes, belonging to the 7 described classes of hormones were DE only in more resistant combinations. Two *pathogenesis-related 1* (*PR-1*) (*Glyma.13G094100.1*; *Glyma.17G066100.1*) involved in SA mediated defense are upregulated within the first 12 hpi. While an *inhibitor of protein-phosphatase* (*IPP2*) (*Glyma.20G157200.4*) is downregulated in more than 8.5 L2FC within the first 12 hpi, and a gene with a BTB/POZ domain belonging to the NPH3 family (*Glyma.13G368300.2*) upregulated at 120 hpi are only DE in more susceptible conditions.

Thirty-four genes encoding different proteins involved in ABA, auxin, GA, JA/ET and SA are DE only at specific timing after *C. truncatum* inoculation. Except for a lipoxygenase (LOX) encoding gene (*Glyma.07G034800.1*) that was early downregulated (within the first 12 hpi); 2 genes involved in ABA biosynthesis, being one a *protein-prhosphatase 2C* (*PP2C*) (*Glyma.19G069200.1*) and the other an allantoinase (*Glyma.13G240600.1*) are only upregulated at 120 hpi; and an *GDSL-like lipase* (*Glyma.11G079400.2*), all the others have a pick of expression at 48 hpi (Figure 6) that is visible within 12 and 48 hpi for JA and ET and within 48 and 120 hpi for genes involved into ABA, Auxin, GA.

6.3.9 Broad transcriptional reprogramming occurs in soybean after infection with *C. truncatum*

Transcription factors (TFs) can act in several levels of resistance of plants to pathogens (Andersen et al., 2018). In this work, 186 genes encoding at least 20 families of transcription factors or involved in transcription factor activities were common DE on among the more resistant and/or more susceptible combinations in at least one timing (Figure 4; 6). From those, 70 belonging to 15 families are DE only in more resistant combinations, among these 40 are only DE in specific timing (Figure 6).

One representant of each of 5 transcriptions factor classes are early modulated (firsts 12 hpi) in more susceptible combinations. A heat shock TF *B3* (*Glyma.19G137800.1*), a *WRKY* (*Glyma.16G054400.1*), a *MYB* (*Glyma.10G006600.1*) and a *NmrA-like negative transcriptional regulator* family protein (*Glyma.04G131100.1*) are overexpressed at 12 hpi (> 4.5 L_2FC) in more susceptible combinations while in more resistant combinations those TFs are only overexpressed at 48 hpi. Also, one *MYC*-type (*Glyma.01G186700.1*) is underexpressed within

the firsts 12 hpi in more susceptible combinations but have not a coordinated time expression in the more resistant ones. Another TF, *YL1* (*Glyma.05G136000.1*) is downregulated in more susceptible combinations within 12 and 48 hpi.

Transcription factors modulated only in specific timings of more resistant combinations include 5 *apetala 2* (*AP2*), one *C2H2*, 7 *MYC*, one *bZIP*, 6 *MYB*, 4 *NAC*, one *GATA*, 5 *RING*-type, one *PLATZ*, one *B-box*, 3 *WRKY*, one *LNK*, one *KNAT*, one *GRAS*, one lateral organ boundaries (LOB), and 5 genes related with transcription factor activity (Figure 6). From those, a *AP2,* a *B-box*, a *MYC,* a *RING*-type*,* 2 *MYB*, 2 *NAC* and one *GRAS* are upregulated between 12 and 48 hpi, while a gene encoding an *LNK* TF is overexpressed within the first 12 hpi; 2 *AP2,* 2 *MYC,* a *PLATZ,* 2 *RING*-type, and one gene with TF activity are upregulated at 120 hpi and all the others are downregulated at 120 hpi (Figure 6).

6.3.10 Soybean defense responses are activated in more resistant combinations upon infection with *C. truncatum*

Around 7% (110) of the subset of genes are involved in reactive oxygen species (ROS) production or regulation. From those, 75% (81) are activated in both conditions and 25.5% (29) are DE only in more resistant combinations. Three genes, including 2 genes encoding peroxidases (*Glyma.11G058100.1*; *Glyma.19G011700.1*) and a cytochrome p450 (*Glyma.13G285300.1*) are early upregulated in more susceptible combinations (within 12 hpi) while in the resistant ones are upregulated later (between 12 and 48 hpi) and with lower levels. The genes encoding peroxidases are downregulated at 120 hpi in more resistant combinations and in in β-*Gm1* while the cytochrome p450 is downregulated only in the resistant ones.

Eleven of the genes only DE at the resistant combinations can be linked to specific timing after inoculation with *C. truncatum*. Including gene encoding a *pyridoxal phosphate (PLP)-dependent transferase* (*Glyma.03g040600.3*)*,* that is the most overexpressed (up to 28 L2FC) gene in more resistant combinations within the first 12 hpi; a gene encoding a germin, that acts as an oxalate oxidase (*PR-15*) (Lane et al., 1993) (*Glyma.02G044500.1*) and a *GTPase* (*Glyma.12G207900.1*) are downregulated at 120 hpi; a germin (*Glyma.03G1689000.1*) is upregulated within 12 and 48 hpi; 2 genes that have peroxidase activity at low pH (*Glyma.17G109400.1*; *Glyma.17G109400.2*), a germin (*Glyma.10G037100.1*) and a dehydrin (HIRD11-like) are upregulated at 120 hpi; 3 cytochrome p450 (p450), 2 of them upregulated between 12 and 48 hpi and downregulated at the same levels at 120 hpi (*Glyma.03G160100.1*; *Glyma.08G140400.1*) and one (*Glyma.12G067000.1*) underexpressed at 120 hpi (Figure 6).

Programmed cell death (PCD) in plants is essential for development and defense mechanisms (Reape et al., 2008). In our study, 43 of the genes involved in PCD were commonly activated in more resistant and/or more susceptible conditions at a specific timing, including those already mentioned in hormone and transcription factor signal transduction. Seven of those, are only DE in more resistant combinations, being 4 DE only at a specific timing. Two of these genes encode a 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase. One (*Glyma.10G063000.1*) is early upregulated (between 0 and 12 hpi), another (*Glyma.12G008700.3*) is upregulated between 12 and 48 hpi (Figure 6). Also, *serpin* considered as a PCD regulator (Bhattacharjee et al., 2015) is upregulated between 12 and 48 hpi and downregulated at the same levels at 120, together with a gene encoding a BON associated protein 2 (BAP2), considered an inhibitor of PCD (Yang et al., 2007).

Among the subset of 1,512 genes, 63 (4%) are genes encoding pathogenesis-related proteins (PR), including *PR-1* and *PR-15*, that were mentioned before. Other PR-proteins include *β-1,3-glucanases* (*PR-2*), *chitinases* (*PR-3*, *PR-4*, *PR-8* and *PR-11*), *thaumatin-like* (*PR-5*), proteinase-inhibitors (*PR-6*), *ribonuclease-like* (*PR-10*) and lipid-transfer proteins (*PR-14*). Among those, 9 (including *PR-2*, *PR-4*, *PR-6* and *PR-14*) are only DE in more resistant combinations, being 5 only DE in specific timing (Figure 6). A *PR-2* (*Glyma.13G048800.1*) and two *PR-6* (*Glyma.13G120600.1; Glyma.18G231400.1*) are upregulated between 12 and 48 hpi, among those *Glyma.18G231400* have not changes in the expression levels at 120 hpi, while the other two are downregulated at this time. The other two PR-proteins (*Glyma.01G078800.1*; *Glyma.16G188800.1*) are PR-14 downregulated at 120 hpi (Figure 6).

Plant enzymes can help to inhibit fungal infection (Oh et al., 2005). Thirty-six genes among the subset of 1,512 were functionally annotated as enzymes. Between those, 13 are only DE in more resistant combinations, and 12 are related to specific time-points. Among those genes 9 are lipases: including two phospholipases C (PLC), one (*Glyma.11G230000.2*) downregulated within 12 hpi; and another (*Glyma.12G030600.1*) upregulated between 12 and 0 hpi. The 3 other lipases have a fungal lipase-like domain, one is upregulated at 48 hpi and downregulated at 120 hpi (*Glyma.03G110600.1*) and the other two, are upregulated only at 120 hpi (*Glyma.03G159000.1*; *Glyma.10G035200.1*). The other 3 enzymes are glycosyl hydrolases, being one upregulated within 12 and 48 hpi (*Glyma.07G236000.1*), one upregulated only at 120 (*Glyma.13G057800.1*), and one downregulated at the same timing (*Glyma.08G150500.1*).

Out of the 1,512 genes, 129 (8.5%) are related to processes involved in plant response against biotic and abiotic stresses, that were not aforementioned in this work. Thirty-seven of those are DE only in more resistant combinations, one is DE only in more susceptible ones and

the other 89 are DE in both conditions. Ten of those genes are DE only in more resistant combinations at specific timing (Figure 6). One gene with a VQ-motif (*Glyma.20G064500.1*) is downregulated at 120 hpi. A wound-induced protein (Wun1-like) (*Glyma.11G234300.1*) is upregulated between 12 and 48 hpi and downregulated later at 120 hpi. Three LURP-one-related (LURP1) (*Glyma.01G129000.1; Glyma.02G154500.1; Glyma.03G041000.1*) are among the defense-related genes. One is upregulated within the first 48 hpi and the other two are downregulated at 120 hpi. Two multidrug and toxic compound extrusion proteins (MATE) (*Glyma.02G089900.1*; *Glyma.03G036200.1*) and a member of the transducing WD40 repeat-like superfamily protein (WD40) alternative transcript (*Glyma.15G126800.2*) are upregulated at 120 hpi. One gene related to stress response (*Glyma.20G044000.1*) upregulated at 120 hpi (Figure 6).

Genes involved in the synthesis of polysaccharides or integrity of the plant cell wall were affected by the infection of *C. truncatum*. In total, 41 genes were shared in more resistant and/or more susceptible conditions in at least one timing. Seven genes can be related to specific timing, 4 of them are upregulated between 12 and 48 hpi, being 2 pectin methylesterase inhibitors (PMEI) (*Glyma.01G138100.1*; *Glyma.01G240300.1*), being *Glyma.01G138100.1* downregulated at 120 hpi, one xyloglucan endotransglycosylase 6 (XTH) (*Glyma.17G065100.1*), while another PMEI (*Glyma.17G036100.1*), a gene encoding a dirigent protein involved in lignin biosynthesis (Burlat et al., 2001) (*Glyma.03G147800.1*) and two genes encoding laccases (*Glyma.11G164000.1*; *Glyma.18G057200.1*) are downregulated at 120 hpi,

6.3.11 Other functions revealed by the analyses that may be involved in soybean response to *C. truncatum*

More than 400 genes, DE, and shared among more resistant and/or more susceptible conditions at specific timing are related with other functions of the plant. Among those, are genes involved in binding, catalytic activity, hydrolases, transferase activity, transporters, oxireductase activity and others. Fifteen genes with domains of unknown function (DUF) are also reported, most of them (10) with a peak of expression at 48 hpi, and one that is highly downregulated only in more susceptible combinations (< -9 L_2FC).

In more susceptible combinations, 6 genes without similarity with annotated databases or with any domains are activated between 48 or 120 hpi. Also, an *armadillo repeat kinesin 3* (*ARK3*) (*Glyma.11G028700.1*) is early downregulated more susceptible combinations, while a kinesin family member 22 (*KIN22*) (*Glyma.18G180900.10*) is upregulated at 120 hpi. An RNA

helicase (*Glyma.11G012200.3*) is upregulated in >11 L2FC at the first 12 hpi, while a gene with and RNA-binding domain (RBD) and an RNA-recognition motif (RRM) (*Glyma.09G217100.1*) downregulated in both treatments with a higher level of susceptibility between 48 and 120 hpi (Figure 6).

6.4 DISCUSSION

Losses of soybean production due to anthracnose are increasing over the years (Dias et al., 2016, 2019; Rogério et al., 2017). Multiple *Colletotrichum* species are reported as causal agents of the disease, being the hemibiotrophic species *C. truncatum* the one with most reports of strains retrieved from symptomatic plants worldwide (Boufleur et al., 2021). Studies performed across the world reported several soybean cultivars with different levels of resistance to the disease (Costa et al., 2009; Nagaraj et al., 2014; Dias et al., 2019). In our work, preliminary studies for screening soybean cultivars with potential resistance to *C. truncatum* were performed and indicated that each interaction between soybean and *C. truncatum* can result in different levels of resistance, suggesting that this interaction is essential for the establishment of the disease.

Pathogenicity assays showed that the same soybean cultivar can have contrasting levels of resistance against different strains of *C. truncatum* (Figure 1). Soybean cultivar *Gm1*, when inoculated with *C. truncatum* strain γ have a more resistant phenotype, while when inoculated with strain β the same cultivar is more susceptible. Cultivar *Gm2* on the other hand is more resistant to strain β and more susceptible to strain γ. The dependency of pathogen strain genotype for the level of resistance in plants was reported before for *Botrytis cinerea* in multiple hosts (Rowe and Kliebenstein, 2008; Corwin et al., 2016b; Zhang et al., 2016), *Sclerotinea sclerotiorum* in sunflower (Davar, 2011), *Pseudomonas syringae* in *Arabidopsis* (Kover and Schaal, 2002) and wild tomato (*Solanum habrochaites*) (Thapa et al., 2015). Besides that, the possibility of linkage among plant resistance and pathogen aggressiveness levels was already raised (Niks et al., 2015; Corwin and Kliebenstein, 2017), however, the genetic basis of this phenomena support the role of pathogen and host variation in the shift of QDR not clear (Corwin and Kliebenstein, 2017).

Besides the potential of destruction of soybean anthracnose, genes involved in soybean resistance against this disease have not yet been investigated. In order to gain insights into mechanisms involved in soybean resistance against *C. truncatum*, we performed an RNAseq analysis to compare the expression patterns of two soybean cultivars inoculated with two strains

of the fungus during the development of the disease (Figure 2). The four treatments were divided into two conditions, based on the level of resistance observed. Treatments with a higher resistance level (γ-*Gm1* and β-*Gm2*) belong to condition A; while the other two treatments (γ-*Gm2* and β-*Gm1*) are more susceptible and belong to condition B, however, they have some variability between them (Figure 1). Our results revealed that the transcriptomic data reflects the biology of the four interactions, were treatments more resistant combinations have similar patterns of changes in gene expression and share up to 38% of DE in each timing; while each one of the more susceptible combinations has a different pattern of expression and share up to 4% of the DE genes within the first 12 hpi, while in the later stages the number of shared genes reach a maximum of 0.4% (Figure 3).

It was shown that unlike biotrophic pathogens, the hemibiotrophic fungus *C. graminicola* induces the expression of defense-related genes in maize during the early stages of infection (Vargas et al., 2012). This behavior was confirmed in other pathosystems involving hemibiotrophic fungi, including rice when inoculated with *M. oryzae* (Bagnaresi et al., 2012) and strawberry when inoculated with *C. acutatum* (Amil-Ruiz et al., 2016). In agreement with published studies, the coordinated modulation of the soybean transcriptome in interactions more resistant interactions, with most of the genes shared among both (72%) having a peak of expression at 48 hpi, suggesting that the asymptomatic phase of the disease is essential for the definition of the levels of resistance of soybean to *C. truncatum*.

More than 400 soybean transcripts were commonly and only DE in both treatments with a higher level of resistance at the same timing with similar levels of changes in expression, among these, 230 can be linked with specific timing after inoculation with *C. truncatum;* while 12 transcripts are associated only with more susceptible treatments, of those, 4 associated with specific timing. These genes are strongly correlated with the plant response upon *C. truncatum* infection, and this correlation was possible due to the contrasting response of the same soybean cultivar upon the change of *C. truncatum* strain; being potential candidate genes to be investigated in further works, as possible play roles in the level of resistance of soybean to anthracnose. Therefore, we focused our efforts to understand the role of the genes that are only modulated during more resistant interactions.

Representants of genes and alternative transcripts belonging to several families and/or pathways involved in recognition, signal transduction, plant defense outputs and other plant processes were DE during the development of *C. truncatum* infection of soybean only in treatments with a higher level of resistance, suggesting that soybean defense against *C. truncatum* goes far beyond pathogen recognition. The involvement of several classes of genes

in plant defense is a typical behavior related to QDR (Corwin and Kliebenstein, 2017), which can involve representants of plant surface and intracellular receptors, enzymes and transporters usually involved in PTI and effector trigger immunity (ETI) defense responses (Niks et al., 2015), which leads to an unclear distinction between both, quantitative and qualitative disease resistance (Poland et al., 2009).

The plant immunity system against a hemibiotrophic pathogen can be explained by the invasion and the spatial invasion models (Cook et al., 2015; Kanyuka and Rudd, 2019), in which it proposed that invading pathogens can be recognized by the plants directly or indirectly, by cell surface immune receptors (CSRIs) or intracellular immune receptors (IIRs), that activated signal transduction and defense pathways (Kanyuka and Rudd, 2019), being a rapid activation of pathogen recognition essential for an accelerated defense response (Haddadi et al., 2016). The interaction between soybean and *C. truncatum* revealed an extensive modulation of genes encoding putative CSRIs or IIRS, being more than 20 of them associated with specific timing of development of *C. truncatum* infection.

Activation of Lectin-RLKs due to pathogen infection was reported before in multiple hosts, including maize in response to *C. graminicola* (Miranda et al., 2017), wheat response to *Zymoseptoria tritici* (Ma et al., 2018) and *Arabidopsis*, where the LecRK-VI.2 RLK is essential for the activation of defense against the hemibiotrophic bacteria *Pectobacterium carotovorum* (Singh et al., 2012). Our results revealed an early downregulation of a gene encoding a Lectin-RLK that occurred only in more susceptible combinations, while another gene encoding a CSRIs with an LRR domain, was upregulated during the same timing only in more resistant combinations. Also, 3 other genes encoding Lectin-RLKs were upregulated during the asymptomatic phase of the disease, while several putative CSRIs were downregulated during the symptomatic phase of the disease. The early upregulation followed by a latter downregulation of RLKs is consistent with other studies reporting the expression of RLKs during plant defense (Decreux and Messiaen, 2005; Lannoo and Van Damme, 2014; Ma et al., 2018). On the other branch of the spatial invasion model system, we have the intracellular recognition of pathogens with NLR receptors (Kanyuka and Rudd, 2019). NLRs with TIR domains were reported before with a role in plant defense against *Colletotrichum acutatum* (Amil-Ruiz et al., 2016) and *Colletotrichum lentis* (Bhadauria et al., 2017). We also observed the activation of few genes encoding IIRs during soybean interaction with *C. truncatum*, two of them overexpressed during the asymptomatic phase of the disease, and underexpressed later, during the symptomatic phase. The modulation of genes encoding CSRIs and IIRs during soybean interaction with *C. truncatum* suggests that both “branches” of the spatial invasion

model are activated and have a role in soybean defense against *C. truncatum,* consistent with the results observed before, during strawberry-*C. acutatum* interaction (Amil-Ruiz et al., 2016). The importance of increase elicitor recognition by plants for improving the management of diverse diseases has already been demonstrated (Boutrot and Zipfel, 2017). Both, CSRIs and IIRs can be transferred among different genotypes, providing a broad spectrum of disease resistance by providing additional recognition capacities in crops (Dangl et al., 2013; Boutrot and Zipfel, 2017, Jones et al., 2016).

After the plant recognizes infection, the recognition signal must be transduced to the plant cell nucleus to initiate several mechanisms involved in plant defense (Jones and Dangl, 2006b; Cook et al., 2015; Andersen et al., 2018). Our results revealed the activation of several signaling pathways in soybean due to *C. truncatum* infection, including MAPKs, calcium fluctuations, TFs families and phytohormones such as JA and ET, SA, GA, CK, Auxin and ABA, with related genes modulated only in condition A. Among them, genes involved in phytohormones pathway and TFs can be associated with the timing of *C. truncatum* infection development in soybean plants.

Ethylene JA, SA, ABA, Auxin and GA play central roles in plant defense against different pathogens (Vargas et al., 2012; Balmer et al., 2013; Gao et al., 2014; Amil-Ruiz et al., 2016; Diniz et al., 2017; Miranda et al., 2017; Andersen et al., 2018). In our work, genes-encoding proteins that have a role in the biosynthesis and/or regulation of these 5 classes of phytohormones can be linked to specific timings of soybean/*C. truncatum* infection during more resistant combinations. In strawberries, both JA and SA are important in defense against *C. acutatum* (Amil-Ruiz et al., 2016). In maize infected with *C. graminicola* increasing levels of JA, ET, SA, ABA, and auxin were reported (Vargas et al., 2012; Balmer et al., 2013; Miranda et al., 2017; Gao et al., 2014).

YAK1 is a member of the Tyr phosphorylation-regulated kinases (DYRKs), an evolutionarily conserved protein kinase first identified in *Saccharomyces cerevisiae* (Yoshida, 2008). Until now, its role in plant defense against biotic stresses is largely unknown. An AtYAK1 gene was recently shown to be involved in ABA signaling and plant growth (Forzani et al., 2019). In our work, a YAK1 homologous gene was highly downregulated during the symptomatic phase of the disease only in more susceptible combinations however, the modulation of other genes related to ABA signaling only during treatments with a higher level to susceptibility to *C. truncatum* was not observed; while several genes involved in ABA signaling were modulated in more resistant ones.

NAC TFs, MYC2 TFs and its suppressor jasmonate-zim-domain protein 1 (JAZ1) are involved in the signaling and production of JA (Kazan and Manners, 2008; Becker et al., 2017; Liu et al., 2017). A *JAMYC/AtMYC2* TF has a key-role in *Arabidopsis* defense gene activation due to JA signaling pathway (Boter, 2004). NAC TFs were overexpressed in resistant canola cultivar when inoculated with the hemibiotrophic fungus *Leptosphaeria maculans* (Becker et al., 2017). Previous studies reported a role of *JAZ* genes in plant resistance to pathogens, such as *Arabidopsis* resistance to the hemibiotrophic bacteria *Pseudomonas syringae* (Demianski et al., 2012) and sugarcane resistance to the biotrophic fungus *Sporisorium scitamineum* (Liu et al., 2017). Our results revealed that the JA signaling pathway is the phytohormone with the most time coordinated expression during condition A, including multiple *JAZ* genes, *NAC* and *MYC2* TFs, indicating a role of JA production in soybean defense against *C. truncatum*, agreeing with a previous study, were the strongest phytohormone activation observed in coffee upon infection of *C. kahawae* was JA (Diniz et al., 2017).

In maize, genes related to MAPK cascade were activated upon infection with *C. graminicola* (Miranda et al., 2017). In our study is important to highlight the early overexpression of a homologous of MKK9 (MAPKK) only in condition A. MKK9 may be involved in camalexin and ethylene biosynthesis via MPK3/MPK6, possible acting as an upstream regulator of MAPK cascade (Xu et al., 2008; Pitzschke et al., 2009). Camalexin is a phytoalexin involved in plant defense response to several pathogens, such as *A. thaliana* defense to *Phytophthora capsici* (Wang et al., 2013) and QDR to *Plasmodiophora brassicae* (Lemarié et al., 2015). While ethylene is widely known to have a role in plant defense against pathogens (Broekgaarden et al., 2015; Andersen et al., 2018). GDSL lipases are reported as important members of plant immunity, being regulated by the MPK3/MPK6 cascade and WRKY downstream TFs (Han et al., 2019) and acting as regulators of the ET signaling pathway (Kwon et al., 2009; Kim et al., 2014), another gene family associated with ET regulation is *AP2* TF (Singh, 2002), part of the *ERF* family and is involved in plant defense against other species of *Colletotrichum* (Amil-Ruiz et al., 2016; Hong et al., 2016; Padder et al., 2016; Miranda et al., 2017). In our work, genes encoding GDSL and AP2 TFs, as other ethylene-encoding genes were coordinately modulated in condition A, indicating a role of ET in soybean defense against *C. truncatum*.

WRKY TFs are involved in the regulation of several defense responses in plants (Seo et al., 2015). *WRKY6, WRKY23* and *WRKY28* and *RING* ZFs are involved in the regulation of SA signaling (Robatzek, 2002; Jing et al., 2009; van Verk et al., 2011; Miranda et al., 2017). *RING* ZF have a role in systemic acquired resistance (SAR) in maize upon infection with *C.*

graminicola (Miranda et al., 2017). *WRKY28* is reported as a regulator of tomato defense against *Phytophthora parasitica* (Peng et al., 2015). In *35S:OsWRKY23 Arabidopsis* mutants, the overexpression of *OsWRKY23* resulted in the increasing of expression *PR-1, PR-2* and *PR-5* genes, increasing the resistance against the hemibiotrophic bacteria *P. syringae* (Jing et al., 2009). These genes are known to be markers for SA-dependent SAR (Molinari et al., 2014). Silencing of MLO genes was commonly reported conferring broad spectrum resistance of plants to powdery mildew (Kusch et al., 2016), however, the deletion of MLO homologous in Arabidopsis unexpectedly leaded to enhanced resistance against *Golovinomyces orontii* (Kuhn et al., 2017) and *P. syringae,* the last one related with SAR (Gruner et al., 2018). In our work, *WRKY23* seems to have a basal expression and is downregulated during the symptomatic phase of the disease, while *WRKY6* and *WRKY28* expression is induced during the early stages of infection, representants of genes encoding PR-1 and PR-2 are induced within the firsts 12 hpi, and a homologous to a MLO12 is overexpressed within 12 and 48 hpi, only in both treatments of condition A. These findings along with the modulation *RING* ZFs and other genes involved in SA regulation suggest a role of SAR in soybean defense against *C. truncatum*. Even if the cross-talk between JA/ET and SA pathways were considered as antagonist in plant defense (Kunkel and Brooks, 2002), our results suggest that these classes of phytohormones act coordinately in soybean defense against *C. truncatum*. The modulation of JA/ET and SA pathways during plant defense against the same pathogen was observed before in soybean when inoculated with *Heterodera glycines* (Zhang et al., 2017).

Transcriptional gene expression control is essential for the fine-tuning of plant defense against pathogens and can be involved from the recognition of the pathogen to the regulation of expression of genes encoding specific defense compounds (Singh, 2002; Seo et al., 2015; Andersen et al., 2018). More than 5000 TFs belonging to 61 families were identified in soybean (Mochida et al., 2009). Our work revealed that soybean inoculation with *C. truncatum* causes a widely transcriptional reprogramming, with more than 60 genes representants of several transcription factor families DE only in condition A, such as *AP2, MYC, MYB, NAC, CRAS, KNAT, LNK* and TFs with zinc finger (ZF) domains, including *C2H2, PLATZ, RING* finger*, GATA*, and a *WRKY.*

The role of TFs in plant defense against biotic stresses was already reported. *C3HC4-type RING* finger was upregulated in strawberry inoculated with *C. acutatum* (Amil-Ruiz et al., 2016). *GRAS* TF is reported as have a role in plant interactions with arbuscular mycorrhiza (Fiorilli et al., 2015; Ho-Plágaro et al., 2019) and its activation can be induced by gibberellins in rice (Day et al., 2004). Transcription factors with ZF domains are known to be involved in

several processes in plants (Li et al., 2013a). The *C2H2-type* have a role in plant defense against abiotic and biotic stresses (Kiełbowicz-Matuk, 2012), and was reported in potato upon infection with *Phythophthora infestans* (Tian et al., 2010), and in *Capsicum annuum* as an enhancer of defense against *C. coccodes* and *Xanthomonas campestris* pv. *vesicatoria* (Kim et al., 2004). Here, genes encoding homologous of *C2H2-type zinc finger* were coordinately downregulated during the symptomatic phase of the disease on condition A, suggesting a role of this type of TFs in plant defense during the asymptomatic phase. In Arabidopsis, a *PLATZ* TF, *ORESARA15* suppressed leaf senescence by modulation of the *growth-regulating factor* (*GRF)* regulatory pathway (Kim et al., 2018). It is important to highlight that in our work, both a *PLATZ* TF and three homologous genes to *GRF5* are overexpressed during the symptomatic phase of the disease, only in condition A; while until now these genes have been reported as responsive to abiotic stresses (So et al., 2015; Kim et al., 2018; Fu et al., 2020), this result suggests that the *PLATZ* TF have a role in the latter stages of soybean response to *C. truncatum* infection.

Genes related to multiple defense responses were activated during the interaction of soybean with *C. truncatum*. In plants, PCD is considered an HR and occurs at the point of penetration of the pathogen limiting growth (Andersen et al., 2018; Balint-Kurti, 2019; Valandro et al., 2020). Overall, activation of PCD occurs due to the recognition of effectors or targets of effectors by NLR genes with CC or TIR domains (Kourelis and van der Hoorn, 2018; Balint-Kurti, 2019), and the regulation of this defense response in plants is performed by the gene *lesion simulating disease* (*LSD1*) (Li et al., 2013b; Wituszyńska et al., 2013), which can interact with several genes described in *Arabidopsis* as the *AtLSD1* deathosome (Valandro et al., 2020). Regulation of PCD also can occur by the level of accumulation of NLRs (Balint-Kurti, 2019); while NLR accumulation is controlled by chaperones and co-chaperones, including *HSP90, RAR1* and *SGT1* and *Skp1* (Catlett and Kaplan, 2006; Zhang et al., 2010). Defense responses induced by HR include SAR (Grant and Lamb, 2006; Wituszyńska et al., 2013), ROS accumulation (Hackenberg et al., 2013; Wituszyńska et al., 2013; Zhao et al., 2018) and synthesis of PR-proteins (glucanases, chitinases and defensin) (Jain and Khurana, 2018). *GATA* family is associated with HR in Arabidopsis (Dangl et al., 1996) and wheat (Guo et al., 2013), and is also associated with rice response to *M. oryzae*, together with other ZF members such as *RING* and *B-box* families (Li et al., 2014). Several genes involved in PCD regulation, including intracellular NLR receptors, *NF-Y, MYB, b-ZIP*, and *GATA* ZF TFs and involved in PCD responses such as SAR, chitinase and ROS accumulation are modulated only in condition A during the asymptomatic phase of the disease indicating a role of HR in soybean defense against *C. truncatum*. Activation of genes involved in PCD also occurred in the early stages of

infection of resistant canola with *L. maculans* suggesting that this early activation of PCD can have a role in limiting lesion sizes after the transition between the biotrophic-necrotrophic phase (Becker et al., 2017).

Along with the role of ROS accumulation in HR, ROS can also be recruited by the plant immune system to create unsuitable environments for pathogen development, acting directly in signal transduction and inhibition of pathogen growth (Lamb and Dixon, 1997). Two genes encoding a *pyridoxal phosphate (PLP)-dependent transferase I* enzyme are among the highest overexpressed genes in condition A during the early stages of *C. truncatum* infection. Little is known about the role of this enzyme in plant defense, but it is related to oxidative stress response in strawberries upon infection with *C. acutatum* (Amil-Ruiz et al., 2016). Representants of several gene classes involved in ROS regulation are also commonly upregulated in treatments with the highest level of resistance to *C. truncatum*, including PR-15, GTPases, peroxidases, genes involved in the phenylpropanoid pathway, *respiratory burst oxidase* (RBOH), GST and p450. These findings suggest a role of ROS regulation during soybean defense against *C. truncatum* and goes in line with other studies reporting an increasing of host resistance due the accumulation of ROS. Including *Arabidopsis* chloroplasts when inoculated with *C. higginsianum* (Schmidt et al., 2020) and resistant genotypes of sorghum when inoculated with *C. sublineolum*, on the other hand, the latter accumulation of ROS in the susceptible genotype, indicates successful pathogenesis (Basavaraju et al., 2009). This result goes in line with our findings, where few genes involved in ROS regulation are only activated during the symptomatic phase of the disease.

Cell wall reinforcing upon pathogen infection is considered an efficient plant defense mechanism (Huang, 2001), and can occur due to lignification of secondary plant cell walls (Andersen et al., 2018). In wheat, silencing of the *LAC* gene *TaLAC4* resulted in increased susceptibility to *Fusarium graminearum* due to a reduction in lignin deposition (Nancy et al., 2020). In soybean, a dirigent (DIR) protein, with a role in the biosynthesis of lignin was overexpressed in a resistant cultivar after inoculation with *P. sojae* (Li et al., 2017). Other classes of proteins involved in the protection of plant cell wall integrity are PMEIs (Lionetti et al., 2017) and XTH (Sharmin et al., 2012). In *Arabidopsis*, AtPMEI10, AtPMEI11 and AtPMEI12 are involved in defense against *Botrytis cinerea* due to the pectin methylesterification of the plant cell wall (Lionetti et al., 2017), while the downregulation of CoXTH1 gene was observed in susceptible jute (*Corchorus olitorius*) after the inoculation with *Macrophomina phaseolina* (Sharmin et al., 2012). The time coordinated modulation of genes involved in cell reinforcement indicates that this mechanism has a role in soybean defense

against *C. truncatum*. While PMEI representants are upregulated during the asymptomatic phase of the disease, their latter downregulation during the symptomatic phase, along with genes involved in lignin biosynthesis may influence the development of symptoms by *C. truncatum*.

Lipid transfer proteins (*PR-14*) are part of the PR-protein family. Transgenic wheat plants with the *TaLTP5* gene, a *PR-14* protein enhanced wheat resistance against *Fusarium graminearum* and *Cochlibolus sativus* (Zhu et al., 2012), while overexpression of another PR-14, *TdLTP4* in *Arabidopsis* enhanced resistance against *Alternaria solani* and *Botrytis cinerea* (Safi et al., 2015). It is important to highlight 5 PR-14 proteins that are modulated only during soybean combinations with the highest level of resistance response, two of them, highly overexpressed during the symptomatic phase of the disease, suggesting a direct antifungal activity to reduce the development of anthracnose symptoms in soybean plants.

Member of the LOR gene family in Arabidopsis, the silencing of LURP1 increased the susceptibility of Arabidopsis plants to *Hyaloperonospora arabidopsidis* (Baig, 2018). An F3H catalyzes the biosynthesis of dihydroflavonols, part of the secondary plant metabolism (Forkmann and Stotz, 1981; Mouradov and Spangenberg, 2014), and was recently reported as an enhancer of Norway spruce (*Picea abies*) resistance against the fungus *Endoconidiophora polonica* (Hammerbacher et al., 2019). The role of WD40 in plant biotic stress response is still unclear (Sharma and Pandey, 2016). As far as we know, a single study showed a putative role of a WD repeat (*TaHOS15*) in a histone deaceacetylase complex in wheat (*Triticum aestivum*), where lower transcript levels of *TaHOS15* leads to increased resistance of wheat to *Blumeria graminis* (Liu et al., 2019). The modulation of LURP1 homologous genes and the high overexpression of an F3H and an WD40 gene observed in condition A suggest a role of these genes in soybean/*C. truncatum* interaction.

The role of MATE in plant immunity was already reported. The overexpression of OsMATE1 and OsMATE2 in Arabidopsis enhanced plant susceptibility to *P. syringeae* (Tiwari et al., 2015), which goes in line with the results observed in condition A, where genes encoding MATE homologous were overexpressed during the symptomatic phase of the disease and may have a role in increasing soybean susceptibility against *C. truncatum*.

An IPP2 protein was downregulated in more than >8.5 L2FC only in more susceptible treatments within the first 12 hpi. The silencing of protein phosphatase 2A (PP2A) gene in tomato plants, increased the resistance of the plants to *Pseudomonas syringae* and *Cladosporium fulvum*, by increasing the expression of genes related to plant defense against biotic stress, such as PR-proteins and genes involved in PCD (He et al., 2004). The

downregulation of an IPP2 protein within the first 12 hpi only in susceptible interactions of soybean with *C. truncatum* suggests a potential role of this protein in increasing the susceptibility of soybean plants to anthracnose.

A couple of genes involved in cellular function coordination seems to have their expression change in more susceptible treatments. Kinesins can have or not an armadillo domain (ARM) (Nebenführ and Dixit, 2018), and two of them have their pattern of expression changed only in more susceptible combinations. ARK3 is homologous of ARK1 in Arabidopsis (Sakai et al., 2008) and acts in preprophase band organization through microtubule association and regulation of the cell cycle (Malcos and Cyr, 2011), being essential for the cells to complete cell division for normal growth (Eng and Wasteneys, 2014). In our work two kinesins were modulated only in more susceptible interactions, suggesting that cellular development functions can be putatively involved in the increased level of susceptibility of soybean to *C. truncatum*.

6.5 CONCLUSIONS

Due to the importance of soybean as a source of human and animal feeds, fungal diseases, such as soybean anthracnose may threaten food biosecurity (Hartman et al., 2011). Plants resistance against pathogens relies on their ability to recognize pathogen molecules, transduce the signal to downstream processes and defensively activate several pathways and products to stop the pathogen attack (Andersen et al., 2018). Gene expression studies among susceptible and resistant interactions of plants with pathogens can help to identify genes potentially involved in plant defense and/or susceptibility (Stutts and Vermerris, 2020). This study provided insights into the highly complex network of genes involved in soybean defense against anthracnose; including key-genes activated only at specific time-points and shared among both treatments that have a more resistant response against *C. truncatum*, that may help to explain the resistance mechanisms underlying this pathosystem. Most of these genes encode proteins known to be involved resistance of plants against biotic stresses.

Based on our results, we suggest that upon recognition of the fungus *C. truncatum* by CSRIs and IRs encoding genes in soybean, treatments with a higher level of resistance to *C. truncatum* modulate several genes related to plant immune responses. Multiple signaling pathways were activated, and extensively transcriptional control of gene expression led to an HR-related with oxidative burst and activation of genes encoding compounds that enhanced plant resistance to pathogens. Functional studies of putative genes and pathways found in this

work are expected to be proved as useful for the improvement of soybean cultivars' resistance to *C. truncatum*.

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SUPPLEMENTARY INFORMATION

Table S1: Functional annotation of genes differentially expressed only in more resistant combinations (condition A)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.14G221500.1	Cell wall related - EXP/EXPR	0,0	0,0	-2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,8
Glyma.01G127100.1	Cell wall related - ligninin - dirigent	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,9	0,0	0,0	-4,6	0,0
Glyma.03G147800.1	Cell wall related - ligninin - dirigent	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-10,9	-3,9
Glyma.11G164000.1	Cell wall related - Ligninin biosynthesis	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,3	-4,7
Glyma.18G057200.1	Cell wall related - Ligninin biosynthesis	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,6	-4,8
Glyma.01G138100.1	Cell wall related - PMEI	0,0	0,0	0,0	0,0	0,0	0,0	6,4	7,7	0,0	0,0	-9,7	-9,5
Glyma.01G240300.1	Cell wall related - PMEI	0,0	0,0	0,0	0,0	0,0	0,0	1,6	2,2	0,0	0,0	0,0	0,0
Glyma.17G036100.1	Cell wall related - PMEI	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,2	-1,5
Glyma.17G065100.1	Cell wall related - XTH	0,0	0,0	0,0	0,0	0,0	0,0	2,9	2,5	0,0	0,0	0,0	0,0
Glyma.07G236000.1	Enzime - Glycosyl hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	2,0	1,7	0,0	0,0	0,0	0,0
Glyma.08G150500.1	Enzime - Glycosyl hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-11,4	-5,0
Glyma.10G017000.1	Enzime - Glycosyl hydrolase	0,0	0,0	2,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,9	-3,3
Glyma.03G110600.1	Enzime - Lipase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,4	-7,2
Glyma.03G159000.1	Enzime - Lipase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	2,5
Glyma.06G003100.1	Enzime - Lipase	0,0	0,0	0,0	0,0	0,0	0,0	2,6	3,3	0,0	0,0	-6,5	0,0
Glyma.10G035200.6	Enzime - Lipase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,3	8,0
Glyma.01G239600.1	Enzime - Lipase - GDXG	0,0	0,0	0,0	0,0	0,0	0,0	2,5	2,4	0,0	0,0	-2,9	0,0
Glyma.11G230000.2	Enzime - Lipase - PLC	0,0	0,0	-8,0	-5,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.12G030600.1	Enzime - Lipase - PLC	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,4	0,0	0,0	0,0	0,0
Glyma.01G215100.1	Enzime - Lipase - PLD1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	0,0	0,0	-4,7	-2,2
Glyma.13G240600.1	Hormone - ABA - Allantoin	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	1,9

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.10G286800.1	Hormone - ABA - GRAM	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,1	-3,0
Glyma.11G083800.1	Hormone - ABA - GRAM	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,2	-4,7
Glyma.16G050700.1	Hormone - ABA - GRAM	0,0	0,0	0,0	0,0	0,0	0,0	2,0	3,0	0,0	0,0	-2,6	-2,3
Glyma.06G310300.1	Hormone - ABA - LEA	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-9,0	-2,9
Glyma.08G021800.1	Hormone - ABA - LEA	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,6	0,0	0,0	0,0	-3,1
Glyma.15G010500.1	Hormone - ABA - LEA	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,9	0,0	0,0	6,3	8,6
Glyma.18G171800.1	Hormone - ABA - other	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,7	-2,0
Glyma.07G253900.4	Hormone - ABA - PP2C	0,0	0,0	0,0	0,0	0,0	0,0	10,4	10,6	0,0	0,0	0,0	0,0
Glyma.19G069200.5	Hormone - ABA - PP2C	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,4	4,1
Glyma.19G176600.1	Hormone - ABA - PP2C	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-1,9	-1,9
Glyma.01G190600.1	Hormone - Auxin - GH3	0,0	0,0	0,0	0,0	0,0	0,0	2,6	0,0	0,0	0,0	-3,5	-4,0
Glyma.03G256200.1	Hormone - Auxin - GH3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,0	-2,4
Glyma.15G017500.1	Hormone - Auxin - IAA	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,0	-1,6
Glyma.19G232400.3	Hormone - Auxin - Other	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-1,8	-2,3
Glyma.19G232400.4	Hormone - Auxin - Other	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,1	-2,2
Glyma.01G137500.1	Hormone - Auxin - SAUR	0,0	0,0	0,0	0,0	0,0	0,0	1,9	2,4	0,0	0,0	0,0	0,0
Glyma.03G029600.1	Hormone - Auxin - SAUR	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,0	-2,1
Glyma.18G300900.1	Hormone - Auxin - SAUR	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,5	-4,1
Glyma.03G172900.1	Hormone - Auxin - WAT1	0,0	0,0	0,0	0,0	0,0	0,0	2,4	1,8	0,0	0,0	-5,6	0,0
Glyma.06G310700.1	Hormone - Auxin - WAT1	0,0	0,0	0,0	0,0	0,0	0,0	2,7	0,0	0,0	0,0	-5,5	-3,7
Glyma.08G182900.1	Hormone - Auxin - WAT1	0,0	0,0	0,0	0,0	0,0	0,0	2,5	2,0	0,0	0,0	-2,3	0,0
Glyma.11G149100.1	Hormone - Cytokinin - CKX	0,0	0,0	0,0	0,0	0,0	0,0	-4,1	-4,1	0,0	0,0	3,1	0,0
Glyma.04G052600.1	Hormone - Cytokinin - LOG	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,0	0,0	0,0	0,0	-2,1

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.02G046000.1	Hormone - ET - Lipase - GDSL	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,6	-7,8
Glyma.06G019200.1	Hormone - ET - Lipase - GDSL	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,2	-1,8
Glyma.08G307200.1	Hormone - ET - Lipase - GDSL	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,6	-7,8
Glyma.10G165600.1	Hormone - ET - Lipase - GDSL	0,0	0,0	5,8	0,0	0,0	0,0	-6,8	-6,5	0,0	0,0	6,7	0,0
Glyma.11G079400.2	Hormone - ET - Lipase - GDSL	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,8	2,3
Glyma.10G241100.1	Hormone - Gibberellin - G2OX	0,0	0,0	2,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,2	-2,8
Glyma.11G003200.1	Hormone - Gibberellin - G2OX	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,2	-3,1
Glyma.06G049900.1	Hormone - JA and ET - ACS	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,1	-7,7
Glyma.07G065700.1	Hormone - JA and ET - ACS1	0,0	0,0	0,0	0,0	0,0	0,0	8,1	8,3	0,0	0,0	-8,4	-8,2
Glyma.01G187800.1	Hormone - JA and ET - F-BOX	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,2	0,0	0,0	-2,2	-2,0
Glyma.17G011800.1	Hormone - JA and ET - F-BOX	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,9	0,0	0,0	-6,0	-8,9
Glyma.20G220200.3	Hormone - JA and ET - F-BOX	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,8	0,0	0,0	-3,6	-2,6
Glyma.13G174100.1	Hormone - JA and ET - GRX480	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,1	0,0	0,0	-8,8	-4,2
Glyma.07G041400.1	Hormone - JA and ET - JAZ	0,0	0,0	0,0	0,0	0,0	0,0	1,9	1,5	0,0	0,0	0,0	0,0
Glyma.09G071600.1	Hormone - JA and ET - JAZ	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,2	0,0	0,0	0,0	0,0
Glyma.11G038600.2	Hormone - JA and ET - JAZ	0,0	0,0	0,0	0,0	0,0	0,0	3,0	2,4	0,0	0,0	0,0	0,0
Glyma.13G112000.1	Hormone - JA and ET - JAZ	0,0	0,0	0,0	0,0	0,0	0,0	2,6	1,9	0,0	0,0	0,0	0,0
Glyma.15G179600.1	Hormone - JA and ET - JAZ	0,0	0,0	0,0	0,0	0,0	0,0	2,5	1,9	0,0	0,0	0,0	0,0
Glyma.16G010000.1	Hormone - JA and ET - JAZ	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,0	0,0	0,0	0,0	0,0
Glyma.07G034800.1	Hormone - JA and ET - LOX	0,0	0,0	-2,4	-2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.02G033700.7	Hormone - SA - ankirin	0,0	0,0	0,0	0,0	0,0	0,0	-9,2	-8,5	0,0	0,0	8,8	0,0
Glyma.12G111400.1	Hormone - SA - ankirin	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,3	-2,1
Glyma.18G247600.1	Hormone - SA - ankirin	0,0	0,0	0,0	0,0	0,0	0,0	2,1	0,0	0,0	0,0	-5,9	-3,3
Glyma.16G025400.1	Hormone - SA - CNGC	0,0	0,0	0,0	0,0	0,0	0,0	3,7	7,2	0,0	0,0	-9,1	-5,8

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.16G218300.1	Hormone - SA - CNGC	0,0	0,0	5,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,0	-4,6
Glyma.19G255500.4	Hormone - SA - CNGC	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,1	-4,9
Glyma.13G094100.1	Hormone - SA - PR-1	0,0	0,0	3,1	2,1	0,0	0,0	-3,0	0,0	0,0	0,0	2,7	0,0
Glyma.17G066100.1	Hormone - SA - PR-1	0,0	0,0	3,9	3,1	0,0	0,0	-3,2	0,0	0,0	0,0	0,0	0,0
Glyma.16G145600.1	Hormone - SA - SAR - MLO	0,0	0,0	0,0	0,0	0,0	0,0	1,8	2,9	0,0	0,0	0,0	0,0
Glyma.01G171700.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,4	-7,8
Glyma.03G108000.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	2,2	0,0	0,0	0,0	-5,0	-4,6
Glyma.05G233500.1	Not annotated	0,0	0,0	-2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,8	2,7
Glyma.08G022000.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	-2,7	-2,7	0,0	0,0	3,1	2,4
Glyma.08G329000.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	-2,7	0,0	0,0	0,0	3,1	2,5
Glyma.09G053300.1	Not annotated	0,0	0,0	8,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-9,2	-8,8
Glyma.12G078700.1	Not annotated	0,0	0,0	6,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,9	-7,0
Glyma.14G055400.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	0,0	0,0	-8,9	-5,9
Glyma.15G032400.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,8	0,0	0,0	-2,2	-2,5
Glyma.15G076100.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	7,9	0,0	0,0	0,0	-8,2	-7,5
Glyma.15G142300.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,7	0,0	0,0	-8,2	-9,0
Glyma.15G268200.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	2,7	3,0	0,0	0,0	-5,3	0,0
Glyma.17G105000.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,4	-2,4
Glyma.17G119000.1	Not annotated	0,0	0,0	0,0	8,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,8	-5,9
Glyma.17G233300.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-1,9	-2,1
Glyma.18G011200.3	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	-10,7	-12,1	0,0	0,0	11,3	11,7
Glyma.18G293400.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,7	2,7
Glyma.20G134000.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	2,9	3,2	0,0	0,0	-4,8	-2,4

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi	48 vs 12 hpi	120 vs 48 hpi

		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.20G157000.1	Not annotated	0,0	0,0	0,0	0,0	0,0	0,0	8,0	7,8	0,0	0,0	0,0	0,0
Glyma.16G182200.4	Other	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,2	7,4
Glyma.05G230200.1	Other - ABIL1	0,0	0,0	0,0	0,0	0,0	0,0	-4,0	-2,6	0,0	0,0	5,2	0,0
Glyma.08G159900.1	Other - ACLA-1	0,0	0,0	-3,8	-2,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.03G144800.3	Other - Actin 11 - Cytoesqueleton	0,0	0,0	0,0	0,0	0,0	0,0	27,6	13,4	0,0	0,0	-28,7	-13,3
Glyma.04G191200.7	Other - ATPase activity	0,0	0,0	0,0	0,0	0,0	0,0	-8,5	-9,8	0,0	0,0	0,0	9,4
Glyma.19G018600.1	Other - ATPase activity	0,0	0,0	0,0	0,0	0,0	0,0	2,3	1,9	0,0	0,0	-2,1	0,0
Glyma.11G247500.1	Other - B12D	0,0	0,0	0,0	0,0	0,0	0,0	2,7	3,1	0,0	0,0	-3,4	0,0
Glyma.06G226500.1	Other - BCL-2	0,0	0,0	7,9	8,2	0,0	0,0	-7,9	-8,4	0,0	0,0	0,0	0,0
Glyma.12G230100.1	Other - binding	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,2	-5,5
Glyma.16G196300.1	Other - Binding	0,0	0,0	0,0	0,0	0,0	0,0	3,6	3,8	0,0	0,0	0,0	0,0
Glyma.07G112000.1	Other - Binding	0,0	0,0	2,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,7	-2,7
Glyma.13G364800.1	Other - Binding	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,4	4,3
Glyma.16G202000.1	Other - Binding	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,3	7,7
Glyma.06G238300.1	Other - Binding - copper	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,2	0,0	0,0	5,2	3,5
Glyma.08G224100.1	Other - BURP domain	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,4	6,7
Glyma.03G001600.2	Other - Catalytic activity	0,0	0,0	3,5	8,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.04G004300.1	Other - Catalytic activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,5
Glyma.07G248000.3	Other - Catalytic activity	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,1	0,0	0,0	-2,6	-2,2
Glyma.13G057800.1	Other - Catalytic activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,1	1,8
Glyma.19G028400.1	Other - Catalytic activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,5	0,0	0,0	3,5	2,5
Glyma.03G002600.1	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,4
Glyma.03G083200.2	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,1	0,0	0,0	0,0	0,0

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.04G180900.4	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	10,2	10,3	0,0	0,0	-9,5	-10,3
Glyma.06G104800.4	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-12,6	-11,6
Glyma.06G319500.1	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	2,6	0,0	0,0	0,0	-2,6	-2,4
Glyma.07G168600.3	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	7,5	7,2	0,0	0,0	0,0	-7,1
Glyma.09G015500.5	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	-10,7	0,0	0,0	0,0	11,3	11,9
Glyma.09G062900.1	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	2,7	0,0	0,0	0,0	-2,9	-2,3
Glyma.09G173400.1	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,7	-1,7
Glyma.14G086300.3	Other - Catalytic activity - Hydrolase	0,0	0,0	10,4	0,0	0,0	0,0	-10,4	-10,3	0,0	0,0	0,0	0,0
Glyma.14G171700.1	Other - Catalytic activity - Hydrolase	0,0	0,0	2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,9	-2,3
Glyma.15G023800.1	Other - Catalytic activity - Hydrolase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,8	0,0	0,0	-2,2	-2,5
Glyma.17G055100.2	Other - Catalytic activity - Hydrolase	0,0	0,0	-10,4	-11,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.08G099600.2	Other - Chaperone	0,0	0,0	0,0	0,0	0,0	0,0	10,4	11,0	0,0	0,0	-10,7	0,0
Glyma.07G109200.1	Other - Chaperone-like	0,0	0,0	0,0	0,0	0,0	0,0	2,7	2,7	0,0	0,0	0,0	-2,6
Glyma.09G007000.1	Other - Chaperonin	0,0	0,0	-8,8	-10,2	0,0	0,0	0,0	0,0	0,0	0,0	8,3	9,4
Glyma.19G229700.2	Other - Chaperonin	0,0	0,0	-1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,5	2,1
Glyma.02G101100.1	Other - CHUP1- like	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-1,8	-2,4
Glyma.11G000800.1	Other - Cyclase	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0	0,0	0,0	-3,2	-2,1
Glyma.06G154700.2	Other - emb2170	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,8	-9,7
Glyma.10G129100.1	Other - epimerase	0,0	0,0	0,0	0,0	0,0	0,0	4,4	3,8	0,0	0,0	0,0	0,0
Glyma.18G025200.1	Other - Extensin	0,0	0,0	0,0	0,0	0,0	0,0	-5,4	-6,8	0,0	0,0	0,0	0,0
Glyma.12G173800.1	Other - FAS1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,1	-2,4
Glyma.07G226400.1	Other - GLR	0,0	0,0	6,5	0,0	0,0	0,0	0,0	3,3	0,0	0,0	-5,9	-5,7
Glyma.07G038400.1	Other - GRF5 - Growth regulation factor	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,3	3,1

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.09G068700.1	Other - GRF5 - Growth regulation factor	0,0	0,0	-1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	2,4
Glyma.11G008500.1	Other - GRF5 - Growth regulation factor	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,4
Glyma.16G032400.1	Other - HMA	0,0	0,0	0,0	0,0	0,0	0,0	1,9	1,9	0,0	0,0	-1,9	0,0
Glyma.09G198300.2	Other - HMGB3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	11,3	11,1
Glyma.07G083800.1	Other - Kunitz domain	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,4	-2,7
Glyma.14G060900.4	Other - L10	0,0	0,0	0,0	0,0	0,0	0,0	8,8	0,0	0,0	0,0	-8,2	-10,5
Glyma.15G172800.2	Other - L18	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,7	-2,7
Glyma.04G170400.3	Other - NAP1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,5	1,8
Glyma.14G095000.7	Other - nucleotide binding	0,0	0,0	9,2	8,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.19G197800.1	Other - Ovate protein family	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,2	-3,2
Glyma.02G105600.2	Other - Oxireductase activity	0,0	0,0	0,0	0,0	0,0	0,0	2,0	3,0	0,0	0,0	0,0	0,0
Glyma.04G086300.3	Other - Oxireductase activity	0,0	0,0	0,0	0,0	0,0	0,0	3,6	2,9	0,0	0,0	-3,4	0,0
Glyma.07G073800.2	Other - Oxireductase activity	0,0	0,0	0,0	0,0	0,0	0,0	8,8	9,2	0,0	0,0	0,0	-9,1
Glyma.11G164700.1	Other - Oxireductase activity	0,0	0,0	0,0	0,0	0,0	0,0	-2,4	-2,7	0,0	0,0	0,0	0,0
Glyma.13G196500.2	Other - Oxireductase activity	0,0	0,0	0,0	0,0	0,0	0,0	3,3	2,2	0,0	0,0	0,0	0,0
Glyma.14G156400.1	Other - Oxireductase activity	0,0	0,0	0,0	0,0	0,0	0,0	8,6	4,8	0,0	0,0	0,0	-8,5
Glyma.18G229100.1	Other - Oxireductase activity	0,0	0,0	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,2	-4,9
Glyma.19G042900.1	Other - Oxireductase activity	0,0	0,0	0,0	0,0	0,0	0,0	2,7	3,1	0,0	0,0	-3,2	0,0
Glyma.01G005100.4	Other - Oxireductase activity - MIOX	0,0	0,0	7,3	0,0	0,0	0,0	0,0	8,8	0,0	0,0	-8,6	-8,7
Glyma.05G224500.1	Other - Oxireductase activity - MIOX	0,0	0,0	-2,5	-2,3	0,0	0,0	0,0	1,9	0,0	0,0	0,0	0,0
Glyma.04G232200.1	Other - P-loop	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-10,4	0,0	0,0	-3,1	-2,0
Glyma.05G212900.1	Other - P-loop	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,8	2,3
Glyma.11G195800.2	Other - P-loop	0,0	0,0	0,0	0,0	0,0	0,0	8,5	9,7	0,0	0,0	-8,8	-9,6

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.13G037000.1	Other - permease	0,0	0,0	0,0	0,0	0,0	0,0	1,7	2,2	0,0	0,0	-3,1	-2,4
Glyma.05G216100.1	Other - Phosphorilase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	1,7
Glyma.09G239900.1	Other - PQQ	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,7	-4,3
Glyma.18G256800.1	Other - PQQ	0,0	0,0	0,0	0,0	0,0	0,0	7,0	0,0	0,0	0,0	-7,2	-6,9
Glyma.06G150300.2	Other - PsaN	0,0	0,0	4,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,8	-4,0
Glyma.06G099400.1	Other - PUP1 - Purine transporter	0,0	0,0	-2,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,8	3,5
Glyma.18G006700.2	Other - REF	0,0	0,0	2,1	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.17G241500.2	Other - Remorim	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	10,3	10,8
Glyma.04G217800.1	Other - RGF9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,1	-7,9
Glyma.13G237400.1	Other - SBP	0,0	0,0	0,0	0,0	0,0	0,0	5,2	7,5	0,0	0,0	-7,9	-4,7
Glyma.04G134100.1	Other - SEC14	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	0,0	0,0	-5,0	-3,8
Glyma.08G007400.1	Other - Sec14p- like	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,5	-7,9
Glyma.05G029800.3	Other - SPFH	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	-2,5	-2,1
Glyma.06G065600.3	Other - SPFH	0,0	0,0	10,5	10,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-9,9
Glyma.20G226500.1	Other - SS18	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,2	3,0
Glyma.05G164800.1	Other - TauE	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,1	-2,4
Glyma.02G126300.1	Other - TCP- 1/cpn60	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,8	7,4
Glyma.01G062600.1	Other - transferase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,0	-4,7
Glyma.04G104500.2	Other - transferase	0,0	0,0	0,0	0,0	0,0	0,0	9,5	3,8	0,0	0,0	0,0	0,0
Glyma.05G033600.1	Other - transferase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,1	0,0	0,0	-9,3	-3,6
Glyma.12G226400.1	Other - transferase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,5
Glyma.01G134600.6	Other - Transferase activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	0,0	0,0	-7,9	-4,7
Glyma.08G010500.1	Other - Transferase activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,8	-2,1

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.12G200000.1	Other - Transferase activity	0,0	0,0	4,7	0,0	0,0	0,0	0,0	4,1	0,0	0,0	-6,8	-1,9
Glyma.02G184200.1	Other - Transferase activity	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,7	0,0	0,0	-6,4	-3,2
Glyma.08G348500.1	Other - Transferase activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,3	0,0	0,0	2,5	3,0
Glyma.17G166500.1	Other - Transferase activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,1	-5,4
Glyma.08G323400.1	Other - transferase MBOAT	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,1	0,0	0,0	4,7	4,4
Glyma.01G099700.3	Other - transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,7	7,9
Glyma.03G113400.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	0,0	0,0	2,2	5,5
Glyma.05G175300.1	Other - Transporter	0,0	0,0	8,7	0,0	0,0	0,0	-8,7	0,0	0,0	0,0	8,1	9,0
Glyma.06G019500.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,0	0,0	0,0	-11,6	-4,4
Glyma.06G131800.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	2,5	2,6	0,0	0,0	-2,2	-2,5
Glyma.08G205800.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,2	7,0
Glyma.08G298900.1	Other - transporter	0,0	0,0	0,0	0,0	0,0	0,0	1,8	1,6	0,0	0,0	-1,4	-1,8
Glyma.11G179100.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,1	-8,1
Glyma.12G084500.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,3	0,0	0,0	0,0	0,0
Glyma.13G340500.3	Other - transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-11,1	-10,8
Glyma.14G051600.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	-4,1	-4,8
Glyma.15G124400.2	Other - transporter	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,6	0,0	0,0	0,0	0,0
Glyma.15G142900.1	Other - transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,2	7,5
Glyma.16G062600.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	1,9	1,9	0,0	0,0	0,0	0,0
Glyma.17G006100.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,0	0,0	0,0	0,0	-1,9
Glyma.20G217400.3	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,7	-3,1
Glyma.01G238800.1	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	3,2	2,9	0,0	0,0	-2,9	0,0
Glyma.08G035300.3	Other - Transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,7	0,0	0,0	-2,9	-4,8

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.15G194600.2	Other - Transporter	0,0	0,0	9,0	9,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-10,3
Glyma.03G156700.1	Other - transporter Sulfur assimilation	0,0	0,0	0,0	0,0	0,0	0,0	2,0	1,6	0,0	0,0	-2,3	0,0
Glyma.20G216200.1	Other - UPF0426	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,5	2,8
Glyma.17G146400.2	Other - VAMP	0,0	0,0	7,1	7,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.13G120600.1	PCD regulator - SERPIN	0,0	0,0	0,0	0,0	0,0	0,0	7,2	7,4	0,0	0,0	-7,5	-7,3
Glyma.06G174800.1	Plant defense - 1 PCD - PLCP	0,0	0,0	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,8	-3,7
Glyma.09G046400.1	Plant defense - 2OG oxygenase - PCD	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,9	0,0	0,0	-4,2	0,0
Glyma.10G063000.1	Plant defense - 2OG oxygenase - PCD	0,0	0,0	5,2	9,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.11G130600.1	Plant defense - 2OG oxygenase - PCD	0,0	0,0	-2,9	-2,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,9
Glyma.12G008700.3	Plant defense - 2OG oxygenase - PCD	0,0	0,0	0,0	0,0	0,0	0,0	2,8	2,4	0,0	0,0	0,0	0,0
Glyma.08G217400.1	Plant Defense - Aquaporin	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	0,0	0,0	-2,5	-2,0
Glyma.07G076100.1	Plant defense - BAP2 - PCD	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,9	-3,6
Glyma.07G185800.2	Plant defense - CNNM	0,0	0,0	-13,9	0,0	0,0	0,0	14,2	14,4	0,0	0,0	-14,5	0,0
Glyma.07G185800.3	Plant defense - CNNM	0,0	0,0	0,0	0,0	0,0	0,0	-3,5	-3,3	0,0	0,0	3,9	0,0
Glyma.02G048600.1	Plant defense - F3H	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,2	8,5
Glyma.01G129000.1	Plant defense - LURP	0,0	0,0	0,0	0,0	0,0	0,0	3,0	2,6	0,0	0,0	0,0	0,0
Glyma.02G154500.1	Plant defense - LURP	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,6	-4,1
Glyma.03G040900.1	Plant defense - LURP	0,0	0,0	0,0	0,0	0,0	0,0	2,0	2,6	0,0	0,0	-4,2	0,0
Glyma.03G041000.1	Plant defense - LURP	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,4	-3,1
Glyma.02G089900.1	Plant defense - MATE	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,0	4,8
Glyma.03G036200.1	Plant defense - MATE	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,7	8,4
Glyma.17G027900.1	Plant defense - MATE	0,0	0,0	5,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,6	-6,3
Glyma.17G030200.1	Plant defense - MLP4	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	-4,1	-2,4

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.17G030300.1	Plant defense - MLP4	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0	0,0	0,0	-5,0	-2,7
Glyma.05G166800.1	Plant defense - other	0,0	0,0	2,4	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.01G091100.1	Plant Defense - Other - ABC2 transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	0,0	0,0	5,1	9,5
Glyma.13G361900.1	Plant Defense - Other - ABC2 transporter	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,6	0,0	0,0	-3,3	0,0
Glyma.15G019200.6	Plant Defense - Other - ABC2 transporter	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,5	0,0	0,0	8,1	9,0
Glyma.01G031500.1	Phenylpropanoid biosynthesis	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	-2,5	-2,0
Glyma.02G034000.1	Phenylpropanoid biosynthesis	0,0	0,0	0,0	0,0	0,0	0,0	1,9	1,9	0,0	0,0	-2,1	0,0
Glyma.07G192700.1	Phenylpropanoid biosynthesis	0,0	0,0	0,0	0,0	0,0	0,0	3,7	0,0	0,0	0,0	-4,9	-6,4
Glyma.09G070300.1	Phenylpropanoid biosynthesis	0,0	0,0	0,0	0,0	0,0	0,0	-1,6	0,0	0,0	0,0	2,0	2,0
Glyma.01G004200.2	Phenylpropanoid biosynthesis - CCoAOMT	0,0	0,0	0,0	0,0	0,0	0,0	2,5	3,6	0,0	0,0	-5,7	0,0
Glyma.01G228700.1	Phenylpropanoid biosynthesis - CHS	0,0	0,0	0,0	0,0	0,0	0,0	2,0	2,2	0,0	0,0	-4,5	0,0
Glyma.20G176100.1	Phenylpropanoid biosynthesis - COMT	0,0	0,0	0,0	0,0	0,0	0,0	-2,0	0,0	0,0	0,0	3,7	2,7
Glyma.07G087400.1	Phenylpropanoid biosynthesis - REF1	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0	-3,2	-2,4
Glyma.20G044000.1	Plant defense - stress response	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,0	6,8
Glyma.02G239800.1	Plant defense - VQ motif	0,0	0,0	0,0	0,0	0,0	0,0	0,0	10,5	0,0	0,0	-3,4	-3,1
Glyma.05G198400.1	Plant defense - VQ motif	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,2	0,0	0,0	-9,4	-4,8
Glyma.20G064500.1	Plant defense - VQ motif	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,6	-2,8
Glyma.01G036800.1	Plant defense - WD40	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-1,9	0,0	0,0	-2,6	-2,6
Glyma.15G126800.2	Plant defense - WD40	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,0	8,9
Glyma.16G116200.3	Plant defense - WD40	0,0	0,0	0,0	0,0	0,0	0,0	-3,5	-8,8	0,0	0,0	4,4	0,0
Glyma.17G074300.1	Plant defense - WD40	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,7	0,0	0,0	-2,5	-2,2
Glyma.01G037200.1	Plant defense - Wound healing	0,0	0,0	0,0	0,0	0,0	0,0	2,0	2,3	0,0	0,0	-3,8	0,0
Glyma.11G234300.1	Plant defense - Wun1-like	0,0	0,0	0,0	0,0	0,0	0,0	1,9	2,3	0,0	0,0	-1,7	-2,1

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.18G022700.1	Plant defense - Wun1-like	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,7	0,0	0,0	0,0	-1,8
Glyma.02G044500.1	Germin - act as oxalate oxidase - PR-15	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,0	-7,8
Glyma.01G078800.1	PR-Protein - PR-14	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,6	-3,4
Glyma.05G055100.1	PR-Protein - PR-14	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,2	0,0	0,0	-2,3	0,0
Glyma.15G119100.1	PR-Protein - PR-14	0,0	0,0	-4,8	0,0	0,0	0,0	0,0	-7,6	0,0	0,0	5,8	8,7
Glyma.16G188800.1	PR-Protein - PR-14	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,4	-2,9
Glyma.17G139800.1	PR-Protein - PR-14	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,8	0,0	0,0	4,1	3,9
Glyma.13G048800.1	PR-protein - PR-2	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,9	0,0	0,0	-3,9	-2,1
Glyma.20G225200.1	PR-protein - PR-4	0,0	0,0	0,0	0,0	0,0	0,0	8,8	9,1	0,0	0,0	-9,1	0,0
Glyma.18G231400.1	PR-Protein - PR-6	0,0	0,0	0,0	0,0	0,0	0,0	3,6	2,9	0,0	0,0	0,0	0,0
Glyma.08G307600.1	Protease - Enzime	0,0	0,0	0,0	0,0	0,0	0,0	1,8	2,1	0,0	0,0	-3,1	0,0
Glyma.19G045800.7	Protease - Enzime	0,0	0,0	0,0	0,0	0,0	0,0	10,8	0,0	0,0	0,0	-10,2	-9,2
Glyma.02G217600.1	Protein kinase activiti - Lectin Pkinase	0,0	0,0	0,0	-5,5	0,0	0,0	4,3	4,8	0,0	0,0	0,0	0,0
Glyma.05G009200.1	Protein kinase activity	0,0	0,0	-2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,0	2,8
Glyma.06G127700.4	Protein kinase activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	11,7	10,6
Glyma.07G088000.1	Protein kinase activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,8	2,2
Glyma.08G318300.1	Protein kinase activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	11,8	10,6
Glyma.10G023300.3	Protein kinase activity	0,0	0,0	0,0	0,0	0,0	0,0	9,7	0,0	0,0	0,0	-10,0	-9,6
Glyma.11G123400.1	Protein kinase activity	0,0	0,0	0,0	0,0	0,0	0,0	10,5	7,1	0,0	0,0	0,0	-1,7
Glyma.16G008500.1	Protein kinase activity	0,0	0,0	3,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,0	-3,6
Glyma.20G105300.1	Protein kinase activity	0,0	0,0	0,0	0,0	0,0	0,0	2,3	4,2	0,0	0,0	0,0	-3,6
Glyma.18G070800.1	SMR	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,2	-2,0
Glyma.15G111300.1	Receptor - CERK1	0,0	0,0	0,0	0,0	0,0	0,0	-2,8	-4,1	0,0	0,0	3,0	0,0

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.05G119600.5	Receptor - - Co-receptor - BAK1/SERK3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,9	0,0	0,0	-7,4	-7,9
Glyma.12G189500.1	Receptor - BRI1 supressor - BSU1-like2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,1	0,0	0,0	-6,5	-12,5
Glyma.19G135800.1	Receptor - CC-NBS-LRR	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-1,9	-2,1
Glyma.02G270700.1	Receptor - CERK1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,4	0,0	0,0	-3,4	-3,8
Glyma.04G127100.1	Receptor - cysteine-rich RLK -CRK	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,3	0,0	0,0	-4,0	0,0
Glyma.13G270200.1	Receptor - Lectin	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,3	-7,8
Glyma.13G303800.2	Receptor - Lectin	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,9	0,0	0,0	-11,6	-10,7
Glyma.06G258400.1	Receptor - Lectin-RLK	0,0	0,0	-1,7	0,0	0,0	0,0	-6,2	0,0	0,0	0,0	6,8	3,6
Glyma.06G262700.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,5	0,0	0,0	-3,9	-2,0
Glyma.07G080700.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,3	0,0	0,0	0,0	0,0
Glyma.07G154100.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,6	3,2	0,0	0,0	-7,1	-3,9
Glyma.07G184000.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,8	-2,0
Glyma.07G188800.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,4	0,0	0,0	-2,1	0,0
Glyma.12G140200.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,8	0,0	0,0	-10,7	0,0
Glyma.12G144500.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,0	8,9	0,0	0,0	-10,3	-8,9
Glyma.16G093900.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	1,8	0,0	0,0	0,0	-3,0	-2,0
Glyma.18G185400.1	Receptor - Lectin-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,2	-1,9
Glyma.10G228600.1	Receptor - LRR	0,0	0,0	0,0	0,0	0,0	0,0	0,0	10,6	0,0	0,0	-7,5	-6,3
Glyma.11G141400.2	Receptor - LRR-Kinase	0,0	0,0	2,3	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.01G004800.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,5	0,0	0,0	0,0	-6,1	-2,9
Glyma.01G007500.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,1	3,0	0,0	0,0	-5,1	0,0
Glyma.02G302600.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,4	-3,2
Glyma.03G165800.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,0	-3,0
Glyma.03G166300.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,7	-8,3

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.07G013700.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,1	-6,3
Glyma.14G144300.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,4	0,0	0,0	-3,5	0,0
Glyma.16G064100.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	1,9	2,2	0,0	0,0	-3,4	0,0
Glyma.16G078900.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	2,9	3,4	0,0	0,0	-5,9	0,0
Glyma.18G198800.1	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	0,0	0,0	-3,1	-2,8
Glyma.18G199000.2	Receptor - LRR-RLK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,3	-6,6
Glyma.11G206700.3	Receptor - RLK-GNK2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,5	-3,0
Glyma.20G137500.1	Receptor - RLK-GNK2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,4	-7,6
Glyma.20G139000.1	Receptor - RLK-GNK2	0,0	0,0	0,0	0,0	0,0	0,0	2,9	0,0	0,0	0,0	-10,6	-11,3
Glyma.20G139300.1	Receptor - RLK-GNK2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,3	-2,4
Glyma.09G210600.1	Receptor - RPM1/RPS3 - CC-NB-LRR	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,5	0,0	0,0	-4,5	0,0
Glyma.18G127900.1	Receptor - TIR-NBS	0,0	0,0	0,0	0,0	0,0	0,0	3,4	0,0	0,0	0,0	-4,1	-3,3
Glyma.02G023900.1	Receptor - TIR-NBS-LRR	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,8	0,0	0,0	-4,2	-2,6
Glyma.13G076200.1	Receptor - TIR-NBS-LRR	0,0	0,0	0,0	0,0	0,0	0,0	8,1	8,2	0,0	0,0	-8,4	-8,1
Glyma.16G210600.3	Receptor - TIR-NBS-LRR	0,0	0,0	-5,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-10,2	-6,9
Glyma.16G214300.1	Receptor - TIR-NBS-LRR	0,0	0,0	0,0	0,0	0,0	0,0	2,5	3,1	0,0	0,0	-4,6	0,0
Glyma.02G100300.1	Receptor - Wall asssociated kinase - WAK	0,0	0,0	0,0	0,0	0,0	0,0	2,1	0,0	0,0	0,0	-2,9	-2,6
Glyma.07G094100.1	Receptor - Wall asssociated kinase - WAK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,7	-7,5
Glyma.13G033500.2	Receptor - Wall asssociated kinase - WAK	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,3	0,0	0,0	-10,2	-10,2
Glyma.07G144000.1	Receptor -LRR-RLP 21	0,0	0,0	0,0	0,0	0,0	0,0	3,0	0,0	0,0	0,0	-6,1	-4,1
Glyma.16G169500.1	Receptor -LRR-RLP 32	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,8	-2,4
Glyma.01G120000.1	Receptor -LRR-RLP 7	0,0	0,0	5,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,5	-4,6
Glyma.06G248700.1	Receptor sensor TIR	0,0	0,0	7,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,0	-8,0

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.12G221600.1	Receptor sensor TIR	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,6	0,0	0,0	-7,9	-7,4
Glyma.03G168900.1	Act as oxalate oxidase - PR-15	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,6	0,0	0,0	0,0	0,0
Glyma.10G037100.1	Act as oxalate oxidase - PR-15	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,1	7,8
Glyma.12G207900.1	ROS - GTPase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,0	-1,8
Glyma.13G293200.1	ROS - GTPase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	0,0	0,0	-2,8	-2,1
Glyma.16G037900.1	ROS - Other	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	1,8
Glyma.03G040600.3	ROS - Oxidative stress response	0,0	0,0	14,0	28,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.06G169800.2	ROS - Oxidative stress response	0,0	0,0	0,0	0,0	0,0	0,0	-1,8	0,0	0,0	0,0	9,9	3,0
Glyma.03G160100.1	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	3,6	4,9	0,0	0,0	-7,3	-3,9
Glyma.03G189900.1	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	-2,1	-2,8	0,0	0,0	3,8	2,7
Glyma.05G152600.1	ROS - p450	0,0	0,0	7,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,7	-7,8
Glyma.08G140400.2	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	10,8	11,1	0,0	0,0	-11,1	-11,0
Glyma.09G048900.1	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	2,8	3,3	0,0	0,0	-5,1	0,0
Glyma.09G049300.1	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	3,1	3,5	0,0	0,0	-5,8	0,0
Glyma.11G108300.1	ROS - p450	0,0	0,0	-2,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2
Glyma.12G067000.1	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,5	-8,9
Glyma.13G173500.1	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,6	0,0	0,0	-3,5	0,0
Glyma.17G227500.2	ROS - p450	0,0	0,0	0,0	0,0	0,0	0,0	4,0	4,0	0,0	0,0	0,0	-7,1
Glyma.20G188000.1	ROS - p450	0,0	0,0	1,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,1	-1,9
Glyma.17G109400.1	ROS - peroxidase activity at low pH	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,8	1,7
Glyma.17G109400.2	ROS - peroxidase activity at low pH	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,9	2,1
Glyma.01G130500.1	Peroxidase	0,0	0,0	6,3	0,0	0,0	0,0	2,6	0,0	0,0	0,0	-6,2	-4,9
Glyma.09G109800.1	Peroxidase	0,0	0,0	0,0	0,0	0,0	0,0	2,8	0,0	0,0	0,0	-5,4	-2,9

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.10G022500.1	Peroxidase	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,0	0,0	0,0	7,7	9,2
Glyma.16G164400.1	Peroxidase	0,0	0,0	-3,7	-2,4	0,0	0,0	3,0	0,0	0,0	0,0	0,0	2,7
Glyma.17G163200.1	Peroxidase	0,0	0,0	0,0	0,0	0,0	0,0	2,0	2,3	0,0	0,0	-2,4	0,0
Glyma.19G233900.1	ROS - RBOH	0,0	0,0	0,0	0,0	0,0	0,0	3,9	3,2	0,0	0,0	0,0	-3,1
Glyma.18G041600.1	ROS - Regulation	0,0	0,0	0,0	0,0	0,0	0,0	3,1	2,9	0,0	0,0	-4,0	0,0
Glyma.13G129000.1	ROS -Plant defense - GST / Thioredoxin	0,0	0,0	0,0	0,0	0,0	0,0	1,8	0,0	0,0	0,0	-3,6	-2,1
Glyma.01G049500.1	Signal transduction - Ca/CaM	0,0	0,0	6,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,8	-7,3
Glyma.04G245000.1	Signal transduction - Ca/CaM	0,0	0,0	3,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,6	-3,8
Glyma.11G157200.1	Signal transduction - Ca/CaM	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,1	0,0	0,0	-6,2	-4,2
Glyma.12G185400.1	Signal transduction - Ca/CaM	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,2	0,0	0,0	-2,0	0,0
Glyma.16G059300.1	Signal transduction - Ca/CaM	0,0	0,0	0,0	0,0	0,0	0,0	2,2	3,4	0,0	0,0	-10,2	0,0
Glyma.03G157800.1	Signal transduction - Ca/CaM	0,0	0,0	3,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,6	-3,8
Glyma.03G232500.2	Signal transduction - Ca/CaM	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-9,5	0,0	0,0	-3,7	-2,8
Glyma.07G004300.1	Signal transduction - Ca/CaM	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,0	0,0	0,0	-2,8	0,0
Glyma.07G226500.1	Signal transduction - Ca/CaM	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,0	0,0	0,0	-7,6	-8,3
Glyma.17G220800.1	MAPK cascade - HK	0,0	0,0	0,0	0,0	0,0	0,0	-2,3	0,0	0,0	0,0	3,4	2,1
Glyma.07G105700.1	MAPK signaling pathway - MKK9	0,0	0,0	0,0	0,0	0,0	0,0	1,7	1,9	0,0	0,0	0,0	-1,8
Glyma.11G243900.1	MAPK signaling pathway - MKP1	0,0	0,0	0,0	0,0	0,0	0,0	1,9	2,0	0,0	0,0	-2,6	0,0
Glyma.15G084100.1	Protein kinase activity - CIPK12	0,0	0,0	0,0	0,0	0,0	0,0	2,5	2,0	0,0	0,0	0,0	-1,9
Glyma.06G202200.1	TF - Heatshock	0,0	0,0	3,3	2,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,2
Glyma.16G071700.2	Transcription factor - LOB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,5	-8,3
Glyma.06G125100.1	Transcription factor - AP2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,7	2,6
Glyma.08G339200.1	Transcription factor - AP2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,1	0,0	0,0	-5,0	-5,4

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.09G117500.1	Transcription factor - AP2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,7	-8,7
Glyma.10G016500.1	Transcription factor - AP2	0,0	0,0	0,0	0,0	0,0	0,0	3,0	2,2	0,0	0,0	-4,0	0,0
Glyma.11G019000.1	Transcription factor - AP2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,7	2,7
Glyma.16G047600.1	Transcription factor - AP2	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,5	0,0	0,0	-2,4	0,0
Glyma.20G168500.1	Transcription factor - AP2	0,0	0,0	0,0	0,0	0,0	0,0	3,8	4,3	0,0	0,0	-5,8	-3,2
Glyma.04G041200.1	Transcription factor - bHLH	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-9,2	-3,5
Glyma.11G186700.2	Transcription factor - bZIP	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,7	-9,2
Glyma.01G224900.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,7	-1,9
Glyma.06G031400.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-5,4	-6,0
Glyma.10G180800.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,6	0,0	0,0	-4,2	0,0
Glyma.12G089100.2	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	11,0	7,4	0,0	0,0	0,0	0,0
Glyma.16G017400.6	Transcription factor - MYB	0,0	0,0	14,2	2,9	0,0	0,0	2,1	0,0	0,0	0,0	0,0	0,0
Glyma.16G023000.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	2,0	2,3	0,0	0,0	-3,7	0,0
Glyma.18G113400.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	5,1	0,0	0,0	0,0	-10,9	-4,2
Glyma.19G164600.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	4,0	2,9	0,0	0,0	0,0	0,0
Glyma.19G178000.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,3	-3,1
Glyma.19G260900.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,8	-5,4
Glyma.19G260900.2	Transcription factor - MYB	0,0	0,0	4,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,6	-5,4
Glyma.19G264200.1	Transcription factor - MYB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,4	0,0	0,0	-4,0	-4,0
Glyma.05G110900.1	Transcription factor - MYC-2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,9	2,5
Glyma.09G150000.2	Transcription factor - MYC-2	0,0	0,0	0,0	0,0	0,0	0,0	3,6	5,3	0,0	0,0	0,0	0,0
Glyma.12G196700.1	Transcription factor - MYC-2	0,0	0,0	0,0	0,0	0,0	0,0	-1,9	-1,9	0,0	0,0	0,0	0,0
Glyma.15G063000.1	Transcription factor - MYC-2	0,0	0,0	4,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,2	-4,4

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.17G155900.1	Transcription factor - MYC-2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,5	2,3
Glyma.18G205100.1	Transcription factor - MYC-2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,3	-4,7
Glyma.20G248100.1	Transcription factor - MYC-2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,0	-2,0
Glyma.U035800.1	Transcription factor - MYC-2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,2	-2,8
Glyma.02G284300.1	Transcription factor - NAC	0,0	0,0	0,0	0,0	0,0	0,0	2,7	3,0	0,0	0,0	-5,1	-3,1
Glyma.12G223600.3	Transcription factor - NAC	0,0	0,0	4,2	3,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,3
Glyma.13G030900.1	Transcription factor - NAC	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
Glyma.14G030700.1	Transcription factor - NAC	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,2	-5,6
Glyma.18G110700.1	Transcription factor - NAC	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,0	-4,0
Glyma.18G301500.1	Transcription factor - NAC	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,2	0,0	0,0	-1,9	-1,5
Glyma.03G109100.1	Transcription factor - WRKY	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,1	-2,1
Glyma.03G208400.1	Transcription factor - WRKY	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,5	0,0	0,0	-2,0	-3,1
Glyma.13G045300.1	Transcription factor - WRKY	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,5	-2,7
Glyma.09G034300.1	Transcription factor - WRKY 23	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-4,4	-3,5
Glyma.05G127600.1	Transcription factor - WRKY 28	0,0	0,0	0,0	0,0	0,0	0,0	3,2	2,6	0,0	0,0	-4,9	0,0
Glyma.04G238300.1	Transcription factor - WRKY 30	0,0	0,0	0,0	0,0	0,0	0,0	2,5	3,3	0,0	0,0	0,0	-3,6
Glyma.06G307700.1	Transcription factor - WRKY 6	0,0	0,0	0,0	0,0	0,0	0,0	2,6	3,1	0,0	0,0	-2,7	0,0
Glyma.09G005700.1	Transcription factor - WRKY 6	0,0	0,0	0,0	0,0	0,0	0,0	2,0	2,2	0,0	0,0	-2,4	0,0
Glyma.15G110300.1	Transcription factor - WRKY 6	0,0	0,0	0,0	0,0	0,0	0,0	2,1	2,3	0,0	0,0	-3,0	0,0
Glyma.04G075400.1	Zinc finger - RING finger/PUB	0,0	0,0	0,0	0,0	0,0	0,0	5,6	6,9	0,0	0,0	0,0	0,0
Glyma.04G153400.1	Zinc finger - RING finger/PUB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-6,2	-6,3
Glyma.06G155100.1	Zinc finger - RING finger/PUB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,1	2,7
Glyma.10G044200.3	Zinc finger - RING finger/PUB	0,0	0,0	0,0	12,6	0,0	0,0	-12,8	-12,9	0,0	0,0	13,9	0,0

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.13G170300.1	Zinc finger - RING finger/PUB	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,8	4,0
Glyma.15G012900.1	Zinc finger - RING finger/PUB	0,0	0,0	0,0	0,0	0,0	0,0	-5,8	-9,7	0,0	0,0	0,0	10,1
Glyma.U029700.1	Zinc finger - RING finger/PUB	0,0	0,0	0,0	0,0	0,0	0,0	1,7	1,6	0,0	0,0	-2,3	0,0
Glyma.20G115600.1	Transcription factor - Zinc Finger - B-Box	0,0	0,0	0,0	0,0	0,0	0,0	1,8	2,4	0,0	0,0	0,0	0,0
Glyma.17G101000.1	Transcription factor - Zinc Finger - CDF3	0,0	0,0	3,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,1	-4,4
Glyma.15G200100.1	Transcription factor - Zinc finger - PLATZ	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,8	3,0
Glyma.01G097900.1	Zinc finger - RING finger - C3HC4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,3	-2,2
Glyma.13G232800.1	Zinc finger - RING finger/PUB	0,0	0,0	0,0	0,0	0,0	0,0	2,4	0,0	0,0	0,0	-9,7	-5,6
Glyma.03G173100.1	Transcription factor - Zinc finger -C2H2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,5	0,0	0,0	-9,6	-3,6
Glyma.06G045400.1	Transcription factor - Zinc finger -C2H2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,7	-2,4
Glyma.10G295200.1	Transcription factor - Zinc finger -C2H2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,7	0,0	0,0	-6,2	-3,3
Glyma.15G033600.1	Transcription factor - Zinc finger -C2H2	0,0	0,0	0,0	0,0	0,0	0,0	2,6	0,0	0,0	0,0	-4,1	-2,3
Glyma.06G008800.1	Transcription factor - Zinc finger -GATA	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,2	-1,7
Glyma.12G008800.2	Transcription factor - Zinc finger -GATA	0,0	0,0	0,0	0,0	0,0	0,0	7,1	0,0	0,0	0,0	-7,4	-8,1
Glyma.03G007600.1	Transcription factor activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,0	7,1
Glyma.12G020900.1	Transcription factor activity	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-11,5	-12,1
Glyma.12G158400.1	Transcription factor activity	0,0	0,0	0,0	0,0	0,0	0,0	3,8	2,0	0,0	0,0	0,0	-7,2
Glyma.12G137700.1	Transcription factor activity - GRAS	0,0	0,0	0,0	0,0	0,0	0,0	2,8	2,9	0,0	0,0	0,0	0,0
Glyma.04G064100.2	Transcription factor activity - KNAT	0,0	0,0	3,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,2	-2,7
Glyma.20G097500.3	Transcription factor activity - KNAT	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,4	-2,7
Glyma.08G179400.3	Transcription factor activity - LNK	0,0	0,0	5,3	5,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.11G154700.10	Transcription factor activity - LNK	0,0	0,0	8,9	10,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-9,5
Glyma.11G154700.9	Transcription factor activity - LNK	0,0	0,0	9,8	10,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-10,6

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.02G124900.1	Unknown function	0,0	0,0	9,6	9,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-9,3
Glyma.04G020700.1	Unknown function	0,0	0,0	0,0	0,0	0,0	0,0	3,3	0,0	0,0	0,0	-4,3	-2,6
Glyma.06G047600.1	Unknown function	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,2	0,0	0,0	0,0	0,0
Glyma.11G242700.1	Unknown function	0,0	0,0	-2,7	-2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.17G233700.1	Unknown function	0,0	0,0	0,0	0,0	0,0	0,0	2,6	2,4	0,0	0,0	-3,0	0,0
Glyma.18G039900.1	Unknown function	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-3,2	-3,4
Glyma.19G160600.3	Unknown function	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,5	0,0	0,0	-9,9	-10,4
Glyma.10G291000.2	Unknown function - 1589	0,0	0,0	0,0	0,0	0,0	0,0	-3,5	0,0	0,0	0,0	3,3	3,2
Glyma.05G121700.1	Unknown function - DUF_B2219	0,0	0,0	7,0	0,0	0,0	0,0	-6,9	-7,5	0,0	0,0	0,0	0,0
Glyma.10G097700.1	Unknown function - DUF1645	0,0	0,0	0,0	0,0	0,0	0,0	2,9	2,8	0,0	0,0	0,0	0,0
Glyma.14G080200.1	Unknown function - DUF1645	0,0	0,0	0,0	0,0	0,0	0,0	2,8	2,9	0,0	0,0	-4,2	0,0
Glyma.17G245100.1	Unknown function - DUF1645	0,0	0,0	0,0	0,0	0,0	0,0	2,3	2,8	0,0	0,0	-5,3	0,0
Glyma.06G304500.1	Unknown function - DUF247	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-7,0	-5,9
Glyma.07G195300.2	Unknown function - DUF3700	0,0	0,0	0,0	0,0	0,0	0,0	11,5	9,1	0,0	0,0	0,0	0,0
Glyma.10G238300.1	Unknown function - DUF4005	0,0	0,0	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-2,6	-2,8
Glyma.20G013400.1	Unknown function - DUF4228	0,0	0,0	0,0	0,0	0,0	0,0	1,8	2,6	0,0	0,0	-4,2	-2,6
Glyma.01G228600.1	Unknown function - DUF4228 domain	0,0	0,0	0,0	0,0	0,0	0,0	3,0	3,2	0,0	0,0	-6,1	0,0
Glyma.02G206000.3	Unknown function - DUF566	0,0	0,0	0,0	0,0	0,0	0,0	-10,4	-10,7	0,0	0,0	0,0	0,0
Glyma.02G024200.1	Unknown function - DUF569	0,0	0,0	0,0	0,0	0,0	0,0	2,2	2,1	0,0	0,0	0,0	0,0
Glyma.11G039800.1	Unknown function - DUF581	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	1,7
Glyma.03G095200.1	Unknown function - DUF632	0,0	0,0	0,0	0,0	0,0	0,0	6,7	7,2	0,0	0,0	0,0	0,0
Glyma.04G140600.1	Unknown function - DUF632	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-8,6	-7,1
Glyma.06G071000.1	Unknown function - DUF642	0,0	0,0	7,6	7,8	0,0	0,0	-7,6	-8,1	0,0	0,0	0,0	0,0

Table S1: (continued)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.07G253600.1	Unknown function - DUF679	0,0	0,0	6,9	0,0	0,0	0,0	0,0	5,4	0,0	0,0	-7,2	-6,9
Glyma.02G005400.1	Unknown function - DUF761	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0	0,0	0,0	-3,2	-3,0
Glyma.18G202500.1	Unknown function - DUF861	0,0	0,0	0,0	0,0	0,0	0,0	5,5	3,7	0,0	0,0	-4,1	0,0
Glyma.07G215000.5	Unknown function - DUF869	0,0	0,0	0,0	0,0	0,0	0,0	-12,5	-12,4	0,0	0,0	0,0	0,0

Table S2: Functional annotation of genes differentially expressed only in more susceptible combinations (condition A)

Transcript Name	Functional annotation	12 vs 0 hpi				48 vs 12 hpi				120 vs 48 hpi			
		γ-Gm2	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*	*γ-Gm2*	*β-Gm1*	*γ-Gm1*	*β-Gm2*
Glyma.13G368300.2	Hormone - Auxin - PCD regulation (SKP1)	0,0	0,0	0,0	0,0	-4,8	0,0	0,0	0,0	5,1	10,3	0,0	0,0
Glyma.09G217100.3	Other - Binding	0,0	0,0	0,0	0,0	0,0	7,3	0,0	0,0	-7,8	-7,7	0,0	0,0
Glyma.20G157200.4	Other - IPP2 - Plant defense	-9,3	-8,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.11G028700.1	Other - kinesin - ARK3	0,0	0,0	0,0	0,0	-7,6	-4,8	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.16G194000.6	Other - PIN	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	9,3	8,4	0,0	0,0
Glyma.11G012200.3	Other - RNA silencing	11,9	11,1	0,0	0,0	-11,8	0,0	0,0	0,0	11,7	0,0	0,0	0,0
Glyma.18G180900.10	Other- kinesin - transporter	0,0	-10,4	0,0	0,0	-8,1	0,0	0,0	0,0	8,1	9,4	0,0	0,0
Glyma.08G266300.2	Protein kinase activity - YAK1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	-11,6	-11,5	0,0	0,0
Glyma.10G228900.1	Receptor - Lectin-RLK	-3,1	-9,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Glyma.07G100700.2	Transcription factor - MYB	0,0	0,0	0,0	0,0	8,8	0,0	0,0	0,0	-7,9	-9,9	0,0	0,0
Glyma.05G136000.1	Transcription factor - YL1	0,0	0,0	0,0	0,0	-9,4	-10,0	0,0	0,0	8,3	0,0	0,0	0,0
Glyma.16G147900.1	Unknown function - DUF3754	-9,1	-8,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

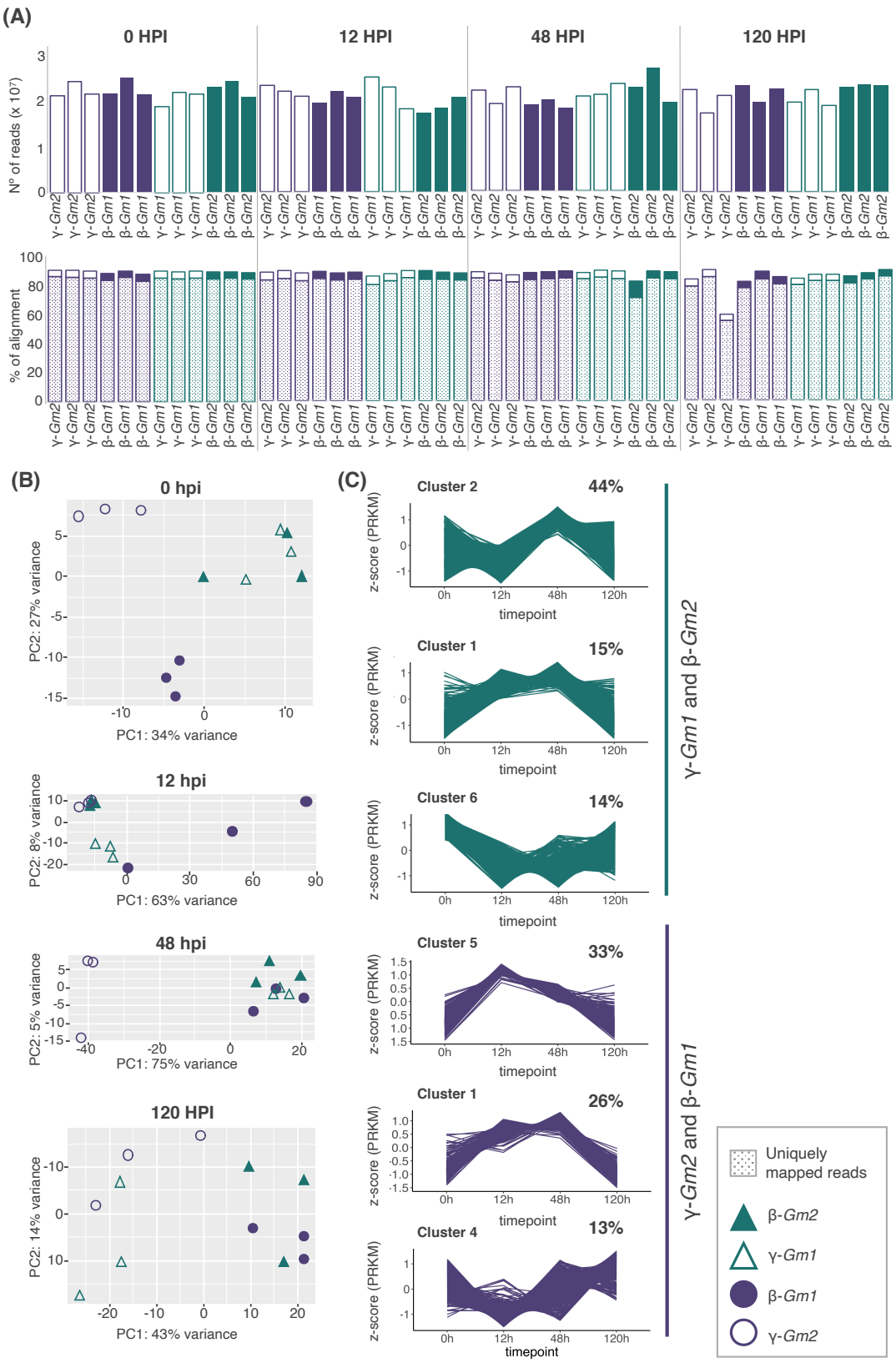

Figure S1: Overview of RNA sequencing data. (**A**) Number of raw reads and percentage of alignment of each library to the soybean reference genome. (**B**) Principal component analysis (PCA) of biological sequenced libraries in each time point in hours post inoculation (hpi). (**C**) *k*-means clustering analysis results and clusters that represent 75% of the genes that clustered in condition A (more resistant) or B (more susceptible).

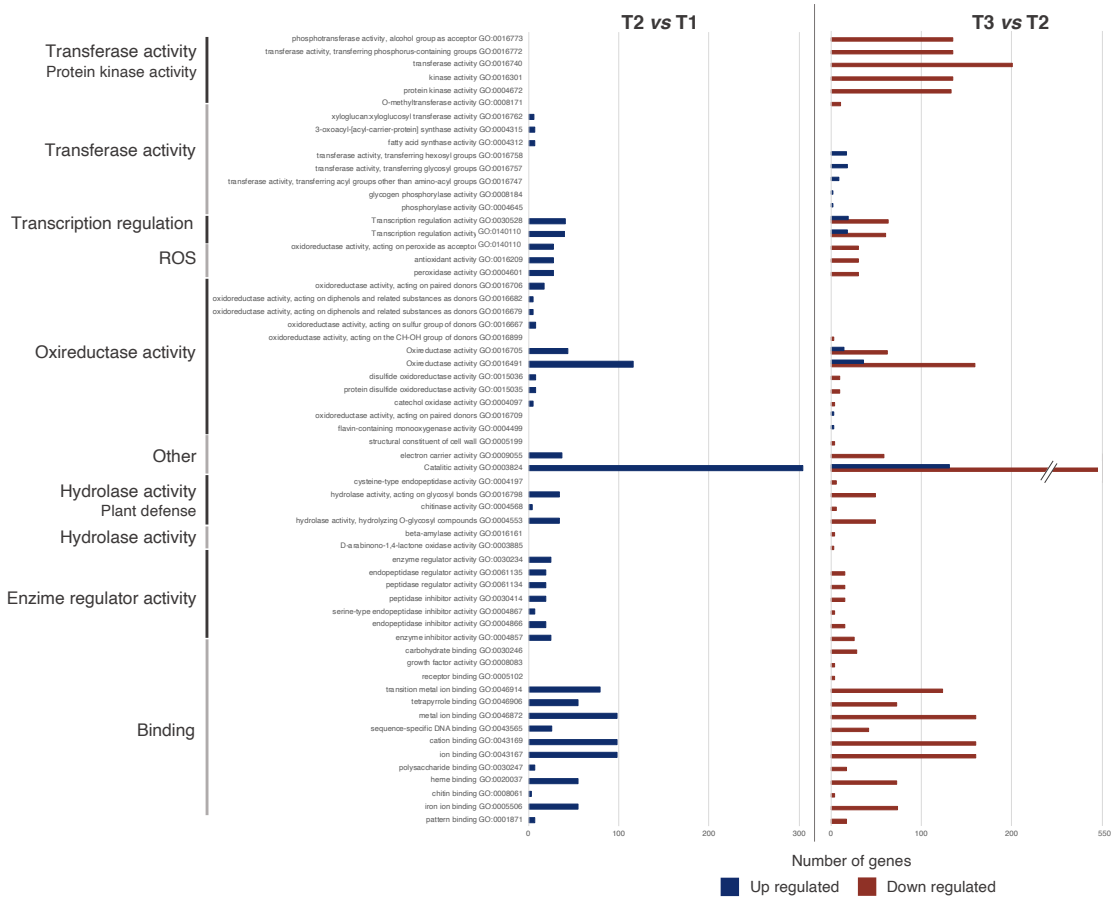

Figure S2: Gene Ontology (GO) enriched molecular functions in condition A (more resistant combinations) up and downregulated genes at 48 *vs* 12 hours post-inoculation (hpi) (T2 *vs* T1) and 120 *vs* 48 hpi (T3 *vs* T2).

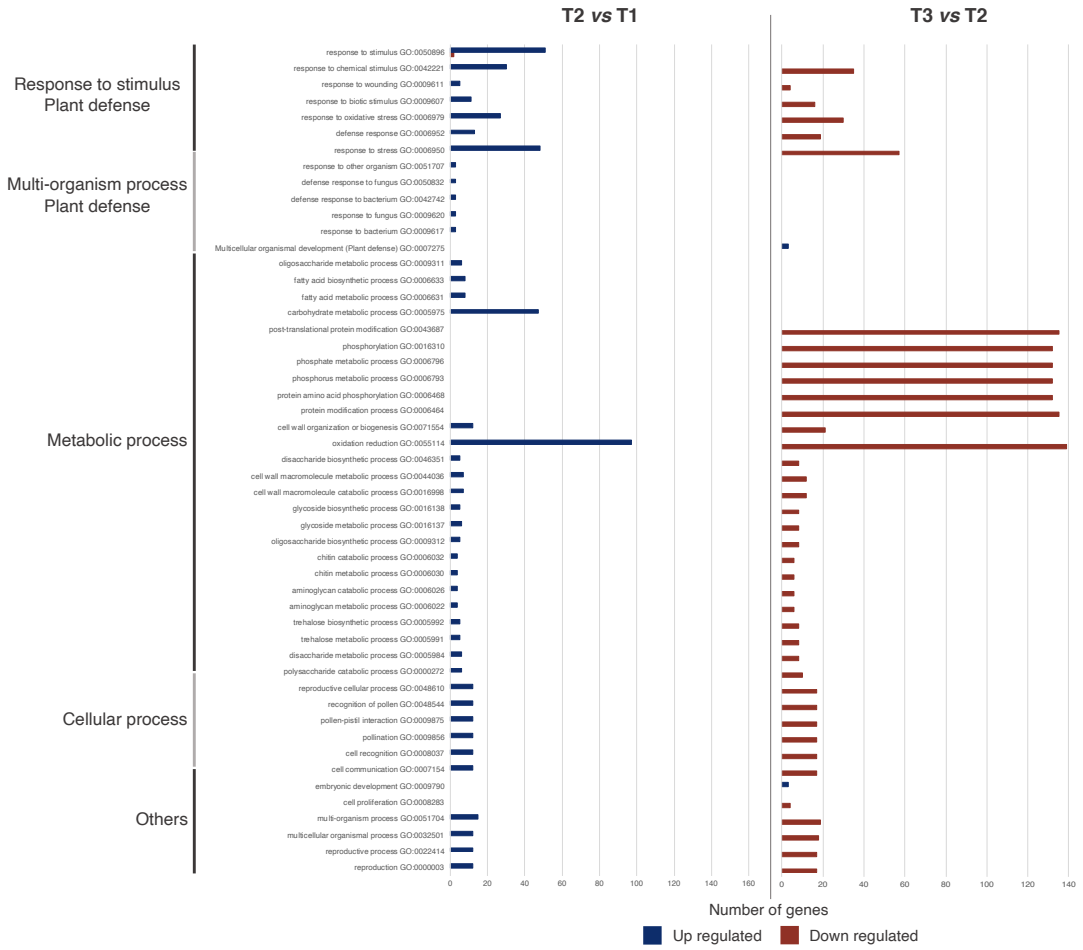

Figure S3: Gene Ontology (GO) enriched biological processes in condition A (more resistant combinations) up and downregulated genes at 48 *vs* 12 hours pos inoculation (hpi) (T2 *vs* T1) and 120 *vs* 48 hpi (T3 *vs* T2).