GENETIC MAPPING, QTLS AND RNA-SEQ: USING APPROACHES IN IDENTIFYING RESISTANCE GENES FOR FUNGAL DISEASES IN PEPPER

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DEDICATION

To my parents, Rinaldo and Jany, with all my love, affection and gratitude.

To my family and all my friends, for the encouragement and love.

I DEDICATE

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SUMMARY

ABSTRACT

BIANCHI, Paola Alvares; D. Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro, April, 2021. GENETIC MAPPING, QTLS AND RNA-SEQ: USING APPROACHES IN IDENTIFYING RESISTANCE GENES FOR FUNGAL DISEASES IN PEPPER. Supervisor: Rosana Rodrigues. Counselors: Helaine Christine Cancela Ramos and Telma Nair Santana Pereira.

Diseases caused by *Colletotrichum* spp. and *Phytophthora capsici* are of major concern in sweet pepper production around the globe. Although resistant cultivars are recommended to control these diseases, there are no commercial genotypes described as resistant to both pathogens. Identifying genes involved in the response of plants to attack by pathogens, using genetic mapping, QTL identification and RNA-seq technology contributes to better understanding of plant-pathogen interaction and can aid in the development of resistant cultivars. This work aimed to investigate *Capsicum-Co. scovillei* and *Capsicum-P. capsici* genetic interactions using different approaches: 1) to generate a genetic linkage map for *Capsicum annuum* var*. annuum,* identify QTLs associated with anthracnose (*Colletotrichum scovillei*) resistance and 2) to study the gene expression of *C. annuum* when infected by *P. capsici*. For the genetic mapping and QTL identification, a population derived from crosses between two *C. annuum* var*. annuum*, UENF 2285 (susceptible to anthracnose) and UENF 1381 (resistant to anthracnose) was used. For the genotyping one plant of each parental, one of the F_1 generation and 170 plants of the F_2 generation were used. Molecular analyses

were carried out using ISSR (Inter Single Sequence Repeats), SSR (Single Sequence Repeats) and AFLP (Amplified Fragment Length Polymorphism) markers. Genetic mapping was obtained using a LOD Score of 4.0 and the QTLs were identified using the R-QTL package. Eleven linkage groups were obtained, with 59 mapped markers and a total length of 1,209.8 cM. Six QTLs with minor effect were found distibuted in the linkage groups 1, 2, 3, 5, 9 and 11, explaining together 23.16% of the variation. For the RNA-seq analysis, two genotypes of *C. annuum* were used: 'Yolo Wonder', susceptible (S) to *P. capsici*, and 'Criollo de Morelos 334', partially resistant (R). Two P. capsici isolates were used: the non-adapted isolate Pc273 (N), and the adapted isolate Pc107 (A). The experiment was performed in triplicate over the period, with each triplet inoculated with an independent inoculum. The assessment of disease progress was made at two different times: 24 and 72hpi (hours post inoculation). Twenty-four RNA samples were extracted from the inoculated plants for the four interactions (RxA, SxA, RxN and SxN), at 24 and 72hpi. The RNA-seq analysis was performed using the DiCoExpress tool. To confirm the changes of the expression patterns observed, primers of four selected genes were designed from the sequenced samples using Primer 3, and Real time qPCR was performed using 12 novel RNA samples collected at 24hpi. A total of 35,884 reads were mapped to predicted genes in *C. annuum.* Around 50% of the total pepper population that expressed genes were analyzed in each project. The differentially expressed genes (DEG) ranged from 2,104 to 7,407 between the three statistical models. Many relevant genes were found in the samples, such as those associated with jasmonic acid expression, ethylene, salicylic acid, genes from the systemic acquired resistance – "*SAR*" family, leucine-like receptors (LLR), argonaute proteins (AGO), dicer-like proteins (DCL) and RNA-dependent proteins. We identified genome regions and potential candidate genes responsible for resistance against both diseases in pepper, and that show promise for providing enhanced understanding of the effects of host resistance to plant pathogens.

RESUMO

BIANCHI, Paola Alvares; D. Sc.; Universidade Estadual do Norte Fluminense Darcy Ribeiro, April, 2021. GENETIC MAPPING, QTLS AND RNA-SEQ: USING APPROACHES IN IDENTIFYING RESISTANCE GENES FOR FUNGAL DISEASES IN PEPPER. Orientadora: Rosana Rodrigues. Conselheiras: Helaine Christine Cancela Ramos e Telma Nair Santana Pereira.

Doenças causadas por *Colletotrichum* spp. e *Phytophthora capsici* são uma grande preocupação na produção de pimentões em todo o mundo. Embora cultivares resistentes sejam recomendadas para o controle dessas doenças, não há genótipos comerciais resistentes a ambos os patógenos. A identificação de genes envolvidos na resposta de plantas ao ataque de patógenos, por meio de mapeamento genético, identificação de QTLs e tecnologia de RNA-seq, contribui para o melhor entendimento da interação planta-patógeno e auxilia no desenvolvimento de cultivares resistentes. Desta forma, este trabalho teve como objetivo investigar as interações genéticas entre *Capsicum-Co. scovillei* e *Capsicum-P. capsici* usando diferentes abordagens, tais quais: 1) gerar um mapa de ligação genética para *Capsicum annuum* var. *annuum* e identificar QTLs associados à resistência à antracnose (*Colletotrichum scovillei*); e 2) estudar a expressão gênica de *C. annuum* quando infectado por diferentes isolados de *P. capsici*. Para o mapeamento genético e identificação de QTLs, a população foi derivada de cruzamentos intraespecíficos entre dois acessos de *C. annuum* var. *annuum*, UENF 2285 (suscetível à antracnose) e o UENF 1381 (resistente à

antracnose). Para a genotipagem da população foi utilizada uma planta de cada parental, uma da geração F_1 e 170 plantas da geração F_2. As análises moleculares foram realizadas usando marcadores ISSR (Inter Single Sequence Repeats), SSR (Single Sequence Repeats) e AFLP (Amplified Fragment Length Polymorphism). O mapeamento genético foi obtido usando um LOD Score de 4.0 e os QTLs foram identificados usando o pacote R-QTL. Foram obtidos 11 grupos de ligação, com 59 marcadores mapeados e comprimento total de 1.209,8 cM. Seis QTLs com efeito menor foram encontrados distibuídos nos grupos de ligação 1, 2, 3, 5, 9 e 11, explicando juntos 23,16% da variação. Para a análise de RNA-seq, dois genótipos de *C. annuum* foram usados: 'Yolo Wonder', suscetível (S) a *P. capsici*, e 'Criollo de Morelos 334', parcialmente resistente (R). Foram utilizados dois isolados de *P. capsici*: o isolado não adaptado Pc273 (N) e o isolado adaptado Pc107 (A). O experimento foi triplicado no período, com cada trinca inoculada com um inóculo independente. A avaliação do progresso da doença foi feita em dois momentos diferentes: 24 e 72hpi (horas pós-inoculação). Vinte e quatro amostras de RNA foram extraídas das plantas inoculadas para as quatro interações (RxA, SxA, RxN e SxN), em 24 e 72hpi. A análise de RNA-seq foi realizada com o auxílio da ferramenta DiCoExpress. Para confirmar as mudanças nos padrões de expressão observados, primers de quatro genes selecionados foram desenhados a partir das amostras sequenciadas usando o Primer 3 e análises de qPCR em tempo real foram realizadas usando 12 amostras de RNA, coletadas 24hpi. Um total de 35884 leituras foram mapeadas para genes previstos em *C. annuum*. Cerca de 50% do total de genes expressos de pimenta foram analisados em cada projeto. Os genes diferencialmente expressos (DEGs) variaram de 2104 a 7407 entre os três modelos estatísticos. Muitos genes relevantes foram encontrados nas amostras, como aqueles associados à expressão do ácido jasmônico, etileno, ácido salicílico, genes da resistência adquirida sistêmica - família "SAR", receptor semelhante à leucina (LLR), proteína argonauta (AGO), dicer- como proteína (DCL) e proteína dependente de RNA. Foram identificadas regiões do genoma e potenciais genes candidatos responsáveis pela resistência contra ambas as doenças em pimentas e pimentões, além de um melhor entendimento do efeito da resistência do hospedeiro a fitopatógenos.

1. INTRODUCTION

The genus *Capsicum* belongs to the Solanaceae family that includes chili and sweet peppers, with Brazil as one of its centers of diversity (Hill et al., 2013). These peppers are considered important vegetables all over the world due to their sensory and nutritional characteristics. They are among the most important constituents of tropical and subtropical cuisine (Welbaum et al., 2015).

The occurrence of several diseases caused by different pathogens is a limiting factor for the production of vegetables, and causes a significant increase in the use of pesticides, increasing the costs of the production and compromising the quality of the final product. Through time, these diseases have shown greater resistance to pesticides, resulting in the frequent use of products with different chemical characteristics that enhance risks to human health and the environment (Damalas and Eleftherohorinos, 2011).

The *Agência Nacional de Vigilância Sanitária - Brazil* stated that, between 2013 and 2015, 74% of the sweet pepper fruit samples had irregularities regarding the presence of unauthorized pesticides and/or with concentrations of residues above the established for the crop, making the sweet pepper one of the foods with the highest levels of contamination by pesticides (ANVISA, 2016).

Among the most important diseases associated with cultivation of *Capsicum* are those caused by the fungus *Colletotrichum* spp. and by the oomycete *Phytophthora*. The occurrence of these diseases can result in considerable losses

to sweet pepper crops in the pre and post-harvest periods, leading to serious economic losses (Kim et al., 2008; Lamour et al., 2012; Töfoli et al., 2015).

Colletotrichum is a phytopathogenic genus prevalent today worldwide and is a destructive plant pathogen, causing diseases in various crops and fruits (Oo et al., 2018). The most economically important aspect of anthracnose in peppers pertains to the lesions of fruits, although *Colletotrichum* spp. can affect many other parts of the pepper plants (Roberts et al., 2001).

Phytophthora spp. can infect pepper on all parts of the plant, including the roots, stems, leaves and fruits. The symptoms consist of brown or black lesions on stems at the soil line and plant wilting. Infected leaves initially show dark green spots that enlarge, followed by necrosis (Kim et al., 2008; Lamour et al., 2012).

The identification of resistant genotypes is an important step towards reducing costs and the environmental impact caused by the excessive application of fungicides (Parlevliet, 2002; Thakur, 2007). The chemical control adopted by producers has resulted in drawbacks over time, such as the emergence of isolates resistant to the fungicides usually used; in addition, biological control is not proving effective, since a few agents are more effective than others (Torres-Calzada and Tapia-Tussel, 2015; Wan and Liew, 2020).

For the development of cultivars that are resistant to diseases, knowledge of the regions of the genome that express these characteristics has become a useful tool for researchers, as well as knowledge of plant response and all mechanisms used by the plants to stop the infection. This recognition induces in plants a batch of molecular mechanisms, including hormone production, oxidative bursts, calcium influx, transcriptomic reprogrammation and sometimes a hypersensitive response (Jones and Dangl 2006; Boller and Felix 2009; Dodds and Rathjen 2010).

The genome of the *Capsicum* species is one of the largest among the Solanaceae family. The first whole-genome sequences of *C. annuum* (CM334) and *C. chinense* (PI159236) were reported by Kim et al. (2014). The study reported that the pepper genome size is ~3–3.5 Gb, is characterized by a high percentage (over 80%) of repetitive elements and includes 35,000 genes.

Different approaches have been used in order to identify regions of the plant genome responsible for the expression of important characteristics for cultivation. Techniques such as genetic mapping, QTL mapping and RNA-seq, for example, allow the identification of significant genes involved in the defense of plants

against pathogens, thus increasing the efficiency of research related to the identification of such genes.

The pepper breeding team at *Universidade Estadual do Norte Fluminense Darcy Ribeiro* (UENF) has been developing multiple initiatives that study the interaction between *C. annuum* and *Co. scovillei*. Researchers there have identified different sources of *C. annuum* that are resistant to *Colletotrichum* spp., the disease inheritance and the complex interactions taking place in this pathosystem (Silva et al., 2014; Bento et al., 2017; Geronimo, 2018; Almeida et al., 2020). The partnership between UENF and the *Universidade Estadual de Londrina* (UEL) has also generated important findings with respect to this interaction, highlighting genes, secondary metabolites and disease inheritance (Baba et al., 2019; Baba et al., 2020; Giacomin et al. 2020).

The *Institut national de recherche pour l'agriculture, l'alimentation et l'environnement* (INRAE) team has been performing different studies focusing on the pathosystem *C. annuum* x *P. capsici*, identifying sources of resistance, inheritance of the disease, genetic mapping, QTL identification and transcriptomic studies (Lefebvre and Palloix, 1996; Thabuis et al., 2004; Bonnet et al., 2007; Maillot, 2018).

This work deals with two aspects of plant-pathogen interaction: genetic mapping in *C. annuum* var. *annuum* for the identification of QTLs related to resistance to *Co. scovileii* in green fruits, carried out at the *Laboratório de Melhoramento Genético Vegeta*l (LMGV – UENF) – Brazil; and the transcriptomic analysis of *C. annuum* infected by *P. capsici* using the RNA-seq technique, performed in the unit *Génétique et Amélioration des Fruits et Légumes* (GAFL – INRAE) - France.

Both approaches generate important information about the genes that control resistance to *Co. scovileii* and *P. capsici* in peppers, as well as a better understanding of the interaction of this pathosystem. Such insight allows breeders and other researchers in the area access to more accurate and efficient methodologies in the identification of sources of resistance to these pathogens, such as selection assisted by markers that are developed specifically for those regions.

2. GOALS

2.1 General goals

Understanding the genetics of plant-pathogen interaction considering the associations between *C. annuum* - *Colletotrichum scovillei* and *C. annuum* – *Phytophthora capsici* as biological models, focusing on generating a linkage map for *C. annuum* var*. annuum,* enabling the identification of QTLs associated with anthracnose resistance and studying the gene expression of *C. annuum* when infected with the oomycete *P. capsici*.

2.2 Specific goals

a) Generating a linkage map based on the genotypic information obtained from the *C. annuum* var*. annuum* population;
b) Identifying QTLs associated with anthracnose resistance in unripe fruits;
c) Analyzing the effects of the interaction between pepper hosts and *P. capsici* isolates at 24 and 72 hours post inoculation on the gene expression pattern;
d) Evaluating the effects of the interaction between the plant and time after inoculation in response to the adapted and non-adapted isolates of *P. capsici*;
e) Comparing the interaction between time after inoculation and *P. capsici* isolate in each host genotype;
f) Performing co-expression analysis on the identified differentially expressed genes in the interaction between pepper host, *P. capsici* isolates and evaluation time.

3. CHAPTERS

3.1 GENETIC MAPPING AND IDENTIFICATION OF QTLs RELATED TO ANTHRACNOSE RESISTANCE IN *Capsicum annuum* var. *annuum*

3.1.1 INTRODUCTION

Most of the worldwide production of peppers is affected by biotic factors such as fungi, bacteria, viruses and other pests. Although several practices and precautions are implemented when planting peppers, anthracnose is probably one of the main constraining factors in the pre- and post-harvest phases (Ridzuan et al., 2018).

Colletotrichum, the causal agent of anthracnose, is a destructive plant pathogen that is prevalent worldwide and responsible for diseases in several cultures. The presence of lesions on fruits is the most important aspect of anthracnose in peppers, although *Colletotrichum* spp. causes damage to many other parts of the plant (Oo et al., 2017).

There are different management strategies for anthracnose control in *Capsicum*, such as crop rotation, application of chemical fungicides and biological control (Ali et al. 2016; Lahkar et al. 2018). Despite all available management

strategies, the use of resistant cultivars is frequently considered the most appropriate method to control plant diseases (Sun et al. 2015).

One of the main objectives of plant breeding programs is to obtain cultivars resistant to diseases. To achieve this goal, it is necessary to find sources of resistance and determine their genetic control. The wide variability as well as the occurrence of pathotypes and their complex interactions with plant hosts, evidenced by the differential response of plant hosts to *Colletotrichum* spp. isolates, has been challenging for plant breeders (Mongkolporn and Taylor 2018).

Nowadays, there are no *Capsicum* cultivars resistant to *Colletotrichum* spp., although some sources of resistance have been identified, such as the accession PBC 932 (*C. chinense*). In this accession, resistance against *Colletotrichum capsici*, expressed at different stages of plant growth, was related to three recessive genes (*co1*, *co2* and *co3*) (Pakdeevaraporn et al., 2005; Mahasuk, et al., 2009; Reis et al., 2009).

Resistance to anthracnose was also identified in UENF 1381, a *C. annuum var. annuum* accession described as highly resistant to anthracnose in the immature stage and moderately resistant in the mature stage (Bento et al., 2017). The *Capsicum* spp. breeding program at UENF has been developing different research efforts on the *Capsicum* x *Colletotrichum* pathosystem, such as selection of resistance sources, disease inheritance, antimicrobial activities and gene identification (Geronimo, 2018; Almeida et al., 2020; Baba et al., 2020; Giacomin et al.; 2020; Pereira et al., 2021).

Genetic linkage map construction has become a necessary tool for molecular genetics and plant breeding programs. It helps to identify the region of a gene, allows several possibilities in mapping studies (Shirasawa et al., 2012) and assist breeders, since one or more markers of the genotype may be associated with one or more controlling genes for qualitative and quantitative characteristics (QTL) (Bhering et al., 2009). The information of these loci has been used in the marker assisted breeding of some species, as well as in studies of synteny, comparative mapping and positional cloning of genes (Shirasawa et al., 2012).

Over the years, different research involving the genetic mapping of *Capsicum* spp. has been developed (Paran et al., 2004; Yi et al., 2006; Li et al., 2015; Zhang et al., 2019; Lee et al., 2020). In addition, researchers have identified QTLs related to many different important traits in pepper (Ben-Chaim et al., 2006; Yarnes et al.,

2013; Mahasuk et al., 2016; Konish et al., 2019), including those related to anthracnose resistance, for example. Knowledge of these regions enables the use of Molecular Marker Assisted Selection and helps breeders to develop, more quickly and effectively, new strategies in their breeding programs.

This work sought to generate a linkage map based on the genotypic information obtained from an intraspecific F_2 population developed from crosses in *C. annuum* var. *annuum* and identify QTLs associated with anthracnose resistance in unripe fruits of *C. annuum* var*. annuum*.

3.1.2 BIBLIOGRAFIC REVIEW

3.1.2.1 Origin, dispersion, botanical and reproductive aspects of the genus *Capsicum*

The genus *Capsicum* has been known since the beginning of civilization and its fruits were used by ancient peoples to improve the visual characteristics and flavor of food. The species that compose it are classified according to level of domestication, and the genus is composed of 38 species (The Plant List, 2020), though new species are continually being described.

Among the contingent species of the genus, five have been domesticated: *C. annuum*, *C. baccatum* var. *pendulum*, *C. chinense*, *C. frutescens* and *C. pubescens*. The species are diploid and can be separated into two groups according to the basic number of chromosomes: one with n = x = 12, and another with n = x = 13 chromosomes; they correspond to the domesticated and wild species, respectively (Moscone et al., 2007).

Based on cross ability and capacity to obtain fertile hybrids, *Capsicum* species are divided into three genic complexes. The *Capsicum annuum* complex brings together the species *C. annuum*, *C. chinense* and *C. frutescens* and their botanical forms; the *Capsicum baccatum* complex brings together *C. baccatum* var. *baccatum* and *C. baccatum* var. *pendulum*; and the *C. pubescens* complex brings together wild species and only one cultivated one, *C. pubescens* (Pickersgill, 1991).

After analyzing archaeological evidence, genetic analyses and plant distributions, researchers have suggested that the species *C. annuum* was initially domesticated in Mexico or Northern Central America (Reifschneider, 2000; Kraft et al., 2014). Subsequently, the species of the *C. annuum* complex had been spread by Spanish and Portuguese navigators across Europe, Africa and Asia by the end of the 15th century, and through the dispersion of seeds by migratory birds (Pickersgill, 1986; Eshbaugh, 1993; Moscone et al., 2007).

While capsicum plants are considered autogamous, cross-pollination can also occur, with rates ranging from 2 to 90%, which can be facilitated by morphological changes in the flower, by the action of pollinating insects and by cultivation practices, among other factors (Carvalho and Bianchetti, 2007; Justino, 2013).

The root system is pivoting, with a high number of lateral branches, reaching depths of 70-120 cm. The height and form of growth of the plants vary according to the species and the cultivation conditions. The leaves are of variable color, size and shape. Nodes with anthocyanin may be observed along the stem length (Carvalho et al., 2017)

The flowers are hermaphrodites; the calyx is composed of five sepals and the corolla has five petals, both of which may vary from six to eight sepals and petals, respectively. The flower is the essential organ for the taxonomy of *Capsicum* species, which is divided into three groups, according to the color of its corolla. *C. baccatum* has white corolla with yellow anthers and *C. pubescens* corolla purple or violet anthers. *C. annuum*, *C. chinense* and *C. frutescens* have corollae that vary between white to greenish yellow and purple to violet anthers, differing taxonomically according to the number of flowers per node and the annular constriction of the calyx (Guerra, 2001).

The fruit is a berry, with great structural and morphological variability, highlighted by its many shapes, sizes, colors and pungencies (Carvalho and Bianchetti, 2007; Zimmer et al., 2012). Three pairs of independent genes control the color of ripe fruits: loci *c1*, *c2* and *y* (Hurtado-Hernandez and Smith, 1985). The locus *y* distinguishes pepper plants with red and yellow fruits and is the same gene for the biosynthesis of capsanthin-capsorubin synthase (CCS) (Lefebvre et al., 1998, Popovsky and Paran, 2000).

Pungency is a characteristic present in the *Capsicum* genus and it is attributed to two capsaicinoids called capsaicin and dihydrocapsaicin that accumulate on the surface of the placenta and are released when the fruit is subjected to any physical damage (Chattopadhyay et al., 2011). The absence of pungency in peppers is controlled by a single recessive gene, *pun-1* or *c* (Wang and Bosland, 2006). Sweet peppers (*Capsicum annuum* var. *annuum*) have large and wide fruits with square to conical shapes, a non-pungent taste and are consumed in salads, cooked or stuffed. Most chili peppers have smaller fruits, varied shapes, a predominantly pungent flavor and are used mainly as a condiment and, in some cases, for ornamental purposes.

3.1.2.2 Economic and nutritional importance

The economic importance of the *Capsicum* genus is due to domesticated species (Perry et al., 2007; van Zonneveld et al., 2015). Among them, *C. annuum* is the most well known. It is cultivated worldwide and is possibly the most widely used species in breeding programs for commercial cultivars (Gonzáles-Péres et al., 2014). It includes open pollinated cultivars, hybrids of sweet peppers and paprika production of the *cayene*, *jalapeño* and other pungent peppers (Ribeiro and Reifschneider, 2008).

Pepper and sweet pepper production occupies almost 2 million hectares worldwide, resulting in a production of more than 32 million tons (FAO, 2015). At the national level, in 2019 around 1 thousand tons of peppers were produced, with the North and Northeast regions being the main producers with 29 thousand tons, corresponding to 70% of the national production. Brazil's sweet pepper production was approximately 254 thousand tons, with the Southeast region being the main producer (49%) (IBGE, 2017).

The cultivation of peppers occurs in all Brazilian regionsnand is very common in family agriculture, a fact that encourages the integration of small farmers and agribusinesses (Rufino and Penteado, 2006). Due to the need for labor for harvesting, the cultivation of peppers contributes to the permanence of workers in rural areas and to increased income for small properties (Sudré et al., 2010).

The fruits are also important sources of natural antioxidants, such as vitamins B, C and E, which represents one of its main functional and nutritional constituents. Peppers have low levels of sodium and cholesterol and have a large percentage of fibers in the raw form, aiding in digestion and the prevention of

intestinal problems (Hanif et al., 2006). They are sold for fresh consumption as green, red, yellow, orange, cream and purple fruit (Frizzone et al., 2001).

In the food and pharmaceutical industries, two classes of chemical substances found in fruits of the genus *Capsicum* are widely used. One of them is carotenoids, with high nutritional value, and the second refers to capsaicinoids, responsible for the pungency of peppers, which are exploited for the production of condiments and analgesics, cosmetics, among other uses (Bosland and Votava, 2012; Arimboor et al., 2015).

Factors such as genotype, fertilizer management, maturity and environmental conditions affect fruit composition and may increase or decrease vitamin concentrations (Bae et al., 2014). In addition, hot and sweet peppers also have antioxidant compounds such as capsaicinoids, ascorbic acid, carotenoids and flavonoids, which are considered biologically active and able to promote health (Rosa et al., 2002; Bae et al., 2014; Khan et al., 2014). Some flavonoids, such as apigenin, also act in increasing the formation of human neurons and in strengthening communication between them (Souza et al., 2015).

3.1.2.3. Anthracnose in *Capsicum* and the genetic control resistance

Anthracnose is a disease caused by *Colletotrichum* fungi. It is considered the most severe fungal disease in sweet and chili pepper crops and is present worldwide, most frequently in tropical and subtropical regions, especially in crops grown during hot and humid periods. It is a disease of complex etiology, caused by different species of the *Colletotrichum* genus (Lobo Jr. et al., 2001; Reis et al., 2009).

In peppers, the disease is characterized by typical symptoms, which consist of sunken and concentric lesions on the fruit, appearing in the field or post-harvest, bearing circular shapes with variable diameter and the formation of an orange spore mass in the center of the lesion (Tozze Júnior et al., 2005).

The *Colletotrichum* genus has several species and is classified as one of the main pathogens in the world. Some of its representative species are *C. capsici, C. acutatum, C. dematium* and *C. coccodes* (Pakdeevaraporn et al., 2005; Than et al., 2008; Diao et al., 2017). Fungi of this genus have circular acervuli, simple conidiophores, hyaline conidia and oval to oblong or falcate features. The host affected epidermis is bounded by an orange-colored mass, a structure called

acervuli, which constitutes one of the main symptoms and signs of the presence of this fungus in plants (Gali et al., 1978).

Colletotrichum spp. use different strategies to colonize host plants according to the stage of fruit development. The fungus usually begins its colonization in a biotrophic and quiescent form in fruit tissue structures (Gan et al., 2012; O'Connell et al., 2012; Prusky et al., 2013; Sahitya et al., 2014). Environmental factors have a direct influence on the development and epidemic spread of anthracnose. The relationships between rainfall intensity, location of plant development and presence of dispersing inoculum can result in different levels of disease severity (Kanto et al., 2014). It is common for the infection to occur during periods of heat (± 27°C) and high humidity (± 80%), as these are considered the ideal conditions for the development of the fungus and, consequently, outbreaks of the disease (Roberts et al., 2001).

The *Capsicum* genus, in general, has characteristics that help it resist disease, ranging from genetic factors to specialized metabolites. During the coevolutionary process, plants and their pathogens developed strategies both to inhibit the attack of pathogens (in the case of plants) and mechanisms to overcome host immunity (in the case of pathogens), thus generating attack and counter-attack strategies (Burdon et al., 2009). However, pathogens have selective advantages over their hosts due to their fast life cycle and enhanced ability to release new genetic pathogenic combinations (Matiello et al., 1997).

The inheritance of resistance in fruits depends on individual *Colletotrichum* species and isolates, the source of resistance and on the stage of ripening of the fruit. Studies have reported five different genes conferring resistance to anthracnose in *Capsicum* genotypes. A few them indicate that resistance to *C. capsici* is controlled by a dominant gene (Lin et al., 2002), while resistance to *C. dematium* is controlled by a partially dominant one (Park et al., 1990). While evaluating the inheritance of *Co. scovillei* resistance in ripe and unripe *C. annuum* fruits, Giacomin et al. (2020) observed that it is expressed independently in the different fruit development stages. In both cases, there are two main genes responsible for resistance with associated polygenic effects.

From a cross between *C. chinense* x *C. annuum*, using as pathogens *C. truncatum*, different genes for each developmental stage were identified: *co1* in unripe mature fruits, *co2* in ripe fruits and a third gene, *co3*, in seedlings

(Pakdeevaraporn et al., 2005; Mahasuk et al., 2009). Nevertheless, the inheritance resistance to *C. gloeosporioides* has been described as dominant or partially dominant (Park et al., 1990).

A gene called *PepEST* that is highly expressed in ripe sweet pepper fruits has been identified; it is capable of preventing the formation of the fungus' appressoria and thus inducing resistance to anthracnose. Although its accumulation was located in the epidermis and in the cortical cell layers of infected ripe fruits, it was rarely observed in epidermal cells of infected immature fruits (Ko et al., 2005). Ko et al. (2016) developed transgenic organisms that overexpress the *PepEST* protein, and observed that these plants exhibited enhanced defenses and resistance to C*. gloeosporioides, C. acutatum* and *C. coccodes*.

Recommendations for anthracnose control in *Capsicum* include using healthy seeds, crop rotation with species that are non-hosts and avoiding the use of Solanaceae, the elimination of alternative hosts and cultural remains, biological control with crop protection in rainy periods, cultural management in order to avoid high humidity and, the most widely used technique, the application of fungicide (Than et al., 2008; Park et al., 2012; Pavan et al., 2016). However, the frequent use of fungicides negatively affects the health of producers and consumers and generates greater expenses and significant environmental impact due to the generation of waste (Sun et al., 2015).

Minimizing the application of fungicides is strongly recommended, and the use of genetic resistant cultivars for the control of anthracnose has been advanced as the most effective method to control plant diseases (Lopes and Ávila, 2002).

3.1.2.4. Genetic mapping

Genetic linkage maps are linear representations of gene positions and/or molecular markers configured in linkage groups. In the context of plant breeding, detailed linkage maps are extremely useful in enabling the complete coverage and analysis of the genome, the location of genes, studies of phylogenetic analyses, the prediction of offspring in experimental crosses, the association of the genes with qualitative and quantitative traits (QTLs), the quantification of the effect of these regions on the studied characteristics and for providing information about gene linkage. (Tanksley, 1993; Ferreira and Grattapaglia, 1998; Schuster and Cruz, 2004; Garcia et al., 2006; Semagn et al., 2006).

The concept of linkage maps emerged in 1910 when Thomas Hunt Morgan realized that in *Drosophila melanogaster* the phenotypic proportions did not coincide with those proposed by Mendel's second law. Based on this observation, Morgan suggested that the distortion of proportions could indicate the grouping of some genes and their occasional permutation between homologous chromosomes (Coelho and Silva, 2005). In 1913, Sturtevant was responsible for creating the first linkage map and suggesting the use of the percentage of recombinants as an indicator of the linear distance of genes for the construction of maps. Such distance is usually given in centimorgans (cM) (Ferreira and Grattapaglia, 1998; Carneiro and Vieira, 2002).

The first genetic map was constructed with the aid of morphological and cytological markers. In the early 1960s, isoenzymes came to be used as biochemical markers, allowing the construction of genetic maps in several species of plants (Carneiro and Vieira, 2002). With the advent of DNA markers in the 1980s, genetic maps have become widely used for a diverse range of different cultures (Ferreira and Grattapaglia, 1998).

Several techniques can be used to construct a genetic map, such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Randomly Amplified Polymorphic DNA), microsatellites (SSR - Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats), AFLP (Amplified Fragment Lenght Polymorphism), SCAR (Sequence Characterized Amplified Regions) and SNPs (Single Nucleotide Polymorphisms) (Silva, 2005). The evolution of these techniques, combined with increasingly complex and specific statistical procedures, has allowed the construction of linkage maps for most plant species of agronomic interest (Carneiro and Vieira, 2002; Slate, 2005).

Genetic maps are constructed from different types and sizes of mapping populations, markers, statistical procedures and computer programs (Ferreira et al., 2006). The efficiency in the process can be affected by differences in genetic distance between markers that can occur due to variations in the degree of recombination observed in different crossings (Liu, 1998).

The construction of genetic maps and the feasibility of their use in plant breeding needs to follow criteria such as simplicity, robustness, transfer and cost-effectiveness (Lorieux et al., 2000). Thus, the methodology of building a genetic map includes procedures and analyses that include choice of contrasting parents,

with the use of segregating populations with the maximum possible linkage disequilibrium an essential feature; population development; choice of the molecular markers to be used; verification of the segregation pattern of each marker locus; analysis of the link between the markers for the formation of the linkage groups; and determining the order and distance of the markers within these linkage groups (Paterson et al., 1991; Tanksley, 1993; Lynch and Walsh, 1998; Carneiro and Vieira, 2002; Collard et al., 2005; Wu et al., 2007; Mollinari, 2009).

Therefore, several computer programs based on different analysis methodologies have been developed, such as “Mapmaker” (Lander et al., 1987), “JoinMap” (Van Ooijein and Voorrips, 2001), “OneMap” (Margarido et al., 2007) and “TetraploidMap” (Hackett et al., 2007). Considering the variables inherent to the process steps, each factor can affect the efficiency of building a map and, as a consequence, it is common to obtain different maps generated for different populations of the same species (Liu, 1998; Paterson et al., 2000).

3.1.2.5. QTL identification

QTLs are regions of the genome or gene loci that are associated with quantitative, polygenic or complex inheritance characteristic controls (Lander and Bolstein, 1989; Khan, 2015). Much of the important agronomic characteristics are polygenic or quantitative inheritance; that is, they are the result of the action of several loci that can exhibit variable effects. A particular QTL can be a single gene or a group of linked genes controlling a particular characteristic. The linkage map analysis in experimental segregating populations is commonly used to dissect the genetic architecture of complex traits (Bazakos et al., 2017).

Mapping a QTL means identifying its position in the genome and estimating its genetic effects, such as the additive effect, dominance effect and other effects present in the model adopted (Toledo et al., 2008). It means making deductions across the genome about the relationships between the genotype and the phenotype of quantitative traits and comprises information about the number and position of loci in the genome under study, in addition to their effects on the characteristic of interest and mode of action (Lannou, 2012).

The identification and location of specific loci mediating quantitative characters is an approach of great importance in plant breeding that aims to expand the knowledge of the genetic inheritance of the characters and identify

molecular markers. These markers can be used in assisted selection for relevant phenotypic characteristics, in addition to leading to a better understanding of the interaction between genotype and phenotype. When the markers linked to the characteristic of interest are identified using computational tools, it is possible to select individuals based on the genotype, a technique known as Marker-Assisted Selection (Bernardo, 2008; Lannou, 2012).

In *Capsicum*, studies have been carried out involving the identification of QTLs. Most have the objective of associating genomic regions with commercial characteristics (Barchi et al., 2009; Lee et al., 2011; Alimi et al., 2013; Ham et al., 2018), but others have sought to identify QTLs associated with disease resistance (Minamiyama et al., 2007; Kim et al., 2011; Moulin et al., 2015; Mahasuk et al., 2016; Siddique et al., 2019).

3.1.3 MATERIALS AND METHODS

3.1.3.1. Obtaining the population and reaction to anthracnose

The F_1, F_2, BC_1 and BC_2 generations were obtained from the crossing between two genotypes of *C. annuum* var*. annuum* from the UENF germplasm bank (Figure 1), identified as UENF 2285 (female parent) and UENF 1381 (male parent). The UENF 2285 genotype is a sweet pepper variety with square-shaped fruit, which is susceptible to anthracnose; the UENF 1381 genotype is a pungent pepper that has been used as a source of anthracnose resistance in the *Capsicum* breeding program developed at UENF.

Seedlings of all generations were produced in a greenhouse located at the UENF campus in Campos dos Goytacazes, RJ, Brazil. Sowing was carried out in polystyrene trays with 128 cells kept in a growth chamber at 28°C. Seedlings with two pairs of permanent leaves were transplanted into 500 mL pots containing a mixture of soil, sand and manure (1:1:1). Crop management followed the recommendations outlined in Filgueira (2012), with some adaptations.

The crossings between the accessions UENF 2285 and UENF 1381 were performed in the early morning or late afternoon when the flower buds were in pre-anthesis. The buttons of the female parents were emasculated and identified. For

the pollen extraction of male parents, the flowers were collected in the morning and dried under incandescent lamps. Afterwards, the pollen was removed and transferred to a gelatin polymer capsule and stored in amber flasks with silica gel in a refrigerator at ± 5ºC for later manual pollination. The emasculated flowers were pollinated and covered with paper bags to avoid contamination (Silva, 2018).

Eighty crosses were carried out between UENF 2285 x UENF 1381, resulting in 24 hybrid fruits. Backcrosses were obtained from 117 artificial crosses between UENF 2285 x F_1 (BC_1) and 66 were obtained from crosses between UENF 1381 x F1 (BC_2) using ten plants from both parents and the hybrid, resulting in 101 fruits of BC_1 and 37 fruits of BC_2. To obtain the F_2 generation, 253 self-fertilizations of the F_1 generation were carried out and the mapping population was composed of 170 individuals from F_2 generation (Silva et al., 2017).

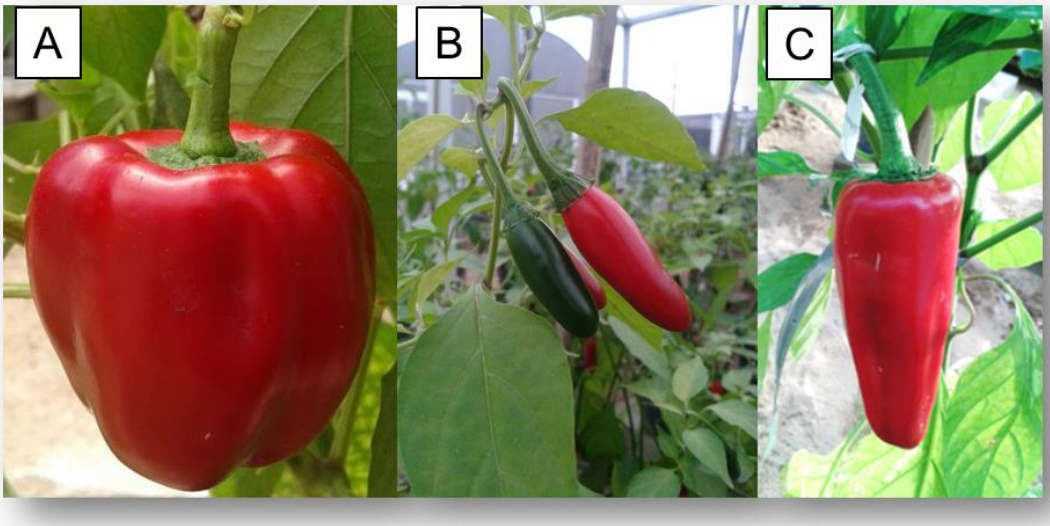

Figure 1. Fruit phenotype of *C. annuum* var. *annuum* parents and hybrid: A) UENF 2285, female parent; B) UENF 1381, male parent; and C) F_1 hybrid from UENF 2285 x UENF 1381. (Silva, 2018).

The isolate used in the inoculations was *Co. scovillei* obtained from a sweet pepper fruit with symptoms of anthracnose, collected by Geronimo (2018) and characterized and classified by Giacomin et al. (2020). Three unripe fruits from each plant of the P_1, P_2, F_1 and F_2 generations were used (Figure 2). The pedicels were removed and disinfestation was performed with immersion in 70% alcohol for

one minute, a 0.2% sodium hypochlorite solution for five minutes and a triple wash in autoclaved deionized water. The fruits were dried and placed in Styrofoam trays. Inoculation was performed with 10 µL of inoculum suspension deposited on a wound made on the fruit's surface with the aid of an entomological needle. After inoculation, the fruits were kept in a humid chamber. Assessment was carried out daily for seven days using a grade scale described by Montri et al. (2009) (Geronimo, 2018).

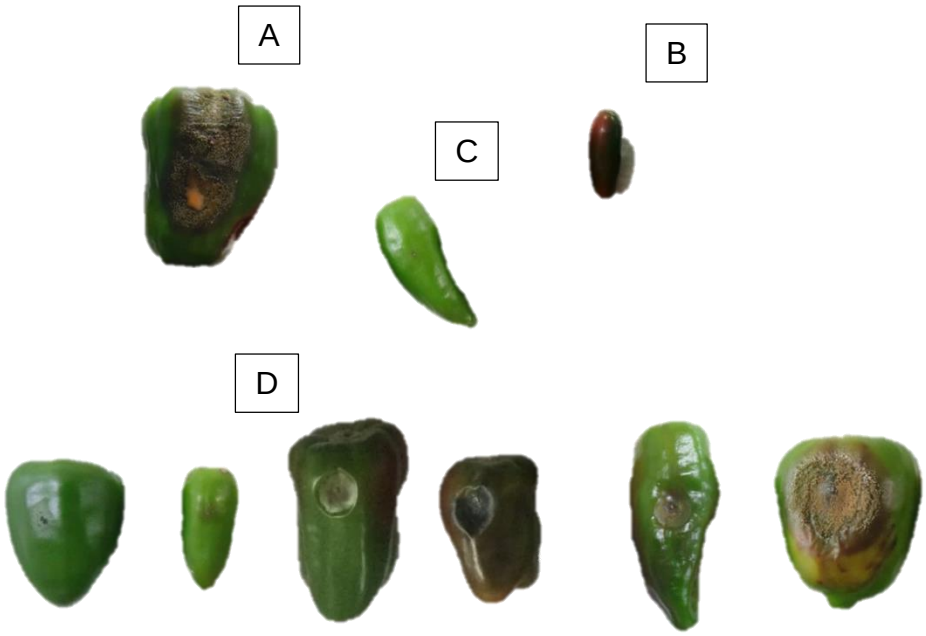

Figure 2. Reaction to anthracnose in immature fruits of *Capsicum annuum* var. *annuum* of the different generations obtained from the intraspecific crossing between UENF 2285 x UENF 1381. **A:** UENF 2285, female parental, susceptible to antrachnose; **B:** UENF 1381, male parental, resistant to anthracnose; **C:** F_1 generation, resistant to anthracnose; **D:** F_2 samples, ranging from resistant to susceptible to anthracnose.

3.1.3.3 Genotyping and construction of the genetic map

3.1.3.3.1. DNA extraction and quantification

Molecular analyses were carried out at the *Laboratório de Melhoramento Genético Vegetal* (LMGV) of the Universidade Estadual do Norte Fluminense Darcy Ribeiro – UENF. Young leaves from generations of the P_1, P_2, F_1 and F_2 were used. About 300 mg of leaf tissue were macerated and transferred to tubes

of 1.5 µL and immersed in liquid N_2 for DNA extraction according to the protocol described by Doyle and Doyle (1987), with modifications (Daher et al., 2002).

After extraction, the integrity and quantity of the genomic DNA were verified via 1% agarose gel using the High DNA Mass Ladder marker (Invitrogen, USA).

3.1.3.3.2. SSR markers

The SSR markers used were selected based on information available in the literature for mapping *C. annuum* (Minamiyama et al., 2006) and *C. baccatum* (Moulin et al., 2015). PCR amplification of 10 ng DNA (2 µL) was performed in a 13 µL reaction containing 1.5 µL dNTP mix (0.2 mM), 1.0 µL MgCl2 (1.9 mM), 1.3 µL PCR Buffer 1X, 0.12 µL Taq DNA polymerase (0.6 U), 6.08 µL of ultrapure water and 1 µL of each primer (5 µM).

The polymerase chain reactions (PCR) were conducted as follows: 4 min at 94ºC, followed by 35 cycles from 1min to 94ºC, 1min at the best anneling temperature according to the primer used, 72ºC for 2 min, and a final extension at 72ºC for 7 min. The amplified fragments were separated on a high-resolution agarose gel (Metaphor 4%) and also using capillary electrophoresis.

A screening using 300 SSR markers in the parental lines and the F_1 generation was the first step in identifying polymorphic markers. The microsatellite markers considered polymorphic among the parents and in the hybrid were selected to be tested on the entire *C. annuum* population, and these primers were used in the mapping analysis.

3.1.3.3.3. ISSR Markers

The ISSR markers for *Capsicum* were selected based on the work developed by Moulin (2013) and from the LMGV stock primers. For the detection of polymorphism among parents, 41 ISSR primers were tested. The amplification reactions were conducted in a final volume of 13 µL, containing 10 ng DNA (2 µL), 1.5 µL dNTP mix (0.2 mM), 1.0 µL MgCl2 (1.9 mM), 1.3 µL PCR Buffer 1X, 0.12 µL Taq DNA polymerase (0.6 U), 6.08 µL of ultrapure water and 1 µL of each primer (5 µM).

A screening of the parental lines and F_1 generation had previously been carried out, and the polymorphic primers were tested on the entire mapping population.

The PCR reactions were conducted as follows: 4 min at 94ºC for initial denaturation, following 38 cycles, each consisting of 94ºC for 1 min, between 48-52°C for 1 min (according to the ideal temperature for amplification of each primer), 72ºC for 3 min and a final extension at 72ºC for 7 min. The amplified fragments were separated by electrophoresis on a 2% agarose gel, stained with 6 µL of the red and blue juice mixture in a 1:1 concentration and subjected to UV light for viewing of the results.

3.1.3.3.4. AFLP markers

The AFLP technique was made in partnership with the *Universidade Estadual de Londrina* (UEL). It was performed following the protocol proposed by Vos et al. (1995), with modifications. The digestion of approximately 500ng of DNA and the ligation of the adapters to the generated fragments were performed in a single reaction. The restriction/ligation reaction was conducted with 1U of MseI restriction enzyme (New England Biolabs, Hitchin, Hertfordshire, UK); 5U of restriction enzyme EcoRI (Thermo Scientific, Waltham, MA, USA); 10X MseI buffer; 1U of T4 DNA ligase (SibEnzime, Academtown, Siberia); 5X T4 DNA ligase buffer; EcoRI (5 µM) and MseI (50µM) adapters; NaCl (0.5M); BSA (1mg/ml); DTT (5mM) and water to a final volume of 30µL.

The samples were incubated for 4 h at 37°C for restriction digestion, followed by 1 h and 15 min at 22°C for the connection of the adapters and for 10 min at 70°C for thermal inactivation of the enzymes. The restriction-binding pattern was verified on a 1% agarose gel and the product was subsequently diluted 4X with ultrapure water.

The pre-selective amplification reaction was performed using 3.5µL of GoTaq® Green Master Mix (Promega, Madison, WI, USA); 4.8µM of the EcoRI and MseI pre-selective primers, containing the base sequences of the respective adapters plus a pre-selective base "A" for EcoRI and "C" for MseI; 3.0µL of the diluted restriction-binding and ultrapure water to complete the volume of 10µL.

In this step, the program consisted of 1 cycle of 72ºC for 2 min, 20 cycles of 94ºC for 1 sec, 56ºC for 30 sec, 72ºC for 2 min and a final cycle of 60ºC for 30 min. Confirmation of pre-selective amplification was performed on a 1% agarose gel and the amplified product was diluted 8X in ultrapure water.

For the selective amplification, an initial screening was carried out with eight combinations of selective EcoRI/MseI primers in a 7% polyacrylamide gel. The

four most polymorphic and reproducible combinations were selected and applied to all samples (6-FAM-EcoRI + ACA/MseI + CAC; NED-EcoRI + AGG/MseI + CAA; VIC-EcoRI + ACT/MseI + CAA; PET- EcoRI + AGC/MseI + CTAG).

The selective reactions were performed for a final volume of 10µL containing 3.5µL GoTaq® Green Master Mix (Promega, Madison, WI, USA); 5µM of MseI primer; 1µM of fluorophore-labeled EcoRI primer (6-FAM, NED, VIC or PET); 2.5µL of the diluted pre-amplification reaction and ultrapure water. The amplification program consisted of 1 cycle of 94°C for 2 min, 65°C for 30 sec and 72°C for 2 min; 8 cycles of 94°C for 1 sec, 64°C for 30 sec (with a decrease of 1°C per cycle) and 72°C for 2 min; 23 cycles of 94°C for 1 sec, 56°C for 30 sec, 72°C for 2 min and a final extension of 60°C for 30 min.

For the resolution of the PCR products, which were obtained by selective amplification, 2µL of each reaction was added to 2µL of ultrapure water, composing a mixture with four primers in 10µL of final volume for each sample. Of this mixture, 1µL was added to 0.2µL of standard size GS-600LIZ (Applied Biosystems, Foster City, CA, USA) and 8.8µL of Hi-Di formamide (Applied Biosystems, Foster City, CA, USA). Then the samples were denatured at 95ºC for 3 minutes and immediately placed on ice and were finally submitted to capillary electrophoresis in an automated ABI 3500 xL Genetic Analyzer system (Applied Biosystems, Foster City, CA, USA).

3.1.3.4. Genetic mapping

Linkage analysis was done using the JoinMap program, version 4.0 (Van Ooijen, 2006). For the analysis, parents and F_1 were excluded. Linkage groups were formed and ranked using an LOD Score of of 4. The recombination frequencies were converted into genetic distance (centiMorgans) using the Kosambi function (1944). The chi-square test [X2 ($p<0.05$, GL=1)] was used to test the hypothesis of Mendelian segregation 1:2:1 for SSR markers and 3:1 for ISSR and AFLP markers. All markers were included in the mapping analysis, even those that did not follow Mendelian segregation.

3.1.3.5. QTL identification

The anthracnose resistance dataset in *C. annuum* var. *annuum* obtained in the work of Georgimo (2018) was used to identify regions with possible QTLs. The Lilliefors test was performed first in order to chek the normallity of the dataset.

The models described by Broman and Sen (2009), in which each genotype of the marker loci was adjusted as an effect of covariates against the measured phenotypic variables, were used to identify the QTLs related to resistance against anthracnose on unripe fruits. Standard interval mapping methods were used, which allowed verification of the position of possible QTLs along the linkage map.

For the quantitative variables, standard interval mapping model estimates using algorithms with maximum probability of existence of QTLs by iterative processes were used. QTL genotype probabilities were verified based on available data from the marker genotypes.

With the H statistic, the presence of QTLs is obtained after the LOD Score estimates, which is a non-parametric statistic that follows the approximate X^2 distribution and can be converted to the LOD = H / (2ln10) statistic. It was adopted as an evidence criterion of possible QTL peaks of LOD Scores with values greater than 2.5. Then, the percentage of the variance explained by the QTL was estimated through the “makeqtl” function of the R-QTL package. The steps developed to reach the objectives are summarized in Figure 3.

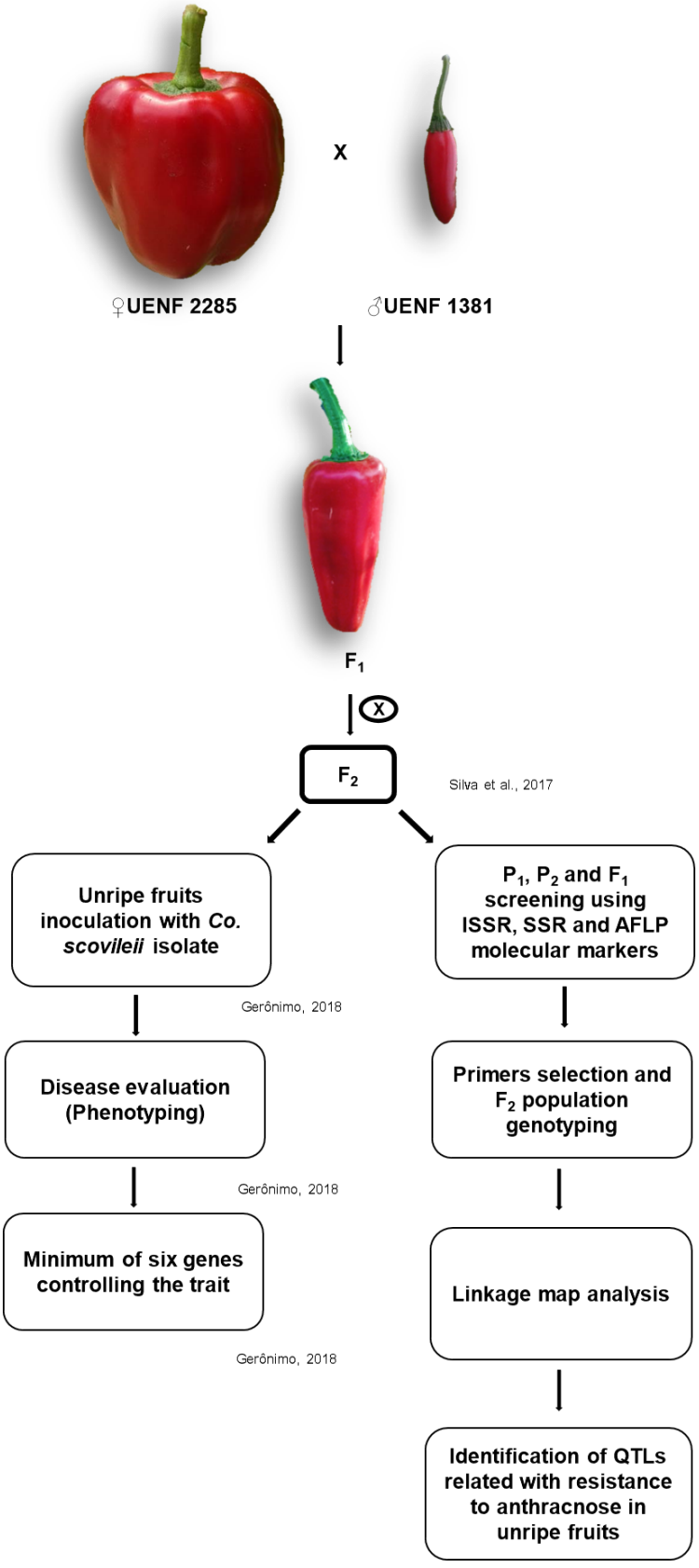

Figure 3. Organization chart of the methodology developed to obtain the intraspecific population of *Capsicum annuum* var. *annuum*, inoculation of immature fruits with the isolate *Colletotrichum scovilleii*, population genotyping, mapping analysis and identification of QTLs.

3.1.4 RESULTS

3.1.4.1. ISSR and AFLP markers

Using the ISSR technique (Figure 4), of the 41 tested primers, 29 did not detect polymorphism in the progeny and 12 demonstrated polymorphism in the studied population. For the AFLP method, 4 pairs of primers were tested; all were selected and generated a total of 331 marks, 110 of which were polymorphic between the parents and the mapping population.

For both ISSR and AFLP markers, several loci were obtained. However, for the mapping, only polymorphic loci between the parents and the population were used; thus, loci that did not obey this condition, being monomorphic, were discarded (Table 1).

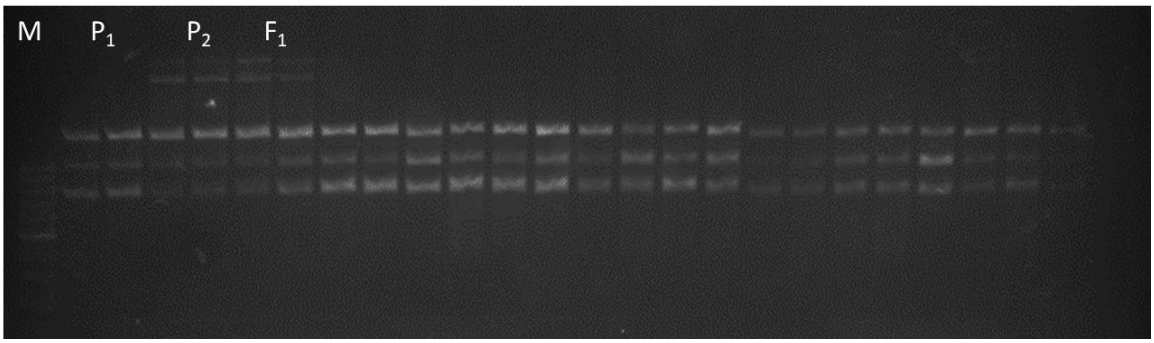

Figure 4. Amplification of DNA fragments using ISSR marker on 2% agarose gel. P_1 = Parental 1; P_2 = parental 2; F_1 = Hybrid; M = molecular weight marker. The following samples are part of the F_2 generation.

Table 1. Characteristics of the ISSR molecular markers that integrated the *Capsicum annuum* var. *annuum* linkage map.

Primer	Sequence (5'-3')	Annealing temperature	Number of amplified loci	Number of polymorphic loci	Linkage group
ISSR 1	$(AG)_8T$	43ºC	4	2	11

AFLP markers were mapped in almost all linkage groups on the map to *C. annuum*, except in linkage group 11, while the only mapped ISSR marker was found in this linkage group. It was possible to observe a concentration of markers of the same type in limited regions of the map, such as, for example, the accumulation of AFLP marks in groups one to ten.

Despite being dominant, the AFLP markers revealed a good number of polymorphic loci that could be mapped and enable the construction of linkage groups. The percentage of marks generated by the AFLP markers was 89.83%, a rate well above that of the microsatellite and ISSR markers, which added up to 10.17%, highlighting the importance of dominant markers for the construction of the map. In addition, they made it possible to allocate microsatellite markers across linkage groups.

3.1.4.2. Microsatellite markers

For the SSR markers (Figure 5), 300 primers developed for *C. annuum* (Minamiyama et al., 2006) were tested, and a total of nine were polymorphic between the parents and the mapping population. It was possible to observe that the distribution of the SSR markers was not uniform in the linking groups, and was only present in three groups (GL1, GL4 and GL11).

Even with a low number of mapped marks, the presence of codominant markers generated relevant information for the mapped population (Table 2).

Table 2. Characteristics of the microsatellite markers that integrated the *Capsicum annuum* var. *annuum* linkage map.

Primer	Sequence (F)	Sequence (R)	Annealing temperature	Number of amplified loci	Number of polymorphic loci	Linkage group
SSR 63	aaacagcaatcccatgaaaacc	gggctttggggagaatagtgtg	58ºC	3	3	11
SSR 83	tgaggcagtggtatggtctgc	cccgagttcgtctgccaatag	58ºC	3	3	1
SSR 131	aagtcatcagctgcaaagacca	ttcaacatgcatccagcttctt	57ºC	3	3	1
SSR 227	cgacgaagtttccgagctcaa	gacacggcgcttctttcctc	57ºC	3	3	11
SSR 271	cctttcacttcagcccacat	accatccgctaagacgagaa	53ºC	3	3	4

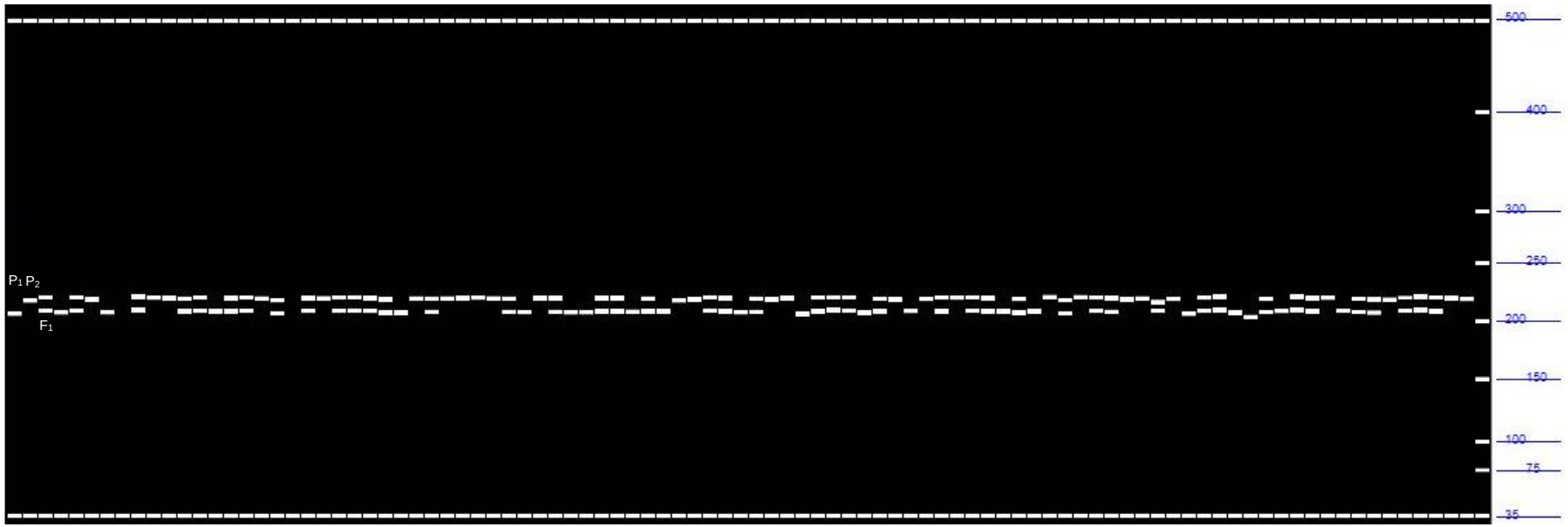

Figure 5. Amplification of DNA fragments using SSR markers on capillary eletrophoresis. P_1 = Parental 1; P_2 = parental 2; F_1 = Hybrid. The following samples are part of the F_2 generation.

3.1.4.3. Genetic mapping for an F_2 population of *Capsicum annuum* var. *annuum*

From the 131 polymorphic markers, 59 composed the linkage map, with 1 being ISSR (1.7%), 5 microsatellite (8.47%) and 53 AFLP (89.83%). The map generated in this work has 11 linkage groups (LG). As a diploid species, with 2n=24, it was expected to obtain 12 linkage groups, the haploid number of the species (n=12).

The linkage group sizes in the map ranged from between 51.9 to 254.1 cM (Figure 6), and the groups had from 3 to 10 markers. There was an average of 104.4 cM on a map with total coverage of 1209.8 cM (Table 3), in a mapping population composed of 170 F_2 individuals.

The distance between the markers varied from 0.7 to 66 cM, with a distance average of 55.01 cM between the markers over the linkage map. Markers with less distance from each other on a map give it more saturation, in addition to increasing the chances of finding QTLs among the mapped regions.

In LG1, 7 markers were mapped, 5 AFLP and 2 SSR, with a length of 254.1 cM and an average of 129.21 cM between the markers. The LG2 consists of 10 AFLP markers arranged at 209.3 cM and an average of 99.87 cM between them. The third linkage group is 141.3 cM in length and consists of 6 AFLP markers, arranged with an average distance of 84.56 cM.

LG4 is composed of 6 markers, including 1 SSR and 4 AFLP; it is 135.4 cM of total length with an average distance of 60.95 cM between each marker. The linkage groups 5, 6, 7, 8, 9 and 10 included only AFLP markers, with 90.9, 78.5, 76.6, 61.3, 56.0 and 54.5 cM, respectively. The last linkage group in the map is composed of 3 markers, 2 SSR and the only ISSR mapped marker, 51.9 cM in length with an average of 20.06 cM between the 3 marks.

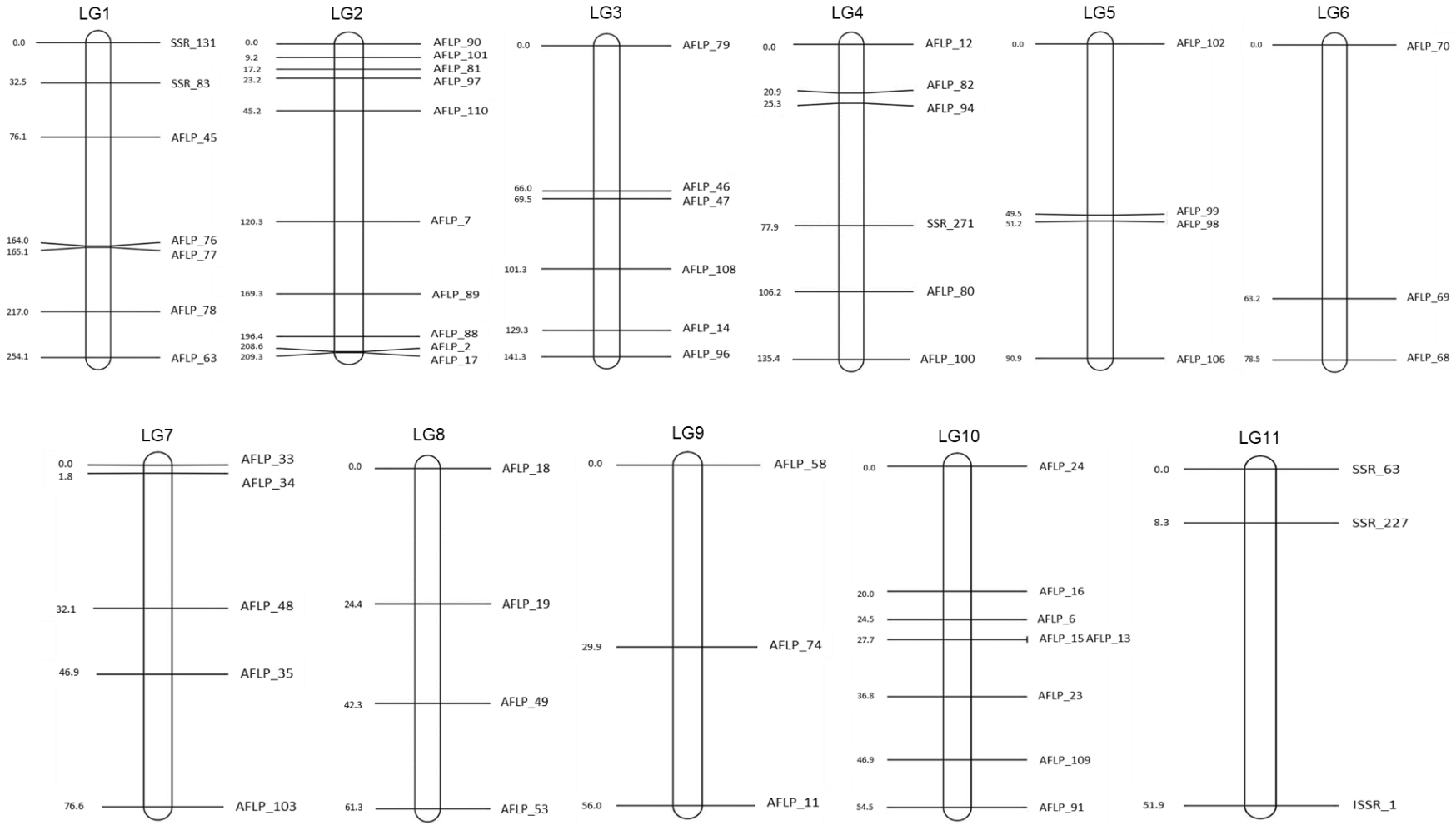

Figure 6. Linkage map of *Capsicum annuum* var. *annuum* constructed with an LOD Score of 4.0, based on 59 molecular markers distributed among 11 linkage groups. The genetic distance between each marker is indicated on the left and the markers are indicated on the right of each group.

Table 3. Number, type of marker, length of each link group (cM) and average distance between marks (cM) in the map of *Capsicum annuum* var. *annuum*.

Linkage group*	Number and type of marker	Length (cM)	Average distance between marks
LG1	7 (2 SSR; 5 AFLP)	254.1	129.21
LG2	10 (10 AFLP)	209.3	99.87
LG3	6 (6 AFLP)	141.3	84.56
LG4	6 (1 SSR; 5 AFLP)	135.4	60.95
LG5	4 (4 AFLP)	90.9	47.9
LG6	3 (3 AFLP)	78.5	47.23
LG7	5 (5 AFLP)	76.6	31.48
LG8	4 (4 AFLP)	61.3	32
LG9	3 (3 AFLP)	56.0	28.63
LG10	8 (8 AFLP)	54.5	26.3
LG11	3 (2 SSR; 1 ISSR)	51.9	20.06
Mapped markers	59	1209.8	55.01
Unmapped markers	72		
Total	131		

Considering the dominant molecular markers mapped, 8 AFLP markers followed the expected segregation (3:1; $p>0.05$), while no ISSR marker followed it; in addition, three SSR markers followed the expected segregation of 1:2:1 ($p>0.05$). Therefore, as this map is not saturated enough, there was a high distortion of Mendelian segregation in the other molecular markers tested.

In this study, 72 loci were not mapped, which indicates that 54.96% of the markers were not linked. The unallocated markers show that the saturation level of the map obtained needs to be increased. However, the available and unbound markers facilitate saturation of the map with the addition of new markers obtained from other studies (Moulin, 2013).

Some markers were removed from the linkage groups that were initially allocated due to the high estimates of recombination fractions, resulting in better ordering of the remaining marks. Rearrangement occurred in linkage groups 4, 6, 7 and 8 and one mark was eliminated in group 6. This procedure is common for the optimization of obtained marks.

3.1.4.4. QTL identification

Phenotypic data obtained from immature fruit stages combined with the molecular markers data were used to perform QTL analysis using the multiple QTL mapping (MQM). The disease evaluation obtained by Geronimo (2018) showed that the AUDPC (Area under the disease progress curve) ranged from zero to 29.5 and plants with AUDPC lower than 9.82 were considered resistant. High AUDPC values indicate greater virulence of the pathogen, while lower values are desirable for this trait. Anthracnose symptoms were observed in all fruits of the susceptible parental line (P_1). The mean values of AUDPC for this immature parent were 15.97, confirming its susceptibility. The fruits of the resistant parental line (P_2) had lower AUDPC values, with an average of 3.44, thus considered highly resistant in immature fruits.

The F_1 generation plants showed symptoms in a more moderate way, tending towards resistance, showing lower AUDPC values, with an average equal to 5.19. The F_2 generation plants possessed a wide variety of symptoms, from fruits without any symptoms to fruits completely compromised by the disease, which characterizes the variability of this generation. The mean AUDPC also tended to the resistant parent, with a value of 7.63.

Six different QTLs of minor effect were identified, which together explained 23.16% of the phenotypic variation, varying from 2.45 to 7.57% (Table 4). The largest effect was displayed by QTL1 in linkage group one; this QTL explained 7.57% of the observed phenotypic variation, with an additive effect of - 0.29. It presented an LOD score of 2.98, and thus was the most importante QTL identified in this study. QTL2, located at 209.3 cM of the LG2, explained 2.45% of the phenotypic variation, with an additive effect of 0.08 and an LOD score of 0.99, the lowest of the six identified QTLs (Table 4).

QTLs 3 and 4, located in linkage groups 3 and 4, were responsible for explaining 2.95% and 4.68% of the variation, respectively, presenting an LOD

score of 1.19 and 1.87. The QTLs are positioned at 98 cM and 86 cM, with an additive effect of 0.19 and – 0.04, respectively (Table 4).

QTL 5, located at position 56.0 cM in LG9, explained 4.40% of the variation with an LOD score of 1.76%. This QTL had an additive effect of 0.17. QTL 6 was identified in LG11 at 51.9 cM, and was responsible for the explanation of 3.41% of the resistance to anthracnose in unripe fruits. This QTL had an LOD score of 1.37 and the additive effect of - 0.01 (Table 4).

The identified QTLs provided an explanation of 23.16% of the phenotypic characteristics evaluated, representing important estimates of the loci position responsible for the resistance of the *C. annuum* var. *annuum* species to *Co. scovileii* in unripe fruits.

Table 4. Characterization of the QTLs identified for resistance to anthracnose in unripe fruits in an F_2 population of *Capsicum annuum* var. *annuum*.

Characteristic	QTL	Linkage Group	Marker*	Position (cM)	LOD score	% of variation	Additive effect
Resistance to anthracnose in unripe fruits	QTL1	LG1	AFLP 45	58.0	2.98	7.57	- 0.29
	QTL2	LG2	AFLP 17	209.3	0.99	2.45	0.08
	QTL3	LG3	AFLP 108	98.0	1.19	2.95	0.19
	QTL4	LG5	AFLP 106	86.0	1.87	4.68	- 0.04
	QTL5	LG9	AFLP 111	56.0	1.76	4.40	0.17
	QTL6	LG11	ISSR 1	51.9	1.37	3.41	- 0.01
Total						**23.16%**	

***** Marker closest to the QTL position on the linkage map

3.1.5 DISCUSSION

The map covered a total of 1209.8 cM in 11 linkage groups. A total of 59 molecular markers are included on the map. Among the 11 linkage groups, the shortest was LG11, with a size of 51.9 cM, while the LG1 was the longest, with 254.1 cM. Linkage groups 6, 9 and 11 featured only three molecular markers each. In contrast, LG2 featured 10 molecular markers representing a heterogeneous distribution in the map.

Kim et al. (2010) identified 19 QTLs associated with resistance to anthracnose in chili pepper (*Capsicum* spp.), in a linkage map using 327 markers composed of 49 SSRs, 175 AFLPs and 100 SRAPs, with a total length of 1896 cM in 13 groups. The average distance per marker was 4.0 cM, and the average distance between the markers per related group was 5.7 to 14.0 cM.

Prince et al. (1993) constructed a pepper linkage map using 192 markers (RFLP and isoenzymes) covering a total of 720.3 cM in 19 linkage groups. Tanksley et al. (1988) developed a genetic reference map for *C. annuum* that contained about 85 RAPD marks obtained from a population consisting of 61 individuals. Subsequently, a number of *Capsicum* genetic maps have been constructed and genotyped with various traditional gel-based marker systems (Lefebvre et al., 1995; Yi et al., 2006; Barchi et al., 2007; Lee et al., 2009; Wu et al., 2009; Mimura et al., 2012).

The size of the *Capsicum* genome is estimated at between 3300 cM and 3600 cM (Moscone et al., 2003; Hill et al., 2013). The linkage map constructed in this work covered 1209.8 cM of the *C. annuum* var*. annuum* genome. Minamyiama et al. (2006) obtained an integrated map for *C. annuum* based on 115 SSR, 228 AFLP, 60 RAPD and one CAPS, with 13 linkage groups, covering about 1042 cM of the genome.

Mimura et al. (2012) obtained a linkage map based on SSR and AFLP markers with coverage of 40.48% of the pepper genome. In contrast, Zhang et al. (2019), using SLAF-seq markers, built a linkage map for *C. annuum* L. with 60.89% coverage of the species's genome. Differences in the techniques, molecular markers and map saturation can directly affect the genome coverage in the resulting linkage map.

The total map length of the present map is somewhat shorter than those of previous studies (Livingstone et al., 1999; Ben-Chaim et al., 2001; Wu et al., 2009; Mimura et al., 2012; Li et al., 2018). However, the map distance calculated by JoinMap is usually shorter than that calculated by different softwares, such as Mapmaker, for example (Bradeen et al., 2001).

As noted in this study, maps developed for *C. annuum* may also present markers with segregation distortions. Zhang et al. (2016) identified segregation distortion in 275 (38.84%) of the 708 markers used in the construction of the genetic map. Lee et al. (2011) found an average distortion of 36.5% of Mendelian segregation for the tested markers. Factors such as structural chromosome rearrangements, deleterious recessive alleles, gametic selection, pre- or post-zygotic selection of allelic combinations, among others, can generate deviations from the expected segregation (Priyamedha et al., 2012).

Segregation distortion, a common phenomenon in genome analysis, is the deviation of the segregation ratio of a locus from the expected Mendelian ratio. These loci are called segregation distortion loci (SDL) or, simply, segregation distorters; they are hidden, but carry an important evolutionary function because they control the viability of individuals bearing different genotypes of the locus. The segregation of marker loci appears to be distorted as a result of the linkage between the neutral markers and the SDL (Xu, 2008).

Intraspecific crosses are used for mapping populations and can generate low polymorphism. Breeding lines of *C. annuum* can also show low levels of polymorphism. Overcoming this low polymorphism is a key challenge for molecular technologies and the AFLP marker is a powerful technique with which to score a number of polymorphic loci in a single experiment (Vos et al. 1995).

AFLP markers were present in almost all the linkage groups, and were responsible for 89.83% of the mapped marks. In other studies developed with *Capsicum*, accumulation of the same type of markers was also observed in a given region (Livingstone et al., 1999; Kang et al., 2001; Sugita et al., 2005). According to Sugita et al. (2005), this concentration of markers is considered common in the genome of *Capsicum* and other solanaceae.

It is known that the ideal number of linkage groups is that referring to the haploid number of the species, in this case 12. However, 11 linkage groups were identified in the present study. According to Carlier (2006), the more we increase

the coverage of a genetic map, the more the number of linking groups approaches the haploid number of chromosomes of species and the more the number of unbound markers approaches zero.

The low saturation of the present genetic map and the non-binding of all the polymorphic marks found may help explain why the number of linking groups is not equivalent to the haploid number of the species. In this case, the low number of markers resulted in a not so saturated map. With few polymorphic markers, the distance between them was great, which failed to allow co-segregation or linkage between many of them.

Based on the *C. annuum* var. *annuum* map, six minor QTLs were identified for resistance to anthracnose in the unripe fruit stage in six different linkage groups: LG1, LG2, LG3, LG5, LG9 and LG11. While analyzing the resistance to anthracnose in unripe fruits in the same population, Geronimo (2018) identified a minimum number of six genes that control the resistance, and the additive-dominant model was sufficient to explain the results obtained.

According to Geronimo (2018), 62.74% of the variation observed in the population for resistance to anthracnoe is due to genetic causes. The genotypic variance was predominantly additive, with a value of 12.32. The additive variance acts directly on the heritability of the trait, representing one of the most important genetic parameters for the breeder that enables positive results in the selection of individuals (Ramalho et al., 1993).

In the analyses carried out in the F_2 generation, while 62.75% of the variation observed is due to genetic causes, 48.47% is attributed to a genetic cause of an additive nature, which indicates that it is possible to fix this characteristic over successive generations of self-fertilization. The results indicate that the epistatic effects are not greatly important in the genetic control of the analyzed trait. For *Capsicum*, this effect expresses great importance, as homozygous plants will be more abundant in the population and the additive genetic effects indicate that the trait will be fixed in subsequent generations (Lobo et al., 2005).

The QTL analyses further indicated that most of the genetic variation was explained by a QTL in linkage group one (7.57%), with an LOD of 2.98. This is a promissing identified locus, responsible for explaining the greatest part of the phenotypic variation in this work and presenting the well-analyzed LOD score.

A minor QTL located in LG2 at 209.3 cM, close to the marker AFLP 17, was identified; it was responsible for explaining 2.42% of the phenotypic variation and had an additive effect of 0.08. While studying QTLs for resistance to anthracnose in two *Capsicum* sources, Mahasuk et al. (2016) found two QTLs corresponding to the resistances to anthracnose in mature green and ripe fruit maturity stages, at the same location of the LG2 (56.9 cM). The LOD values of the QTLs were 3.25 and 4.21, with ability to explain total phenotypic variation of 19.5 and 18.2% and additive effects of 0.52 and 1.68, respectively.

An interspecific map between *C. annuum* and *C. chinense* identified QTLs for anthracnose resistance in linkage groups 3 and 5 (Sun et al., 2015). Different from the QTLs identified in this work in the LG3 and LG5, those authors found a QTL located at 41.8 cM for the first linkage group with an LOD of 2.3 that explained 2.93 of the resistance at the mature fruit stage. For linkage group 5, six QTLs were found, located between 0.0 cM and 1.6 cM, with an LOD score ranging from 2.65 to 32.26, explaining between 9.31 and 62.38% of the variation for resistance in mature stage fruits (Sun et al., 2015).

When mapping QTLs for resistance to *Colletothricum* spp. in pepper, Lee et al. (2010) identified three QTLs in linkage group 9, positioned at 10 and 10.06 cM. The QTLs explained between 57.48% and 78.91% of the variation, presenting an LOD ranging from 13.36 to 15.98, substantially higher values than the QTLs found here. Mahasuk et al. (2016) also found a minor QTL for resistance to anthracnose in LG9 with an LOD score of 3.41, with an ability to explain phenotypic variation of 11.3 % and the additive effects of 14.65. In the present work the QTL identified in LG9 explained 4.4% of the phenotyipic variation with an LOD score of 1.76.

Kim et al. (2010) identified a total of 19 loci of quantitative characteristics in two QTLs with greater effect, with phenotypic variations of 16.4%, and 16 smaller QTLs. Five of these smaller QTL were responsible for 60.73% of phenotypic variations in resistance to anthracnose, and the LOD score of 4 was selected.

Alternatively, markers with significant segregation distortion have been used for genetic map construction, and these distorted markers may have affected the QTL analysis (Wen and Zhang, 2013). The values of the LOD Score statistic for most QTLs, obtained using the R/‘Package QTL’ Program, are critical ones, which can be justified by the low saturation of the linkage map and the inheritance of the characteristic.

3.1.6 CONCLUSIONS

A genetic map with 11 linkage groups was obtained for an F_2 population of *C. annuum* var. *annuum*, including one ISSR, five microsatellites and 53 AFLP markers, covering 1209.8 cM of the species genome.

Six QTLs with minor effects, related to anthracnose resistance in unripe pepper fruits, were identified in six different linkage groups. Together they explained 23.16% of the phenotypic variation for the trait.

The inclusion of more molecular markers is necessary for greater saturation of this map, rendering it greater reliability and allowing the obtainment of the number of groups referring to the haploid number of *C. annuum* var. *annuum*. A higher saturation of the linkage map will allow the capture of more QTLs, thus increasing their magnitude and reallocation in the linkage map to explain the character, and as more QTLs are identified, higher efficiency indexes may enable the selection assisted by molecular markers.

3.2 TRANSCRIPTOME ANALYSIS IN *Capsicum annuum* L. INFECTED BY *Phytophthora capsici* Leon. USING RNA-SEQ TECHNOLOGY

3.2.1 INTRODUCTION

Plants have adapted to respond to various conditions of environmental stress, activating specific molecular and physiological changes (Atkinson et al., 2013). This activation is the timely perception of stress to respond quickly and efficiently, in which a complex signaling cascade is activated by constitutive basal defense mechanisms in plants (Andreasson, et al., 2010). After exposure to stress, specific ion channels and kinase cascades are activated. Reactive oxygen species (ROS), phytohormones like abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), accumulate, and a reprogramming of the genetic machinery results in adequate defense reactions, increasing plant resistance to minimize biological damage (Laloi et al., 2004; Fujita et al., 2006; Spoel et al., 2008; Velázquez et al., 2009).

The soil-borne fungal-like oomycete *Phytophthora capsici* (Leon.) is one of the most devastating pathogens for pepper production. *P. capsici* can produce a wide variety of symptoms depending on the specific plant part involved and the developmental stage of the crop. Since *P. capsici* is a soilborne pathogen, symptoms usually first develop at the soil line in the roots and crown; however, infection can occur at any plant part where water splashes soil onto the plant. The most common symptoms on peppers are crown rot and fruit rot. Under wet

conditions, disease tends to manifest itself as wilting of the plants (Erwin and Ribeiro, 1996; Walker and Bosland, 1999; Lamour et al., 2011; Mansfeld et al., 2017).

Different sources of resistance have been reported, including *C. annuum* 'Criollo de Morellos 334' (CM334), PI201232, PI201234 and Perennial lines. Among these, the most resistant is CM334, which has been widely used in breeding programs (Lefebvre and Palloix, 1996; Thabuis et al., 2004; Bonnet et al., 2007; Candole et al., 2010; Liu et al., 2014; Wang et al., 2016; Arpaci and Karatas, 2020). Resistance to this pathogen in peppers is a highly complex trait. It is polygenic and influenced by several factors, including environmental conditions, virulence of the isolates and the source of resistance (Rehrig et al., 2014).

Resistance to *Phytophthora* in the Solanaceae family is quantitative in nature (Vleeshouwers, et al., 2011; Chen et al., 2014). Many important loci generally have small effects and common SNPs distributed throughout the genome with effects below detectable levels of significance are responsible for a large part of the heritability of this complex characteristic (Yang et al., 2010; Boyle et al., 2017). In addition, many QTLs have been identified in pepper associated with resistance against *P. capsici* (Lefebvre and Palloix, 1996; Thabuis et al., 2003; Mallard et al 2003; Minamiyama et al., 2007; Truong et al., 2012; Rehrig et al., 2014).

In several species, the co-localization of NB-LRR genes and QTLs has been reported, indicating that homologous genes could control resistance to both simple and complex inherited resistance. One major effect of QTL resistance and two resistance genes to *P. capsici* have been reported in pepper: a complex cluster of QTLs called *Pc5.1*, *Pc5.2* and *Pc5.3*, common to several resistance sources, *CaPhyto* from PI201234 and PhR10 from CM334, on chromosomes P5 and P10 (Mallard et al., 2003; Wang et al., 2016; Xu et al., 2016).

RNA-Seq is an approach developed to characterize the whole transcriptome profile that uses deep sequencing technologies. It provides a much more accurate measurement of the levels of transcripts and their isoforms than other methods, such as the cDNA and EST markers, for example (Wang et al., 2009). In plants, RNA-seq has been used to investigate global expression profiles and reveal the signal transduction pathways involved in the resistance network under various stresses (Chen et al., 2015; Hrdlickova et al., 2016). This approach

can reveal the precise location of transcription boundaries, has very low background signal because DNA sequences can be unambiguously mapped to unique regions of the genome and the results of RNA-Seq also show high levels of reproducibility (Wang et al., 2009).

In recent years, numerous studies have focused on transcriptome profiles using RNA sequencing (RNA-Seq) technology. Liu et al. (2017) generated a large-scale transcriptome profile data set for an elite pepper breeding line through high throughput mRNA sequencing and established a “pepper-Hub” public data platform for pepper research. There are some reports on transcriptional profiling and analysis of gene function in peppers and other plants (Lu et al., 2012; Li et al., 2016; Hu et al., 2016). Transcriptional levels are only a predictor for protein expression as they do not account for post-transcriptional processes such as translational regulation or protein stability (Liu et al., 2019).

This work aimed to analyze the effects of the interaction between pepper hosts and *P. capsici* isolates at 24 and 72 hours post inoculation. It evaluated the effects of the interaction between plant and time after inoculation in response to the adapted and non-adapted isolates of *P. capsici*, comparing the interaction between time after inoculation and *P. capsici* isolate in each host genotype and performing co-expression analysis on the identified differentially expressed genes in the interaction between pepper host, *P. capsici* isolates and evaluation time.

3.2.2. BIBLIOGRAFIC REVIEW

3.2.2.1. *Phytophthora capsici* in *Capsicum* and genetic control resistance

Pepper late blight, caused by the oomycete *Phytophthora capsici* Leonian, is one of the most serious causes of damage and loss in pepper production worldwide (Babadoost, 2005; Lu et al., 2017). It is a pathogen capable of infecting different crops of agronomic interest, including Solanaceae, Cucurbitaceae and Fabaceae. However, it exhibits local adaptation in certain geographic regions and isolates show varying levels of aggression, depending on the infected host (Granke et al., 2012; Lamour et al. 2012).

Species of the *Phytophthora* can reproduce sexually and/or asexually during their development cycle. For sexual reproduction to occur there must be a perception of mating factors, which trigger the differentiation of male and female sexual structures called antheridia and oogonia, respectively. The fertilization of oogonium by antheridium leads to the production of an oospore with thick and resistant walls (Erwin and Ribeiro, 1996; Lamour et al., 2012). Sexual reproduction has several evolutionary advantages for the pathogen, including ensuring high levels of diversity and heterozygosity (Granke et al., 2012; Lamour et al., 2012).

In asexual reproduction, reproduction takes place by means of asexual sporangia that release mobile zoospores, under conditions of high temperatures in areas with water accumulation. When submerged, each sporangium can release 20 to 40 biflagellate zoospores, which can initiate an infection. Although it is less effective for conservation, asexual reproduction promotes rapid dispersion during epidemics to the detriment of genetic diversity (Hausbeck and Lamour, 2004; Lamour et al., 2012).

Oomycetes are responsible for root rot and are capable of attacking most organs, including the crown, leaves, flowers and fruits, and can manifest themselves at any stage of plant development. In the field, the disease appears in the form of ridges and the symptoms are rotting of the neck and root system and sudden wilting; in more severe cases, it can cause necrosis in aerial parts and lead to the death of the plant (Santos and Goto, 2004; Marto et al., 2007; Pavan et al., 2016).

Resistance to *P. capsici* in species of the Solanaceae family is quantitative in nature and race specific (Vleeshouwers et al., 2011; Chen et al., 2014). In peppers, studies on the genetics of resistance to this pathogen identified several loci of quantitative characteristics (QTLs). While the genotype Criollo de Morellos (CM334) has resistance to all tested isolates of *P. capsici*, this is not a commercial cultivar (Thabuis et al., 2003; Quesada-Ocampo and Hausbeck, 2010; Lamour et al., 2011).

Studies of resistance to *Phytophthora*, in different species of Solanaceae, have reported the co-location of simple resistance genes and QTLs, indicating that homologous resistance genes control this characteristic. Two race-specific resistance genes have been reported in peppers: *CaPhyto* from PI201234 and

PhR10 from CM334 on chromosomes P5 and P10, respectively (Wang et al., 2016; Xu et al., 2016).

Major and minor resistance QTLs to *Phytophthora* have been mapped on several chromosomes (Thabuis et al., 2003; Bonnet et al., 2007; Truong et al., 2012). The major QTLs were identified near the P5 chromosome in several studies, regardless of the sources of resistance or isolates of *P. capsici* (Bonnet et al., 2007; Mallard et al., 2013; Liu et al., 2014; Rehrig et al., 2014). Several minor and isolate-specific QTLs have been reported, but their positions are variable, depending on the genetic background and *P. capsici* isolate (Thabuis et al., 2004; Truong et al., 2012; Rehrig et al., 2014).

While studying candidate genes for *P. capsici* resistance in *C. annuum*, Siddique et al (2019) identified 14 significant QTLs, and among three PcRR (*P. capsici* root rot) isolates, two of these QTLs on chromosome P5 showed major effects. They identified *SAR8.2* in *QTL5.2*, a systemic acquired resistance (SAR)-related gene known as *CASAR82A*, located in the phloem and epidermal cells of infected pepper leaves and stem tissues. The involvement of the pepper SAR8.2 gene in pathogen infection and environmental stress responses and its use as a marker of abiotic elicitors suggests it might also be an important candidate gene for PcRR resistance.

3.2.2.2. Plant defense against pathogens

Several pests and diseases constantly affect plants, such as diseases caused by pathogenic microorganisms, which are responsible for significant agricultural losses. However, during evolution plants developed biochemical and morphological mechanisms for their defense (Wit et al., 2007). As a result, plants have two major groups in their defense system: constitutive or preformed defenses and induced defenses.

Constitutive defenses are those naturally present in plants, functioning as chemical and physical barriers. These preformed defenses protect plants, at first, whether or not an attack or aggression occurs (Heath, 1997; Heath, 2000; Van Loo et al., 2006). The defenses induced are those evident after the perception of the invasion of the pathogen, or when the plant suffers injury. They can derive from hypersensitivity response, tissue lignification and activation of chemical barriers in which there is an increase in the concentration or synthesis of various

AMPs and proteins related to pathogenesis, also known as PR proteins (Heil, 2010).

Plants can be immune to most potential pathogens and have the ability to reduce the disease severity of actual pathogens. In the both forms, the recognition of the potential pathogens occurs by way of chemical cues, originally named elicitors, which for the last few years have been referred to as pathogen-associated molecular patterns (PAMPs) (Bittel and Robatzek, 2007). Since these molecular patterns exist in all organisms, they should actually be designated as Microbe-Associated Molecular Patterns (MAMPs) (Mackey and McFall, 2002), which are recognized by pattern recognition receptors (PRRs) at the cell surface. Activation of these PRRs leads to active defense responses, (MAMP/PAMP-triggered immunity), both in basal and non-host resistance (Jones and Dangl, 2006).

Not all MAMPs recognized by plants play a role in pathogenicity, but they correspond to molecules essential for microbial life. Some examples are fungal chitin and ergosterol, the main structural components of cell walls and upper fungi membranes. Although some microbial molecules are not in accordance with the classical understanding of MAMPs or effectors, they are important for pathogenicity and, after the host's perception, induce a hypersensitivity reaction (Rotblat et al, 2002; Bittel and Robatzek, 2007).

The production of several cellular messengers is initiated soon after the perception of PAMP or MAMP. During the first few minutes after the pathogen is detected, inflows of Ca2 + ions cause depolarization of the membrane (Boller and Felix, 2009). This ion is an omnipresent secondary messenger in the signaling of plant cells and induces the production of reactive oxygen species (ROS) and nitric oxide (NO). The oxidative "explosion" contributes to the defense of the host against the pathogen (Boller and Felix, 2009; Frederickson Matika and Loake, 2014; Seybold et al., 2014).

After the initial cell change reactions to the pathogen, the production of phytohormones associated with biotic stresses is observed (Boller and Felix 2009). These hormones include salicylic acid (SA), which is generally associated with interaction with biotrophic pathogens, as well as jasmonic acid (JA) and ethylene (ET), which are associated with interactions with necrotrophic pathogens (Loake and Grant, 2007; Bari and Jones, 2009; Wasternack and Hause, 2013).

They play the role of drivers, inducing and regulating the reprogramming of gene expression (Eulgem and Somssich, 2007; Moore et al., 2011; Wu et al., 2012; Frederickson Matika and Loake, 2014).

3.2.2.3. RNA-seq approach

RNA molecules are essential components of all living cells. Understanding the identity and abundance of these molecules in a given cell under specific conditions is the ultimate goal of RNA research. High-throughput approaches that allow large-scale interrogation of RNA sequences emerged in the early 1990s (Hrdlickova et al., 2016).

Transcriptome annotation is usually performed using slower and more expensive methods of cloning complementary DNA (cDNAs) or express sequence tagging (EST) libraries followed by capillary sequencing (Marra et al., 1999; Souza et al., 2000). Both techniques involve a high cost and have limited production, offering a glimpse into the true complexity of cell type-specific splicing and transcription. From the analysis of these data, the basis for the programs used for RNA-seq data was obtained (Wang et al., 2008; Guttman et al., 2009; Garber et al., 2011).

RNA-Seq has become the method of choice for studying gene expression. Compared to other methods, such as those based on DNA microarray, RNA-Seq offers a high level of data reproducibility through lanes and flow-cells, which reduces the number of technical replicates for the experiments, while offering less background noise and greater dynamic range for detection. Even more relevant, the RNA-Seq directly reveals the identity of the sequence, a factor essential for the analysis of unknown genes and newly transcribed isoforms (van Dijk et al., 2014; Han et al., 2015; Hrdlickova et al., 2016).

In general, the RNA-Seq technology is used in the analysis of differential expression involving some specific conditions, which can be summarized in five steps. First, the RNA samples are fragmented into cDNA and sequenced on a high-performance platform. After that, the small sequences generated are mapped to a genome or transcriptome. In the third step, the expression levels for each gene or isoform are estimated. Next, the mapped data is normalized and differentially expressed genes (DEGs) are identified. Finally, the relevance of the data produced is assessed in a biological context (Wang et al., 2009; Oshlack et al., 2010; Zhang et al., 2014; Li et al., 2015; Costa-Silva et al., 2017).

3.2.3. MATERIALS AND METHODS

3.2.3.1. Genotypes, environment, isolates and disease evaluation

Two genotypes of *C. annuum* were used: 'Yolo Wonder', susceptible (S) to *P. capsici*, and Criollo de Morelos 334, partially resistant (R). Two isolates different in their level of aggressiveness in peppers were used: the non-adapted to infect pepper isolate Pc273 (N), and the adapted to infect pepper isolate Pc107 (A), resulting in four biological interactions: RxA, SxA, RxN and SxN. This step was carried out at the *Génétique et Amélioration des Fruits et Légumes* (GAFL) unit at INRAE – Avignon/FR.

The inoculations were performed as described in Lefebvre and Palloix (1996), in which seven week old apexes were removed from the pepper plants with a razor blade before a 4-mm diameter and a mycelium was plugged, previously grown on a V8 media for seven days at 22°C, was put on the fresh section of the cut stem. A $4cm^2$ piece of aluminum foil was used to cover the mycelium for one day and ensure high humidity at the inoculation site. The inoculated plants were transferred to a 12/12 hour light/dark cycle growth chamber at 24/22°C for the assay. The disease symptoms are illustrated in Figure 1.

Figure 1. Symptoms of disease caused by *Phytophthora capsici* including: leaf blight and lesions (A), fruit rot (B), stem rot (C) and root rot (D). Majid et al., 2016.

The experiment was performed in triplicate during the period, with each triplet inoculated with an independent inoculum in order to produce three true biological

replicates. The assessment of disease progress was made at two different times: 24 and 72hpi (hours post inoculation).

3.2.3.2. RNA samples and mapping of reads

Twenty-four RNA samples were extracted from the inoculated plants for the four interactions (RxA, SxA, RxN and SxN), in 24 and 72hpi. Each sample consisted of six stem fragments collected in bulk, with each fragment having a 5 mm long cut under the stem necrosis. The samples were ground in liquid nitrogen with a mortar and cold pestle. The total RNA was extracted using the QIAGEN RNeasy Plant Mini kit. Library preparation and Illumina sequencing were done at the Institute of Plant Sciences Paris-Saclay (IPS2, France).

All stages of the experiment, from growth conditions to bioinformatic analyses, were managed in the CATdb database (Gagnot et al., 2008), according to MINSEQE's minimum information on a high-throughput sequencing experiment. The RNA-seq project was submitted to the international repository NCBI GEO.

3.2.3.3. RNA-seq analysis

In order to perform RNA-seq analyses, biological models must first be designed. Our analyzed data set consisted of four biological interactions (RxA, SxA, RxN and SxN) X 2 times (24 and 72hpi) X 3 replicates = 24 samples. Thus, such analysis requires factors to be analyzed two by two. Our primary data set was divided in half, and 12 samples were analyzed at a time. For this, three biological models were defined:

- **Biological Model 1 – Interaction Host x Isolate for each evaluation time**
- **$Log(Y_{ijkl}) = \mu(Y) + H_i + I_j + Rk + (HI)_{ij} + Epsilon_{ijkl}$**
    - $Log(Y_{ijkl})$: log-number of reads for l, in host i, isolate j and replicate k
    - $\mu(Y)$: Average number of reads
    - H_i: Host i
    - I_j: Isolate j
    - R_k: Replicate k
    - $(HI)_{ij}$: Pairwise interaction between host i and isolat j
    - $Epsilon_{ijkl}$: residual for l

This biological model was designed in order to measure the effect of the host, the effect to the isolate and the effect of the interaction between host and

isolate on the number of reads of each gene. As we tested this model independently at two times after inoculation, it was necessary to divide the whole dataset into two different projects: project capT1, which analyzes these effects at Time1, at 24hpi, and project capT2, which evaluates the effects at Time 2, at 72hpi.

- **Biological Model 2 – Interaction Host x Time for each isolate**
- **$Log(Y_{ijkl}) = \mu(Y) + H_i + T_j + R_k + (HT)_{ij} + Epsilon_{ijkl}$**
    - $Log(Y_{ijkl})$: log-number of reads for l, in host i, time j and replicate k
    - $\mu(Y)$: Average number of reads
    - H_i: Host i
    - T_j: Time i
    - R_k: Replicate k
    - $(HT)_{ij}$: Pairwise interaction between host i and time j
    - $Epsilon_{ijkl}$: residual for l

This biological model was designed in order to compare the interaction between host and time in each isolate, and was divided into two different projects: project capA, which analyzes the host-time interaction when the genotypes are infected for the adapted isolate A, and the project capN, which evaluates the interaction when the genotypes are infected for the non-adapted isolate N.

- **Biological Model 3 – Interaction Times x Isolate in each host**
- **$Log(Y_{ijkl}) = \mu(Y) + I_i + T_j + R_k + (IT)_{ij} + Epsilon_{ijkl}$**
    - $Log(Y_{ijkl})$: log-number of reads for l, in isolate i, time j and replicate k
    - $\mu(Y)$: Average number of reads
    - I_i: Isolate i
    - T_j: Time j
    - R_k: Replicate k
    - $(IT)_{ij}$: Pairwise interaction between isolate i and time j
    - $Epsilon_{ijkl}$: residual for 1

This biological model was designed in order to compare the interaction between times of evaluation and isolate in each host, and was divided into two different projects: project capR, which analyzes the isolate-time interaction in the resistant accession (R), and the project capS, which evaluates the interaction in susceptible accession (S).

RNA-seq analysis was performed using DiCoExpress (Lambert et al., 2020), a tool developed for R software (R Core Team, 2019) described in Box 1. The ‘counts per million’ (CPM) cutoff strategy was used to remove low-expression genes. For the evaluated data set, the use of CPM = 1 was determined with FDR = 0.001 because the number of filtered genes was the most reliable after filtering of all biological models. A gene was declared differentially expressed when its p-value was less than 0.05.

Box 1. The DiCoExpress tool (Lambert et al., 2020)

DiCoExpress is a tool implemented in R with a set of directories where data, scripts and results are organized. During the first step the quality of raw data before and after the normalization methods is checked, which consists of filtering and normalizing the raw data, removing the genes with no expression and with low counts. The rest of the genes are kept for posterior analyses.

The second step is the differential analysis in which differentially expressed genes are obtained after the design of the contrasts, which are all estimated, and the likelihood ratio test (LRT) is performed for each contrast considered. The probabilities of significance (p-values) generated by the LRT are adjusted by the Benjamini-Hochberg procedure (BH). The "Alpha_DiffAnalysis" is the cutoff used on FDR values to decide if a gene is differentially expressed or not.

The third step is the Venn intersection union, which compares several lists of DEGs and plots Venn diagram between them, according to the contrasts previously described. This comparison allows one to perform the fourth step, the co-expression analysis. Following the recommendations accompanying the DiCoExpress tool package, a filter function is used to remove the genes with low mean normalized counts. The remaining genes are analyzed and mixture models are estimated in a first loop to define a second one. The best model is the one that minimizes the Integrated Completed Likelihood (ICL).

The last step is an Enrichment analysis, which use the hypergeometric test to characterize a functional genes list. This function determines the annotation terms underrepresented, and those overrepresented in the gene list when compared to a reference list. All of these steps are detailed described in Lambert et al. (2020).

Seven contrasts were designed for each of the three biological models (Table 1), automatically using comparisons between two factors, where the log of the gene expression is modelled by all the factors describing the experiment.

3.2.3.4. Gene validation

RNA samples were extracted from the inoculated plants for the four biological interactions at 24hpi. Each biological interaction was performed in triplicate, producing a total of 12 samples. Each sample consisted of three pooled stem fragments separated in three biological replicates for the genotypes, with each fragment 5-mm long and cut under the stem necrosis. Samples were frozen in liquid-nitrogen.

To confirm changes in the expression patterns observed, primers of three selected genes were designed from the sequenced samples using the Primer 3 (Untergasser et al., 2012). The genes were selected according to the expression pattern, comparing the genotypes and isolates as a function of time. The RNA was treated with DNAse and reverse transcribed to cDNA. Real time qPCR were performed using SYBRGreen. The steps developed to reach the objectives are summarized in Figure 2.

Table 1. Contrasts designed for each biological model in an experiment for RNA-seq analysis of *Capsicum* - *Phytophthora capsici* interaction.

Contrasts **Biological model 1**	**Description**
[R-S]	Pepper DEGs[1] between the two hosts independent of the isolate
[A_R-A_S]	Pepper DEGs between the two hosts when infected by the adapted isolate
[N_R-N_S]	Pepper DEGs between the two hosts when infected by the non-adapted isolate
[A-N]	Pepper DEGs whatever the host genotype when we contrast the isolate
[R_A-R_N]	Pepper DEGs in the resistant host when we contrast the isolate
[S_A-S_N]	Pepper DEGs in the susceptible host when we contrast the isolate
[R_A-R_N]-[S_A-S_N]	Difference in the pepper DEGs between the two hosts when infected by the isolates
Contrasts **Biological model 2**	**Description**
[T1-T2]	Pepper DEGs between the two times independent of the genotype
[R_T1-R_T2]	Pepper DEGs between the two times in the resistant host
[S_T1-S_T2]	Pepper DEGs between the two times in the susceptible host
[R-S]	Pepper DEGs whatever the host genotype when we contrast the time
[T1_R-T1_S]	Pepper DEGs in 24hpi when we contrast the host genotype
[T2_R-T2_S]	Pepper DEGs in 72hpi when we contrast the host genotype
[T1_R-T1_S]-[T2_R-T2_S]	Difference in the pepper DEGs between the two hosts with both times evaluated

[1] DEGs – Differentially Expressed Genes

Table 1 – Cont.

Contrasts **Biological model 3**	**Description**
[T1-T2]	Pepper DEGs between the two times independent of the isolate
[A_T1-A_T2]	Pepper DEGs between the two times in the adapted isolate
[N_T1-N_T2]	Pepper DEGs between the two times in the non-adapted isolate
[A-N]	Pepper DEGs whatever the isolate when we contrast the time
[T1_A-T1_N]	Pepper DEGs in 24hpi when we contrast the isolate
[T2_A-T2_N]	Pepper DEGs in 72hpi when we contrast the isolate
[T1_A-T1_N]-[T2_A-T2_N]	Difference in the pepper DEGs between the two isolates with both times evaluated

[1] DEGs – Differentially Expressed Genes

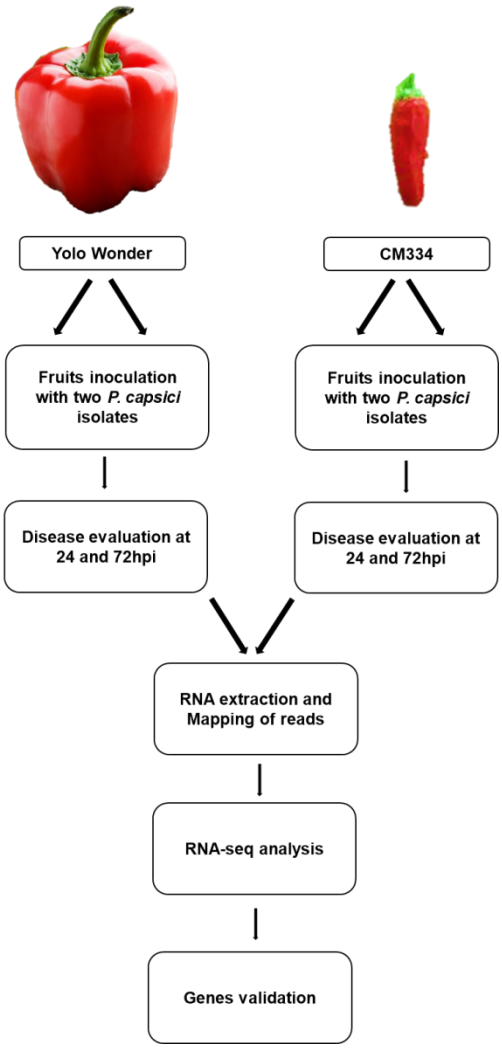

Figure 2. Organization chart of the methodology developed to analyze the transcriptomic data of *Capsicum annuum* inoculated with two different isolates of *Phytophthora capsici* and evaluated at two timepoints.

3.2.4. RESULTS

3.2.4.1. Quality control

Approximatelly 50% of the expressed *Capsicum annuum* L. genome was analyzed in this work. A total of 35,884 reads was mapped to predicted genes in *C. annuum* by a library for the 24 samples from the four biological interactions. Genes without any or low counts were excluded during the normalization step and only reliable genes were kept. The Trimmed Mean of M-values (TMM) and the

Counts per Million (CPM) normalized values were homogeneous in each evaluated project. The estimate of transcriptional activity of genes under *P. capsisi* infection revealed a high proportion of associated filtered reads (Table 2).

Table 2. Summary of reads aligned to the reference *C. annuum* genome for 24 libraries in pepper genotypes inoculated with *P. capsici* adapted and non-adapted isolates at 24 and 72 hpi.

Condition	Nb reads	Nb of genes with at least 1 read
T1 x A x R_1	24.142.105	23.671
T1 x A x R_2	23.264.920	23.275
T1 x A x R_3	32.162.839	23.500
T1 x A x S_1	24.854.479	23.047
T1 x A x S_2	23.805.129	22.924
T1 x A x S_3	23.560.658	22.661
T1 x N x R_1	22.983.077	23.229
T1 x N x R_2	27.489.884	23.311
T1 x N x R_3	24.635.257	22.782
T1 x N x S_1	27.301.533	22.913
T1 x N x S_2	23.249.425	22.170
T1 x N x S_3	25.491.542	22.365
T2 x A x R_1	25.899.150	23.928
T2 x A x R_2	44.647.346	23.701
T2 x A x R_3	19.639.962	23.057
T2 x A x S_1	27.056.684	23.080
T2 x A x S_2	30.972.996	23.083
T2 x A x S_3	23.786.886	22.432
T2 x N x R_1	27.949.264	23.757
T2 x N x R _2	25.086.660	23.518
T2 x N x R_3	20.954.047	22.822
T2 x N x S_1	34.839.324	23.649
T2 x N x S _2	26.035.189	22.886
T2 x N x S _3	24.630.715	22.881
Min	19.639.962	22.170
Max	44.647.346	23.928
Mean	26.434.961	23.110
STD	5.142.276	462

T1 – 24hpi; T2 – 72hpi; A – Adapted isolate; N – Non-adapted isolate; R – Resistant genotype; S – Susceptible genotype; Min – Minimum; Max – Maximum; STD- Standard deviation

Around 50% of the expressed pepper genes were analyzed in each project (Table 3), with 17,793 genes analyzed in project capT1, 18,346 in capT2, 18,046

in capA, 18,202 in capN, 14,123 in capR and 17,239 in capS. The high quality of the data insured consistency between the high number of reads and the following steps. The percentage of analyzed genes ranged from 39.35% to 51.12% among the six projects. The p-value (<0.05) was checked in all six projects in order to verify that these genes were differentially expressed.

Table 3. Number of discarded and analyzed genes after the normalization method on the raw counts of *Capsicum annuum* var. *annuum.*

Project	N° of discarded genes	N° of analyzed genes	% of analyzed genes
capT1[1]	18,091	17,793	49.58%
capT2[2]	17,538	18,346	51.12%
capA[3]	17,838	18,046	50.28%
capN[4]	17,682	18,202	50.72%
capR[5]	21,761	14,123	39.35%
capS[6]	18,645	17,239	48.04%

[1] Comparing the interaction between host and isolate at 24hpi; [2] Comparing the interaction between host and isolate at 72hpi; [3] Comparing the interaction between host and time in adapted isolate; [4] Comparing the interaction between host and time in non-adapted isolate; [5] Comparing the interaction between time and isolate in resistante host; [6] Comparing the interaction between time and isolate in susceptible host.

3.2.4.2. Biological Model 1 – Interaction Host x Isolate for each evaluated time

3.2.4.2.1. Differential Analysis

To identify DEGs, paired comparisons were made independently between host and isolate. In Model 1, evaluating this interaction at 24hpi and 72hpi, 4,740 and 7,094 DEGs were obtained, respectively. The DEGs were separated into up- and downregulated groups for each contrast (Figure 3). In capT1, this number ranged from 8,003 up to 5,291 downregulated genes. In capT2, there were 11,066 upregulated genes and 9,500 downregulated genes.

With respect to the capT1 project (Figure 4a), the larger number of DEGs was identified when we contrasted the host genotypes whatever the isolate

(Contrast A) with 1,351 DEGs. When we compare the two hosts inoculated with each isolate individually, it's possible to observe that the amount of DEGs is almost four times greater for interaction with the adapted isolate (Contrast B) when compared with the interaction with non-adapted isolate (Contrast C). Contrasting the hosts and the isolates together (Contrast D), only one gene was diferrentially expressed.

Analyzing this interaction at 72hpi (Figure 4b), contrasting the hosts whatever the isolate (contrast A), 947 genes were diferrentially expressed. The number of DEGs between the two hosts infected by the adapted isolate was almost 3 times greater than at 24hpi, with 678 DEGs (B), while the number of genes when the hosts were infected by the non-adapted isolate decreased by half (n=33 DEGs) (C). Contrasting the hosts and the isolates together (Contrast D), eight genes were differentially expressed.

Checking the list of DEGs obtained for each project, genes with important functions against disease were expressed at 24hpi and 72hpi in the peppers. One gene similar to *RPP13 disease resistance protein* was highlighted (Figures 5a), presenting a higher average expression in the resistant genotype infected by the adapted isolate when compared with that infected by the non-adapted isolate, showing the same expression level pattern in the susceptible genotype with a higher mean than genotypes infected by the adapted isolate.

Two genes similar to *XA21 Receptor kinase-like protein* were differentially expressed 24hpi in the peppers (Figures 5b and 5c), the "CA.PGAv.1.6.scaffold787.6" gene was overrepresented on the resistant genotypes, while the "CA.PGAv.1.6.scaffold149.47" showed the opposite, being more expressed at 24hpi on the susceptible genotypes. Genes similar to *TLP Thaumatin-like protein* (Figure 5d) and *RLK1 G-type lectin S-receptor-like serine/threonine-protein kinase* (Figure 5e) were also expressed.

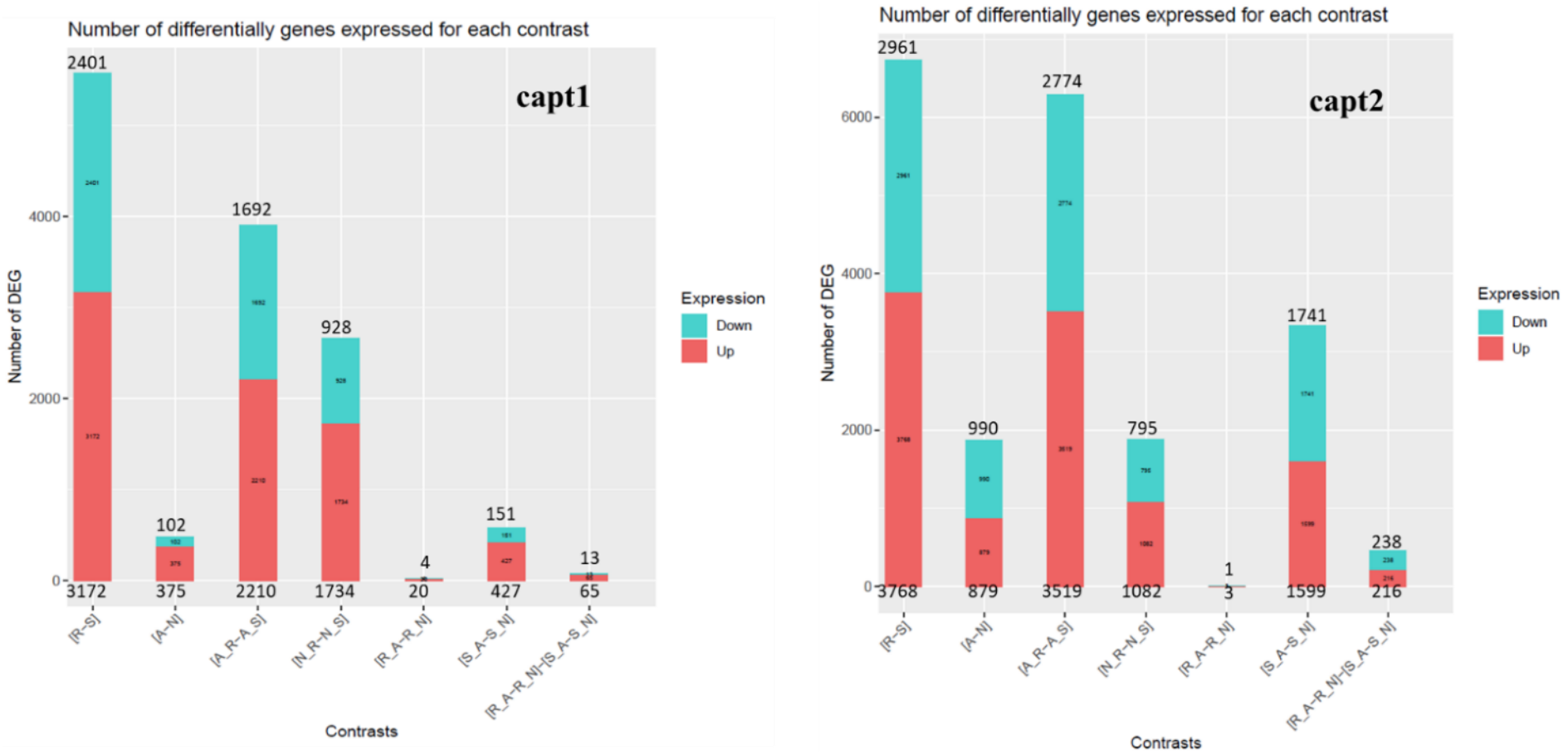

Figure 3. Up- and downregulated genes expressed between the seven contrasts in the projects capT1 (Interaction between Host and Isolate at 24hpi) and capT2 (Interaction between Host and Isolate at 72hpi) for the *Capsicum annuum* genotypes infected by *Phytophthora capsici* isolates. The Y-axis shows the number of up- and downregulated DEGs. On the X-axis, the seven contrasts analyzed in both projects are described.

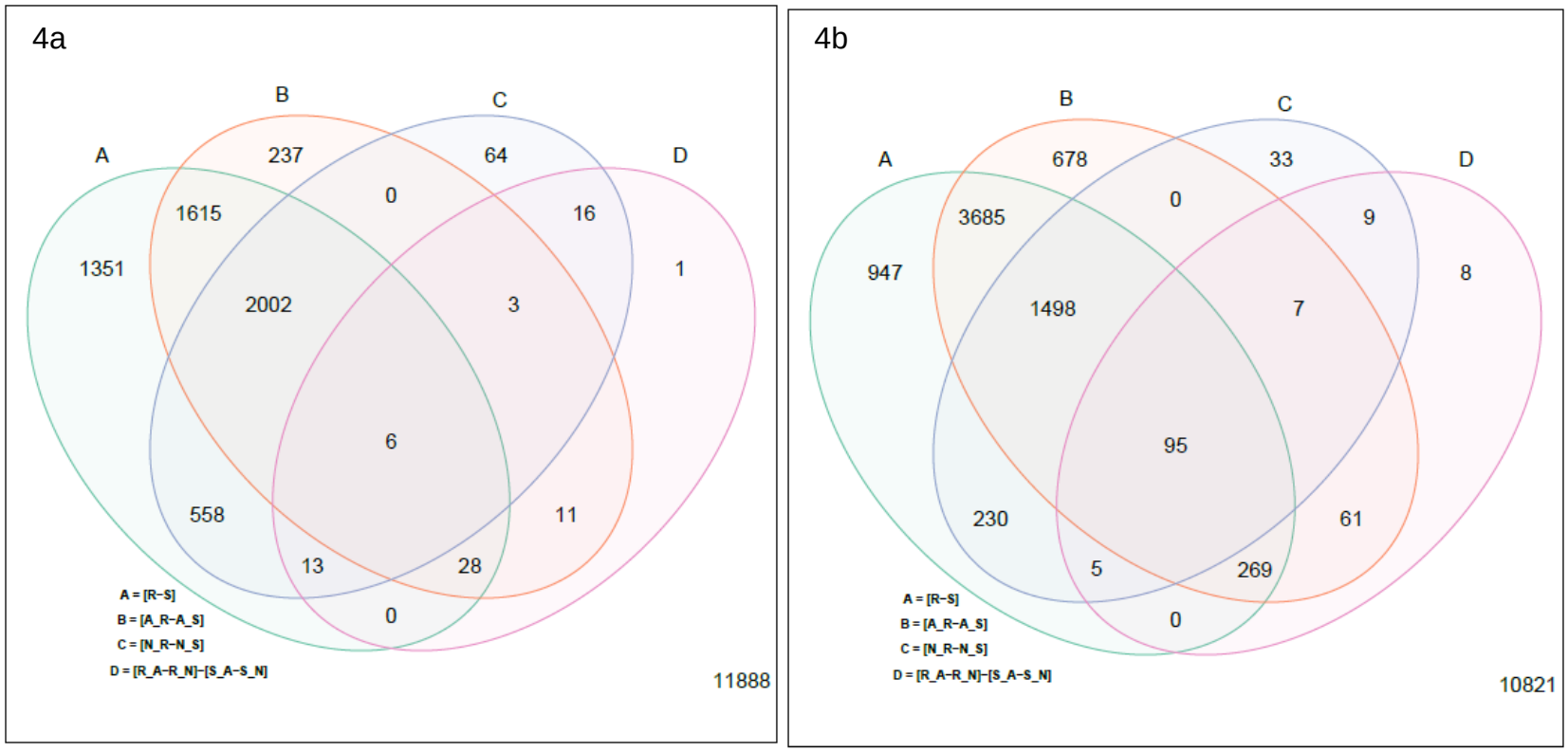

Figure 4. Venn diagram illustrating the number of DEGs in *Capsicum annuum* infected by *Phytophthora capsici* isolates at 24hpi (Figure 4a) and 72hpi (Figure 4b) in four different contrasts between host and isolate. **A:** Pepper DEGs between the two hosts independent of the isolate; **B:** Pepper DEGs between the two hosts when infected by the adapted isolate; **C:** Pepper DEGs between the two hosts when infected by the non-adapted isolate; **D:** Difference in the pepper DEGs between the two hosts when infected by the isolates.

Relevant genes were also expressed at 72hpi. The gene CA.PGAv.1.6.scaffold115528 had a similar pattern in its expression level for the susceptible genotypes, with a higher average when compared with Time 1 (Figure 6a). For the resistant genotypes, the expression level decreased in Time 2 no matter which isolate was used. The standard deviation remained low in both analyses, which indicates data uniformity.

The gene CA.PGAv.1.6.scaffold149.47 was also expressed in Time 2, showing a different expression level when compared with Time 1 (Figure 6b). In this case, it's possible to see an increase in the expression level at 72hpi when we have the resistant genotype infected by both isolates and a decrease when the susceptible hosts were inoculated with the adapted and non-adapted isolate.

At Time 2 genes similar to *RPP13 disease resistance protein RPP13*, *XA21 receptor kinase-like protein* and *At4g27190 disease resistance protein* were also expressed. They presented an important expression level between the 4 biological conditions, varying according to the host x isolate interaction (Figures 6c, 6d and 6e).

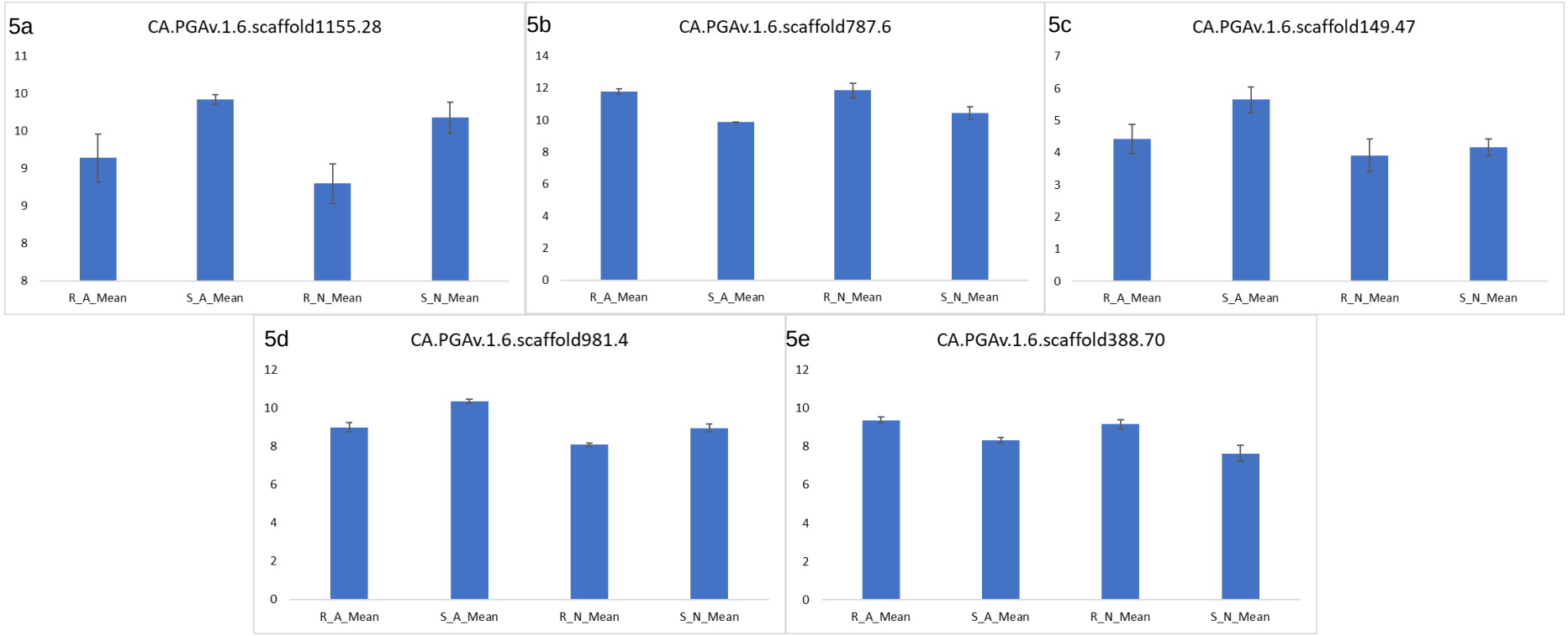

Figure 5. Levels of gene expression in *Capsicum annuum* when infected by adapted and non-adapted isolates of *Phytophthora capsici* at 24hpi with the four biological conditions: **R_A:** resistant host with adapted isolate; **S_A:** susceptible host with adapted isolate; **R_N:** resistant host with non-adapted isolate; **S_N:** susceptible host with non-adapted isolate.

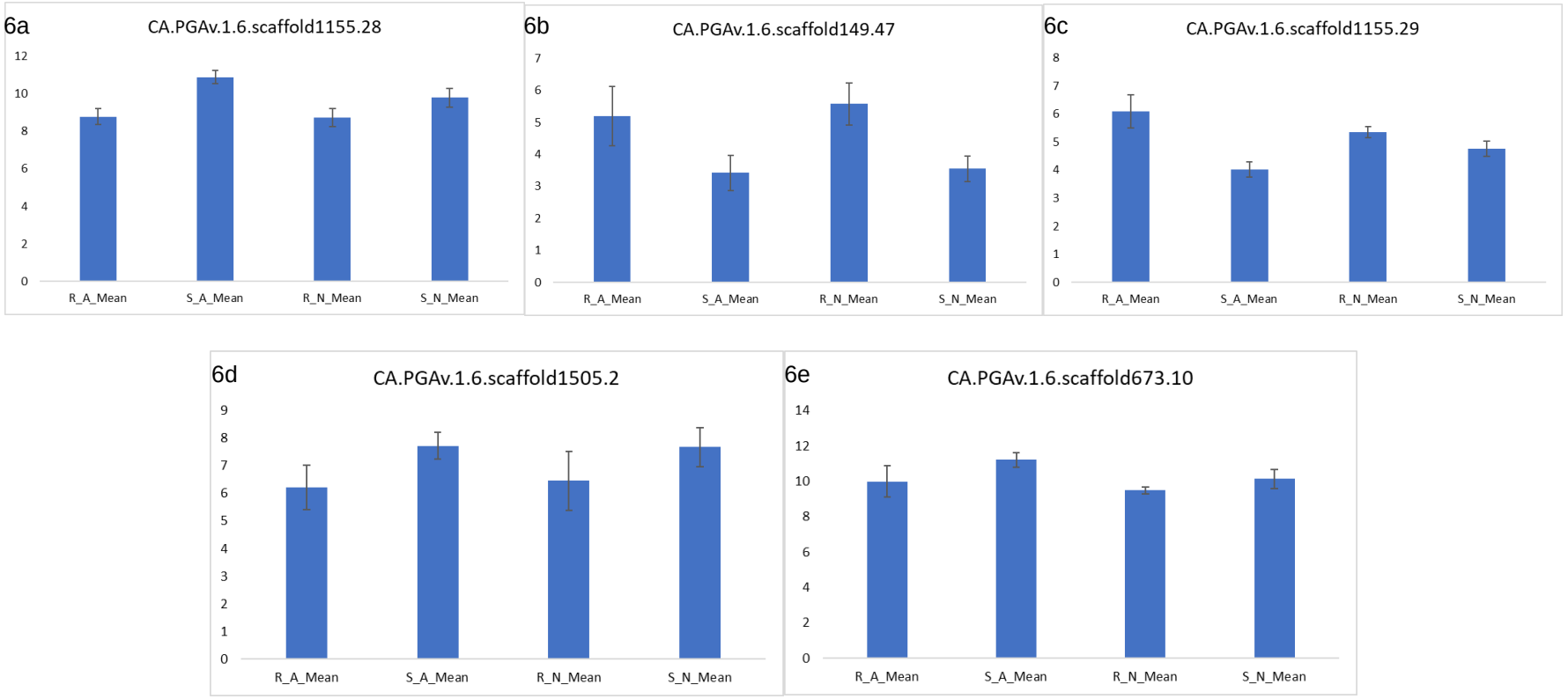

Figure 6. Levels of gene expression in *Capsicum annuum* when infected by adapted and non-adapted isolates of *Phytophthora capsici* at 72hpi with the four biological conditions: **R_A:** resistant host with adapted isolate; **S_A:** susceptible host with adapted isolate; **R_N:** resistant host with non-adapted isolate; **S_N:** susceptible host with non-adapted isolate.

3.2.4.2.2. Co-expression

The performance of a co-expression analysis makes it possible to cluster genes according to their average expression profiles over all samples based on a Venn diagram.

The ICL curve has a clear minimum which is a marker of a good quality clustering analysis. While for capT1 ten clusters of co-expressed genes were found (Figure 7a), for capT2 16 groups were found (Figure 7b).

For capT1, groups 1, 5, 8 and 10 (663, 257, 617 and 168 genes, respectively) contain genes with low expression in resistant and susceptible genotypes inoculated with adapted isolate and with higher expression levels in resistant genotypes infected by the non-adapted isolate.

The groups 3, 4, 7 and 9 (643, 112, 613 and 270, respectively) clustered genes with the opposite behavior of the previously mentioned groups. They contain genes with higher expression in resistant and susceptible genotypes infected by adapted isolates and low-expression genes in the other two biological interactions, most notably in group 4, which contains genes with lowest expression in resistant and susceptible genotypes inoculated by the non-adapted isolate.

Group 2 (238 genes) had a greater difference in terms of interaction between resistant genotypes with the adapted and non-adapted isolate, while the susceptible genotypes presented a small difference in expression between the isolate's infection. Group 6 (239 genes) showed a very similar expression between the interactions RxA, RxN and SxA, and the lowest expression in SxN.

For capT2, groups 1, 2, 5, 6, 11 and 12 (227, 390, 667, 123, 527 and 455 genes, respectively) contain more highly expressed genes in both genotypes contaminated by the adapted isolate than when contaminated by the non-adapted. Groups 4, 7, 8, 10, 13, 14 and 16 (532, 114, 322, 379, 349, 384 and 833 genes, respectively) summarized the genes overexpressed in resistant genotypes inoculated by the non-adapted isolate.

On the other hand, groups 9 and 15 (582 and 281 genes) contain those genes with the opposite expression level to the interaction RxN. Group 3 (143 genes) contains genes expressed homogeneously in the 4 biological interactions.

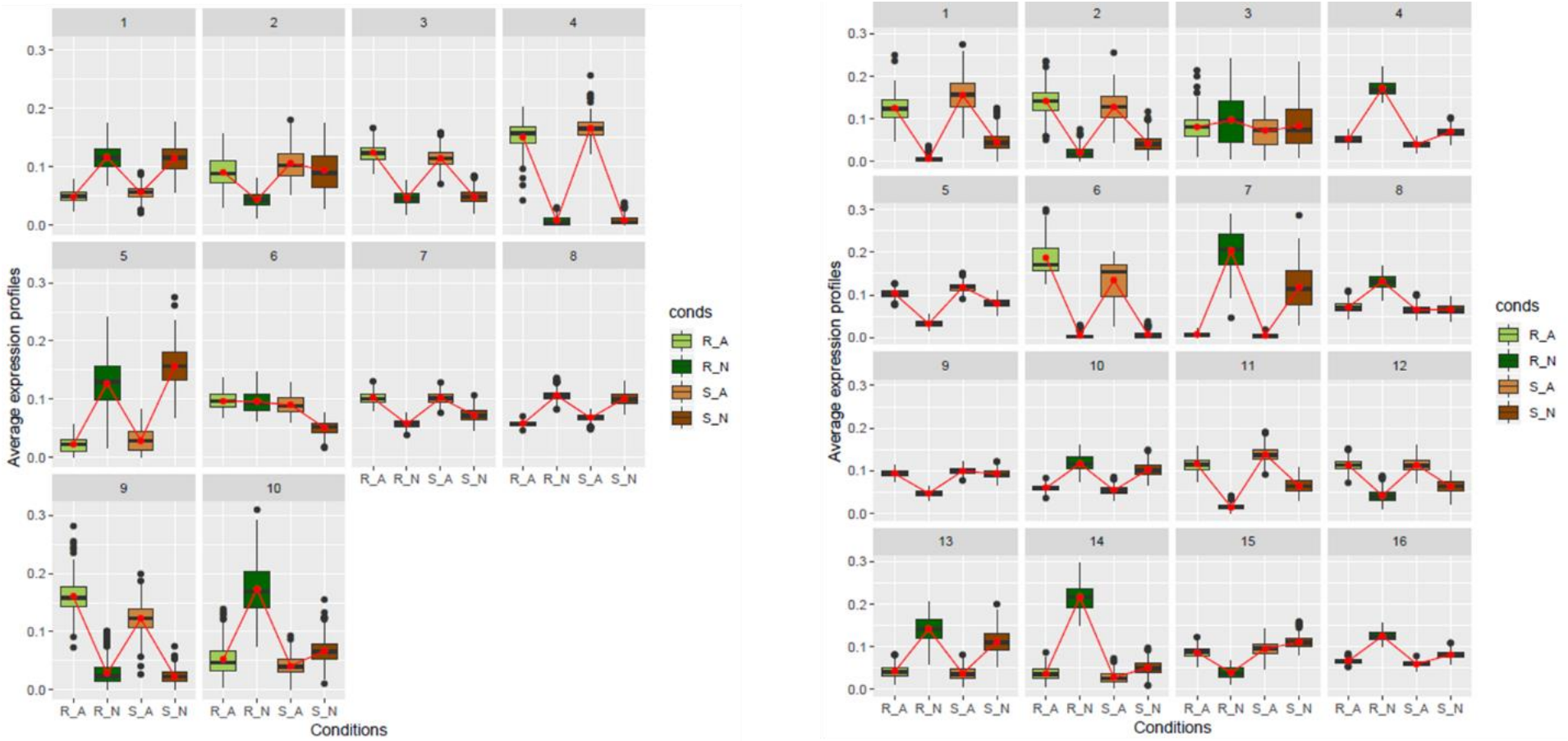

Figure 7. Co-expression of the differentially expressed genes in *Capsicum annuum* infected by two different isolates of *Phytophthora capsici* at two timepoints: 24hpi (Figure 7a) and 72hpi (Figure 7b). **R_A:** resistant host with adapted isolate; **R_N:** resistant host with non-adapted isolate; **S_A:** susceptible host with adapted isolate; **S_N:** susceptible host with non-adapted isolate.

3.2.4.3. Biological model 2 - Interaction Host x Time for each isolate

3.2.4.3.1. Differential Analysis

In this model, evaluating the interaction between plant and post-inoculation times when using adapted and non-adapted isolates, 7,407 and 5,631 DEGs were obtained, respectively. The up- and downregulated values were estimated for each contrast (Figure 8). The number of these genes varied according to the contrast and the isolate used. With isolate A, the number ranged from 926 to 7,547 genes among the seven contrasts. With isolate N, this variation was between 296 and 6,494 differentially expressed genes.

The number of upregulated genes using isolate A was greater than the number of downregulated genes, corresponding to 12,947 and 11,096, respectively. On the other hand, the number of downregulated genes was greater than the number of upregulated genes in four contrasts when we used isolate N, with 10,889 down- and 9,860 upregulated genes.

For the capA project (Figure 9a), when we contrast the two host genotypes whatever the time post inoculation, we see a greater number of genes (n=1,099) (Contrast A). Analysing Contrasts B and C, with comparisons between the hosts in Time 1 and Time 2 (24 and 72hpi, respectively), it is possible to see the higher number of genes at 72hpi (Contrast C). No genes correlated between both periods in this case. Contrasting the hosts and evaluation times together (Contrast D), 34 genes was differentially expressed.

The number of genes between the two hosts, whatever the time post inoculation, in project capN (Figure 9b), is lower than the previous project, with a total of 850 DEGs (Contrast A). The difference in genes expressed in Times 1 and 2 (Contrasts B and C) is small, but follows the standard of project capA, with fewer genes expressed at 24hpi than 72hpi. Contrasting both the hosts and the two evaluation times together (Contrast D), 32 genes were differentially expressed.

The difference between the amounts of expressed genes under the infection conditions could be due to the pathogen’s attack mechanism and the plant’s recognition of it. The adapted isolate is more successful during the infection process, while the plant has a defense machinery that tries to stop it as soon as its can, causing the higher number of genes expressed in this situation.

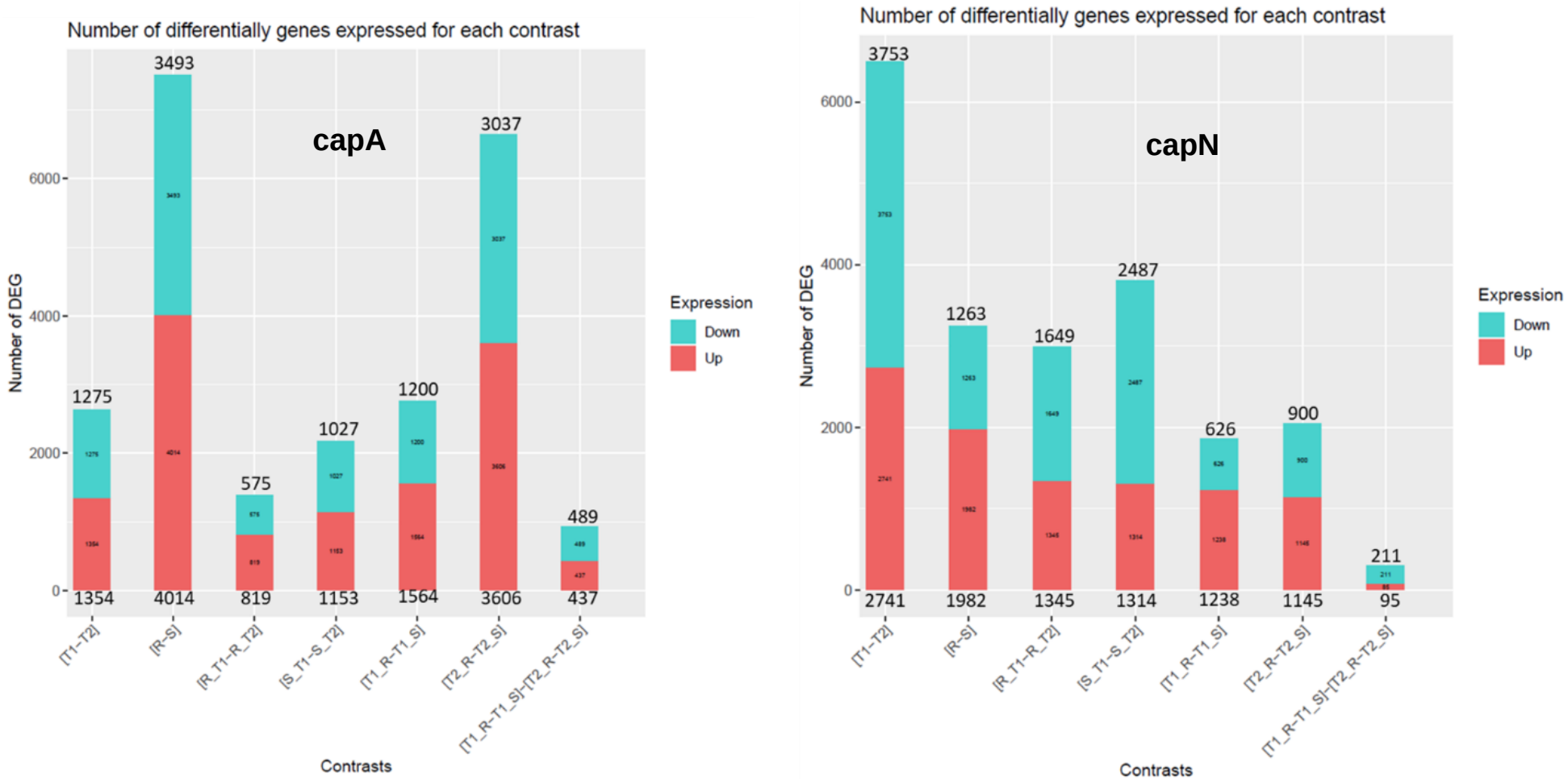

Figure 8. Up- and downregulated genes expressed between the seven contrasts in the projects capA (Interaction between host and time of evaluation infected by the adapted isolate) and capN (Interaction between host and time of evaluation infected by the non-adapted isolate) for the *Capsicum annuum* genotypes. The Y-axis shows the number of up- and downregulated DEGs. On the X-axis, the seven contrasts analyzed in both projects are described.

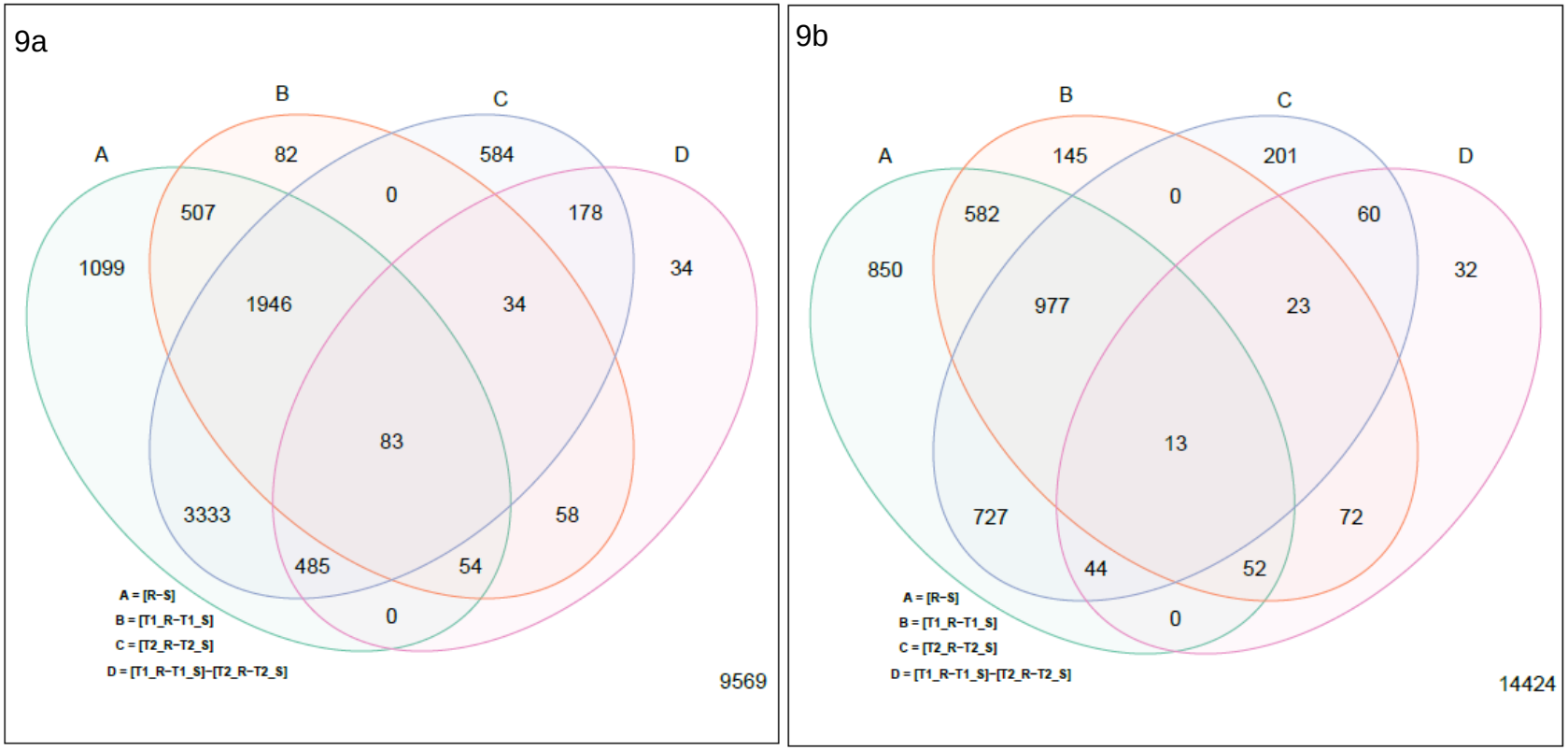

Figure 9. Venn diagram illustrating the number of DEGs in *Capsicum annuum* in the interaction with time of evaluation infected by the adapted (Figure 9a) and the non-adapted isolates of *Phytophthora capsici* (Figure 9b) in four different contrasts between host and time of evaluation. **A:** Pepper DEGs between the two hosts independently of the evaluaion time; **B:** Pepper DEGs between the two hosts evaluated at 24hpi; **C:** Pepper DEGs between the two hosts evaluated at 72hpi; **D:** Difference in the pepper DEGs between the two hosts evaluated at 24 and 72hpi.

With respect to project capA, five genes were highlighted (Figure 10). In the gene “CA.PGAv.1.6.scaffold411.21” (Figure 10a), the difference in the expression between the contrasts is visible, with more expression in this gene at Time 1 (24hpi) in both genotypes than in Time 2 (72hpi).

Figure 10b shows the expression of the gene similar to *RPP13L3 Putative disease resistance*; it is more highly expressed in susceptible genotypes than in resistant ones, whatever the time post inoculation. The opposite is true for the gene shown in Figure 10d, a gene similar to *GH3.5 Jasmonic acid-amido synthetase JAR1*, which is more highly expressed in resistant genotypes than in susceptible ones, at both evaluation times.

The gene similar to *RLK7 Receptor-like protein kinase* (Figure 10c) has an interesting pattern of expression between genes, being over expressed in resistant genotypes, at both times, and more expressed at Time 1 than at Time 2. The gene “CA.PGAv.1.6.scaffold1524.13” (Figure 10e) had a high expression in the resistant genotype at both evaluation times and nearly nonexistent expression in the susceptible hosts. It could be a relevant gene for resistance against oomycete infection, especially because it is a gene from the *RPP13* family, a gene known for resistance against pathogens in peppers.

A few genes expressed during the infection caused by the non-adapted isolate are exemplified in Figure 9. The gene “CA.PGAv.1.6.scaffold1155.28” (Figure 11d), similar to *RPP13 protein,* was expressed in the four contrasts, with higher expression in susceptible genotypes at both times of evaluation, as expressed in project capA. Gene “CA.PGAv.1.6.scaffold322.58” (Figure 11a) followed the same pattern and was more highly expressed in susceptible hosts than in the resistant ones.

The gene “CA.PGAv.1.6.scaffold818.8” (Figure 11b), as in the previous project, showed higher expression in the resistant genotypes at both evaluation times compared to the susceptible genotypes. This is another example of a relevant gene for resistance to oomycetes that was expressed in both evaluated projects.

The resistant and susceptile genotypes showed higher expression of the gene “CA.PGAv.1.6.scaffold1631.6” (Figure 11c) at 24hpi than at 72hpi. The opposite, however, happened for the gene “CA.PGAv.1.6.scaffold608.26” (Figure 11e), where at 24hpi there was less expression for the gene than at 72hpi, and the gene showed low expression at Time 1 for susceptible genotypes when compared with the other three contrasts.

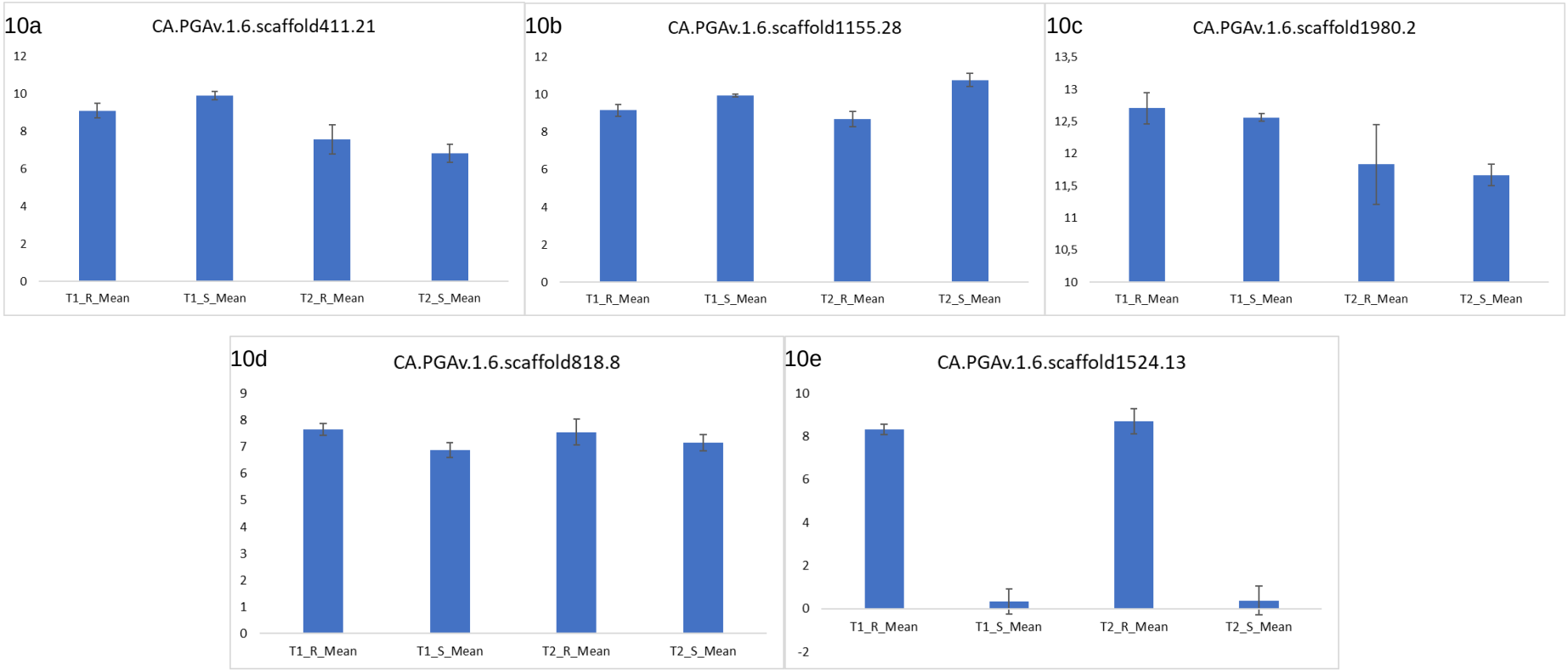

Figure 10. Levels of gene expression in *Capsicum annuum* in the interaction with evaluation the time of when infected by the adapted isolate of *Phytophthora capsici* with the 4 biological conditions: **T1_R:** 24hpi in resistant host; **T1_S:** 24hpi in susceptible host; **T2_R:** 72hpi with resistant host; **T2_S:** 72hpi in susceptible host.

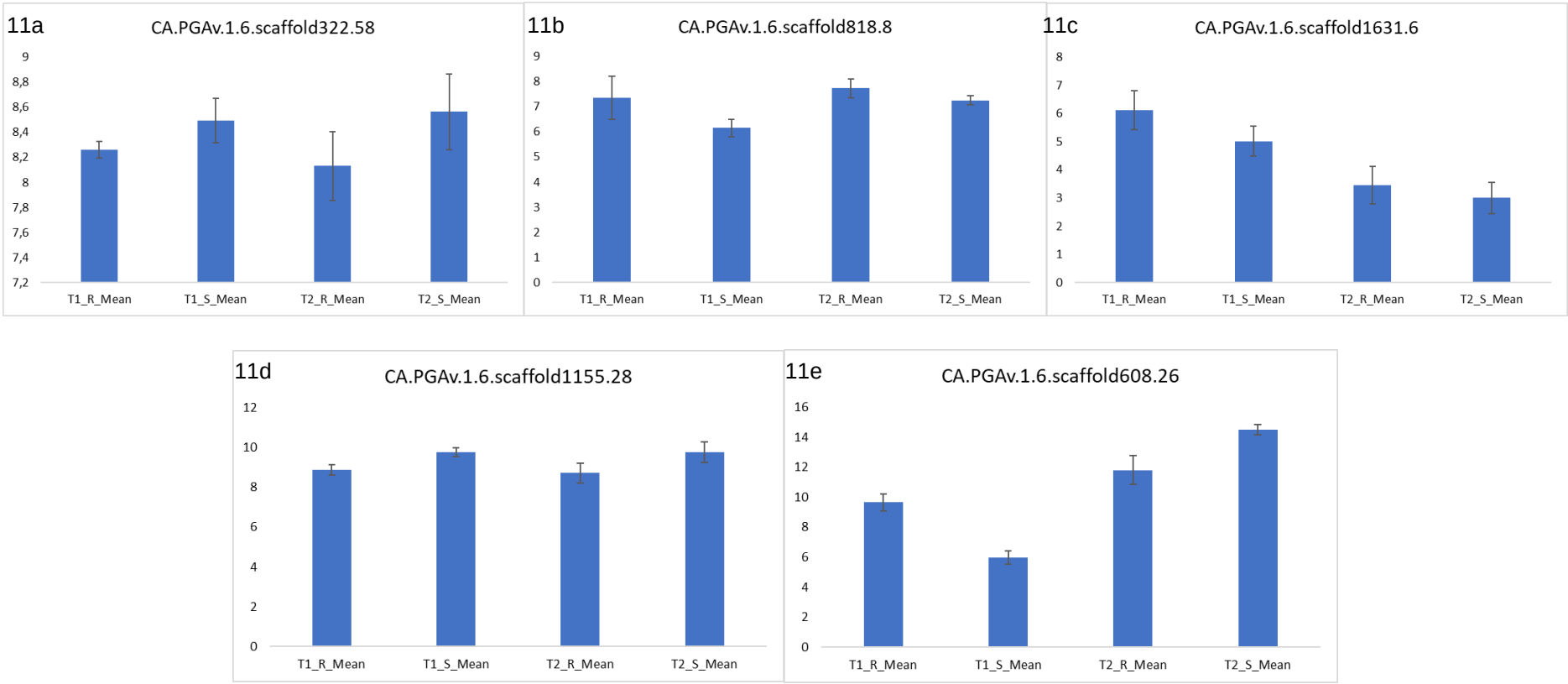

Figure 11. Levels of gene expression in *Capsicum annuum* in the interaction with the time of evaluation when infected by the non-adapted isolate of *Phytophthora capsici* with the 4 biological conditions: **T1_R:** 24hpi in resistant host; **T1_S:** 24hpi in susceptible host; **T2_R:** 72hpi with resistant host; **T2_S:** 72hpi in susceptible host.

3.2.4.3.2. Co-expression

According to the ICL curve, 17 clusters of co-expressed genes were found for capA (Figure 12a). Groups 1, 7 and 9 consist of the genes with high levels of expression for the susceptible genotypes evaluated at 24 and 72hpi. Each group contained 196, 527 and 232 genes, respectively.

Clusters of genes with high expression averages for the resistant genotypes assessed at 24 and 7hpi were observed in groups 3, 4, 10 and 12. The number of genes in each group was 180, 113, 242 and 554, respectively. This behavior was the opposite of that observed for the previously mentioned gene clusters characterized by genes expressed on the susceptible genotypes.

Group 2 only had clusters of high expression genes for the susceptible genotype evaluated at 72hpi, while the other three treatmens presented genes of low average expression, compared with the treatment T2_S. That group contained 166 genes. In contrast, group 16 (230 genes) was composed of genes with the exact opposite pattern, where the genes of high expression were visible on the resistant genotype evaluated at 24hpi.

Groups 5 and 13 presented a similar pattern for the average expression of the genes for each contrast, clustering 600 and 554 genes, respectively. The same happened in groups 6 and 17 (424 and 576 genes, respectively), but with the opposite standard for the two previously mentioned groups.

Groups 11 and 15 contained 266 and 253 genes, respectively, with the higher expression genes clustering in the contrasts with the both host genotypes evaluated at 72hpi. The opposite took place, however, in group 8, with the most highly expressed genes (n=266) in both host genotypes evaluated at Time 1, and in group 14, which had a high number of genes expressed in the contrasts T1xR, T1xS and T2xR and a low number of genes in the last contrast. This group had 252 genes.

For the capN project genes, the best number of clusters according to the ICL curve was 16 (Figure 12b). Groups 1, 2 and 13 had clusters of over expressed genes for both times in resistant host genotypes, with 454, 116 and 371 genes, respectively.

Groups 3 and 5 (229 and 334 genes, respectively) had the genes with highest average expression in susceptible genotypes evaluated at both times, with genes more heterogeneously expressed in group 3. The groups 4, 8, 9 and 15

(669, 165, 222 and 452 genes, respectively) contained the over expressed genes in the contrast T1xS, but with a small diference for the treatment T1xR and a bigger difference in the average for the contrast T2xR.

The genes in groups 10 and 11 showed a small difference in average expression between the four contrasts, with 454 and 512 genes, respectively, in the two groups. Clusters 6, 7 and 16 have more highly expressed genes in resistant genotypes evaluated at 72hpi, with group 16 being the one with the biggest difference in the level of expression in this contrast compared with the other 3 contrasts. The groups had 461, 448 and 225 genes, respectively.

Groups 12 and 14 showed the opposite trend for the gene level expression in each contrast. Cluster 12 (244 genes) had low expression genes at Time 1 for both hosts when compared with Time 2; in contrast, cluster 14 (278 genes) had low expression genes at Time 2 for both hosts when compared with Time 1.

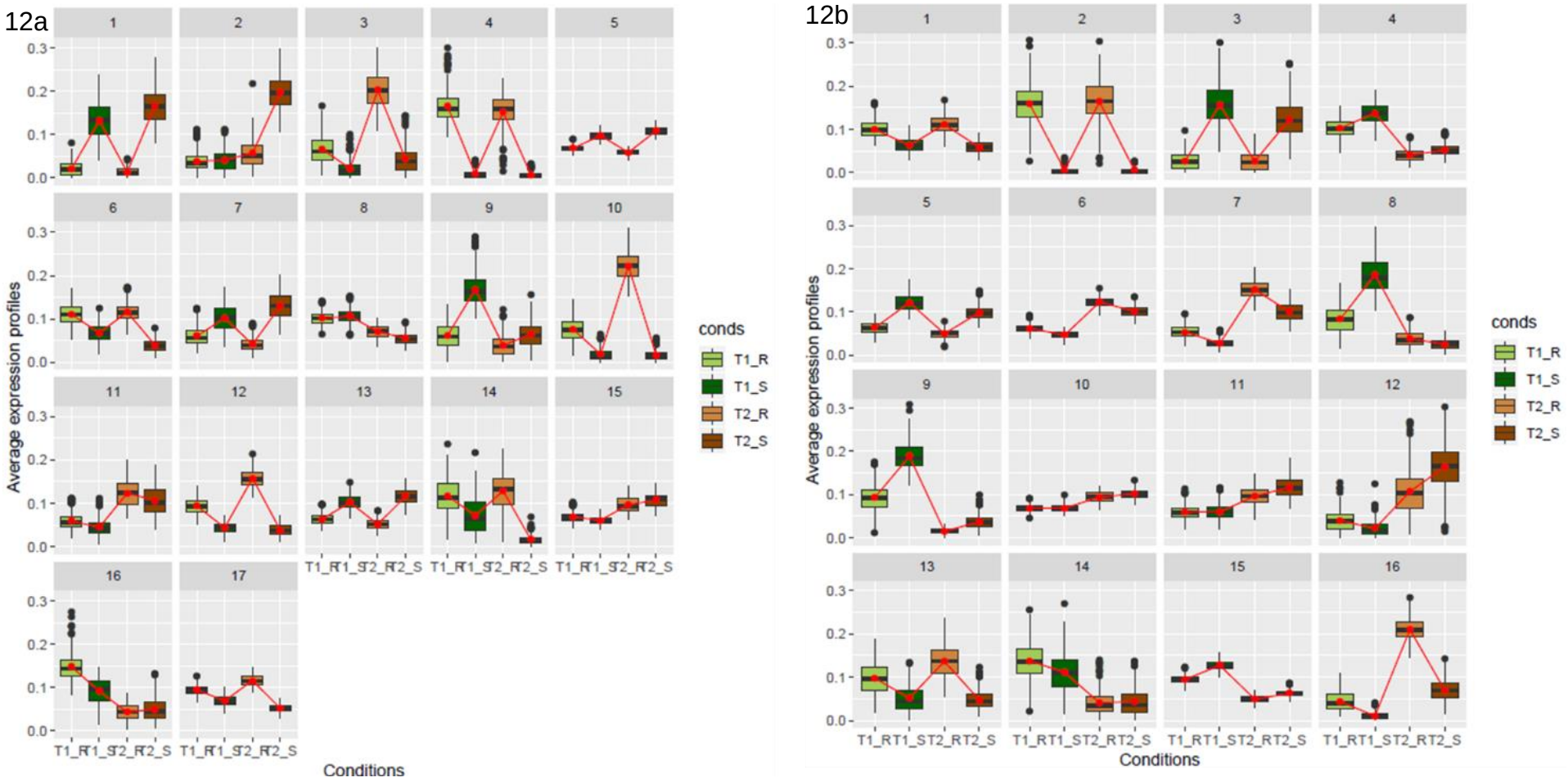

Figure 12. Co-expression of the differentially expressed genes in *Capsicum annuum* at two timepoints, according to the isolate of *Phytophthora capsici* used: adapted (Figure 12a) and non-adapted (Figure 12b). **T1_R:** 24hpi in resistant host; **T1_S:** 24hpi in susceptible host; **T2_R:** 72hpi with resistant host; **T2_S:** 72hpi in susceptible host.

3.2.4.4. Biological model 3 – Interaction Times x Isolate in each host

3.2.4.4.1. Differential Analysis

Evaluating the interaction between isolate and time in each genotype used, resistant and susceptible, 2,104 and 6,912 DEGs were obtained, respectively. The DEGs were separated into up- and downregulated groups for each contrast (Figure 13). The number of these genes varied according to the contrast and genotype used. For the resistant ones, a total of 3,420 up- and 3,559 downregulated genes were expressed. With the susceptible genotype, there were 9,985 upregulated and 11,287 downregulated genes.

The project capR presented four contrasts with a higher number of upregulated genes and three contrasts with more downregulated genes, while the capS showed five contrasts with a higher number of downregulated genes and two with more upregulated genes. There were more non-identified genes in capR than in the other projects analyzed for the 3 biological models.

In capR (Figure 14a), it is possible to observe that 1,121 genes were expressed when we contrast both times no matter the isolate (contrast A), and when we consider just the isolate non-adapted in both times (contrast C), there are more expressed genes than when using the adapted isolate (contrast B). No gene was expressed in both situations (contrast B and C). Contrasting both isolates and the two times of evaluation together (contrast D), no gene was differentially expressed.

For capS (Figure 14b), there were also more expressed genes in the contrast between the times with the non-adapted isolate (C) when compared with the adapted isolate at both times (B), and again no gene was expressed in these two contrasts at the same moment. Contrasting both isolates and the two times of evaluation together (contrast D), 263 genes were differentially expressed.

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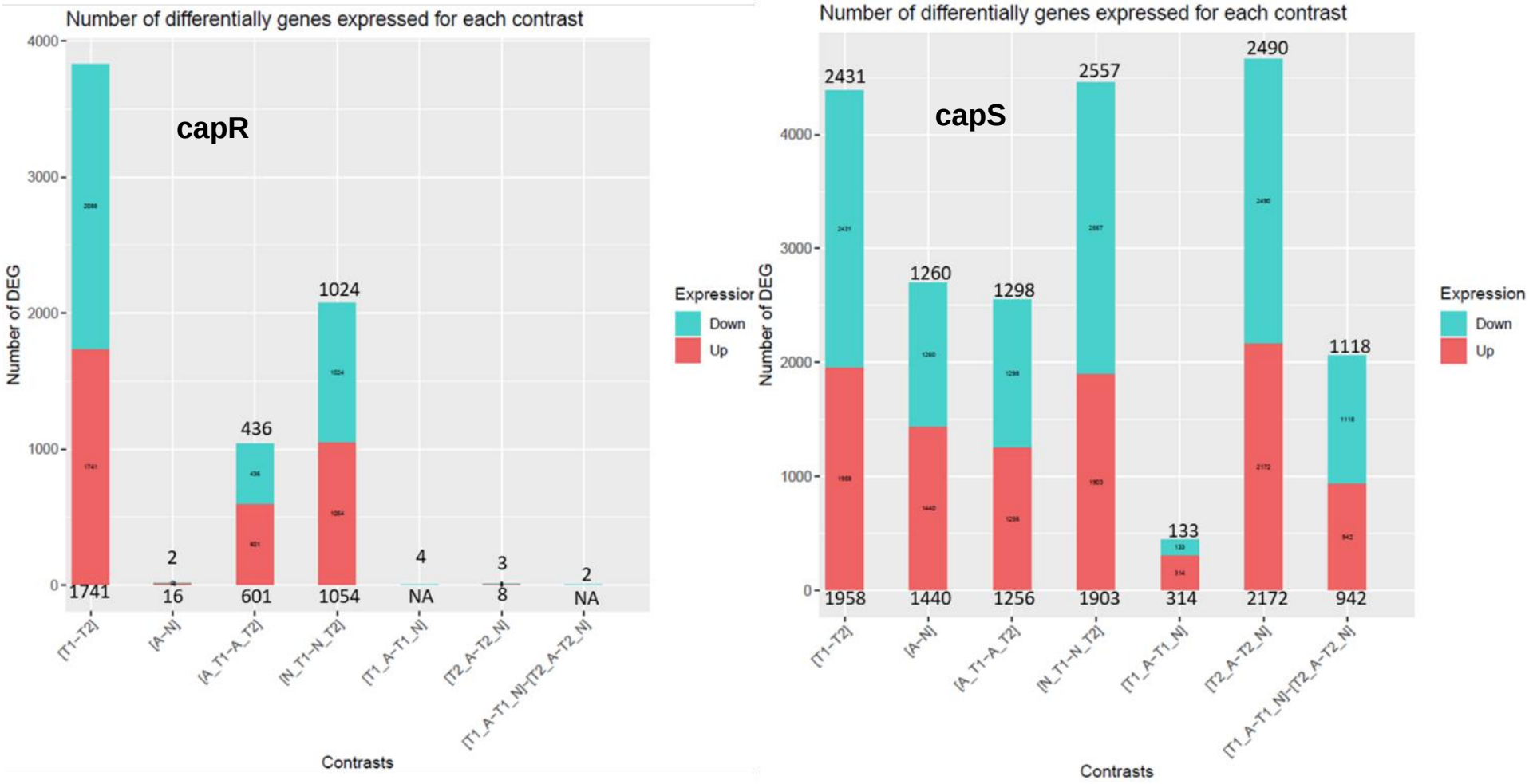


Figure 13. Up- and downregulated genes expressed in *Capsicum annuum* between the seven contrasts in the projects capR (Interaction between time of evaluation and isolate type in resistant host) and capS (Interaction between time of evaluation and isolate type in susceptible host). The Y-axis shows the number of up- and downregulated DEGs. On the X-axis, the seven contrasts analyzed in both projects are described.

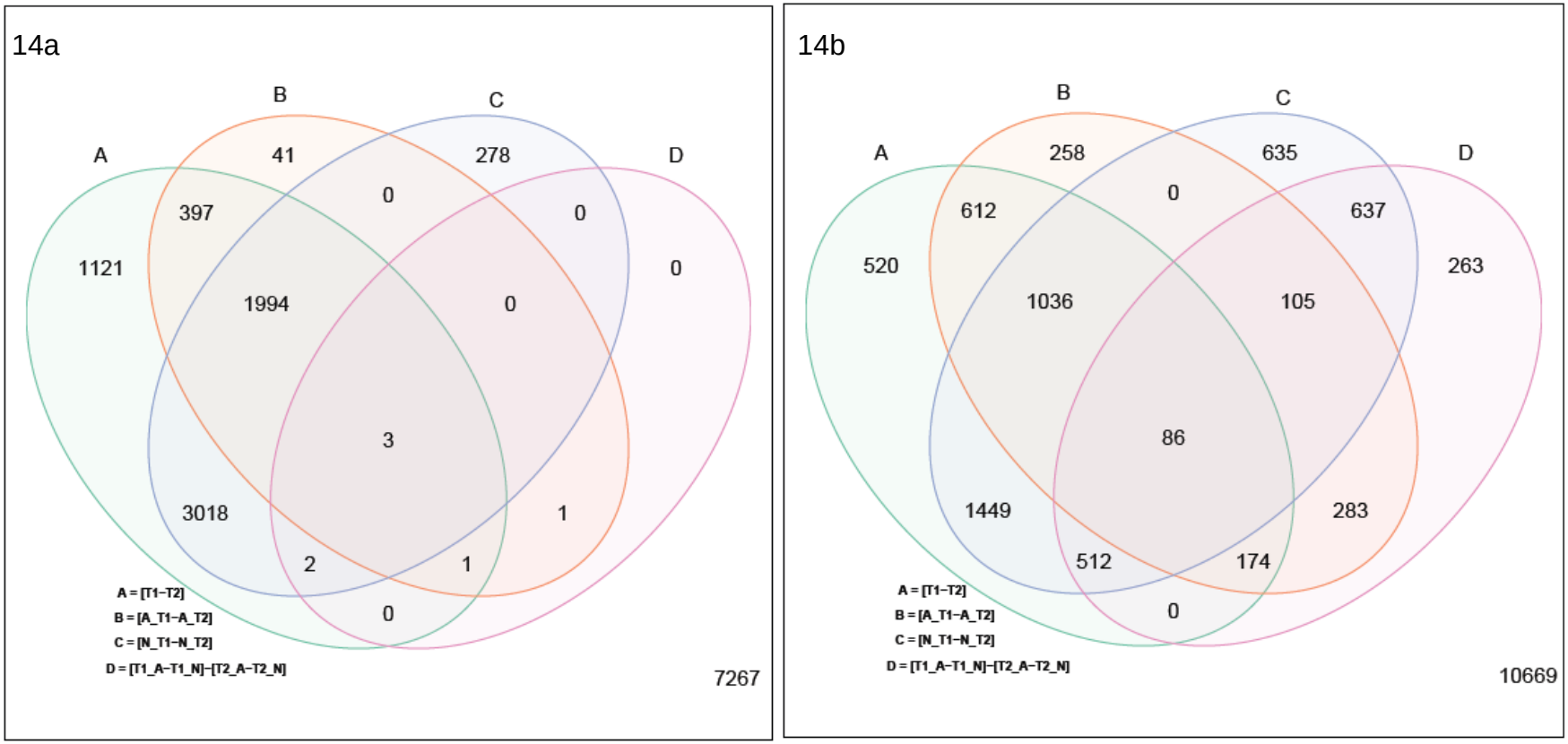


Figure 14. Venn diagram illustrating the number of *Capsicum annuum* differentially expressed genes in the interaction between host and type of *Phytophthora capsici* isolate in resistant (14a) and susceptible genotypes (14b) in four different contrasts. **A:** Pepper DEGs between the two times of evaluation independently of the isolate; **B:** Pepper DEGs between the two times of evaluation with the adapted isolate; **C:** Pepper DEGs between the two times of evaluation with the non-adapted isolate; **D:** Difference in the pepper DEGs between the two times of evaluation with the two isolates of *P. capsici*.

More than 14,000 genes were analyzed in the project capR and different relevant genes were highlighted (Figure 15). The gene “CA.PGAv.1.6.scaffold124.1” (Figure 15a), similar to *At5g63930 probable leucine-rich repeat receptor-like protein kinase*, was expressed with an average that was quite similar to the four analyzed contrasts, showing lower expression and a higher standard deviation for the contrasts at Time 2.

The gene “CA.PGAv.1.6.scaffold941.21” (Figure 15b), similar to *R1A-10 Putative late blight resistance protein homolog R1A-10,* presented higher expression levels in the contrasts at Time 1, while the gene “CA.PGAv.1.6.scaffold818.8” (Figure 15c), similar to *GH3.5 Jasmonic acid-amido synthetase JAR1*, had a homogenous expression level in the four contrasts.

In Figure 15d we have the gene “CA.PGAv.1.6.scaffold1155.28”, with a clearly higher level of expression at Time 1 contrasted with the adapted isolate, while the gene “CA.PGAv.1.6.scaffold160.43” was more highly expressed at Time 2 contrasted with the same isolate. These genes are similar to *RPP13L3 putative disease resistance* and *R1B-16 putative late blight resistance protein.*

In capS many important genes were expressed, including “CA.PGAv.1.6.scaffold533.13” (Figure 16a), which similar to *SABP2 Salicylic acid-binding protein 2* was more highly expressed at Time 2 with the non-adapted infection than in the other contrasts, with a lower standard deviation for the 4 conditions.

The gene “CA.PGAv.1.6.scaffold65.72” (Figure 16b) was more highly expressed at Time 2 with both isolates than at Time 1, having the highest expression at 72hpi infected by the non-adapted isolate. The gene “CA.PGAv.1.6.scaffold1155.28” (Figure 16c) also had higher expression at Time 2, but had a higer level during the infection with the non-adapted isolate.

The genes “CA.PGAv.1.6.scaffold58.22” and “CA.PGAv.1.6.scaffold388.70” (Figures 16d and 16e), similar to *RDR1 RNA-dependent RNA polymerase 1* and to *RLK1 G-type lectin S-receptor-like serine*, respectively, also showed more pronounced expression at Time 2at Time 1.

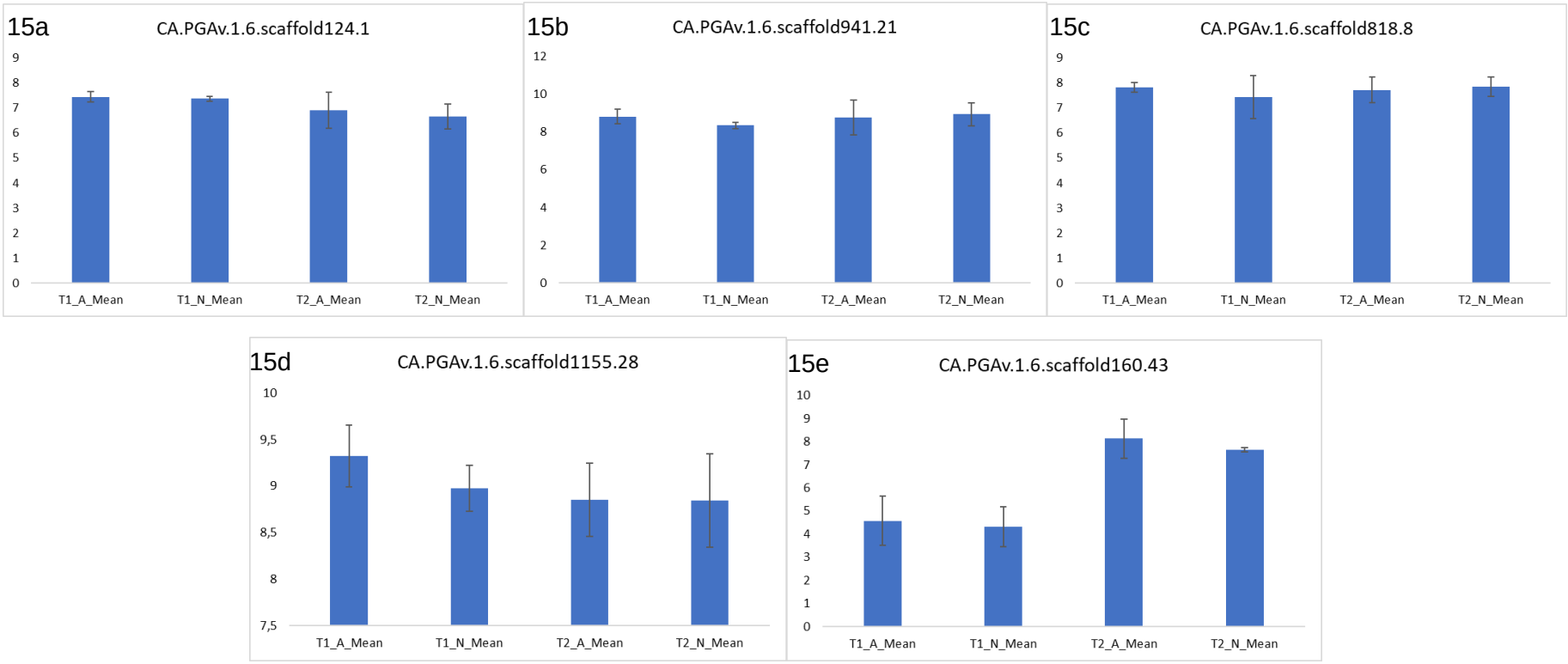


Figure 15. Levels of gene expression in *Capsicum annuum* according to the interaction between time of evaluation and type of isolate in resistant genotype under 4 biological conditions: **T1_A:** 24hpi with adapted isolate; **T1_N:** 24hpi with non-adapted isolate; **T2_A:** 72hpi with adapted isolate; **T2_N:** 72hpi with non-adapted isolate.

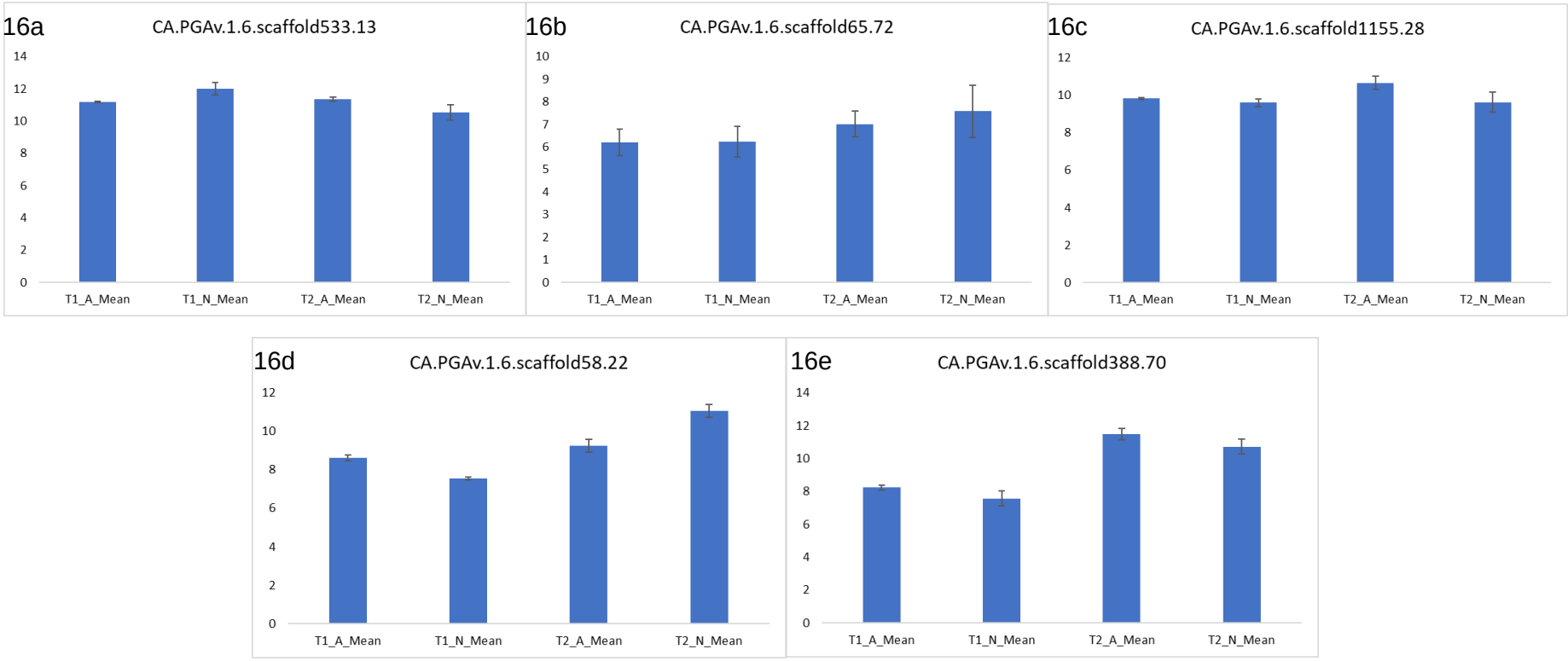


Figure 16. Levels of gene expression in *Capsicum annuum* according to the interaction between time of evaluation and type of isolate in susceptible genotype under 4 biological conditions: **T1_A:** 24hpi with adapted isolate; **T1_N:** 24hpi with non-adapted isolate; **T2_A:** 72hpi with adapted isolate; **T2_N:** 72hpi with non-adapted isolate.

3.2.4.4.2. Co-expression

Analysing the co-expression between the differentially expressed genes as a function of the host genotype (Figure 17), 12 clusters were established for the project capR (Figure 17a) and 14 groups for were established for the project capS (Figure 17b). These genes presented differences in the expression standard related to the groups.

For capR, groups 1, 5, 8, 10 and 11 (316, 660, 474, 766 and 576 genes, respectively) had clusters of high expression genes in the contrasts at Time 2 (72hpi) infected by each isolate, with a large difference in the expression average from the genes expressed at Time 1 (24hpi) contrasted with both isolates. The contrast T2_N contained the genes with the highest expression profile compared with the other three contrasts in the mentioned clusters.

Groups 2, 3, 4, 6 and 12 have a profile in which the contrast between 24hpi and non-adapted isolate showed genes with a higher average expression. These groups contained 446, 486, 642, 346 and 402 genes, respectively. Group 7, with 338 genes, showed a profile in which the higher expression genes are those expressed at T2_A and the lower expression ones are those expressed at T1_A, presenting a significant contrast between both times for the same isolate.

However, cluster 9 (288 genes) contained the most highly expressed genes in T1_A than in the others contrasts, especially when compared with the contrast T2_A, where we can see a higher difference in the average expression profile between them, thus presenting the complete opposite trend as that of group 7.

In capS, groups 5, 9 and 12 (488, 605 and 274 genes, respectively) present the same profile of gene expression, in which the contrast T2_A had the lower expression average compared with the other groups. Clusters 7, 8 and 11 had 643, 731 and 329 genes, respectively, coexpressing in the same standard, with the genes expressed in T2_A presenting a higher average in comparison with the other contrasts.

The genes in the contrast between Time 1 and non-adapted isolate in groups 2, 10 and 12 (489, 468 and 274 genes, respectively) had the highest level profile expression compared with the other three contrasts for those groups, different from groups 4 and 13, in which genes of lower expression clustered in the same contrast, containing 244 and 435 genes, respectively.

Clusters 1, 3, and 14 had the genes with a higher average expression profile at Time 2 with non-adapted isolates compared with the other three contrasts in the same group. Those groups had 678, 399 and 750 genes, respectively. Group 6 had the pattern of genes expressed in T2_A with a higher average, clustering 379 genes around the four contrasts.

3.2.4.5. General view

Among the three biological models studied in this research more than 14 thousand genes of *C. annuum* were evaluated, or more than 40% of the expressed genome of the species. A total of 35,884 raw counts were analyzed using the RNA-seq approach. However, not all of these reads presented enough counts to be analyzed, and because of this the quality control was performed in the dataset, resulting in half of those reads being used for the further analysis.

Regading biological model 1, more genes were expressed at 72hpi than at 24hpi for the resistant and susceptible genotypes infected by the adapted isolate, while more genes were expressed at Time 1 than at Time 2 for the host genotypes contaminated by the non-adapted isolate.

In biological model 2, a higher level of expressed genes was obtained in projects capA and capN in both genotypes at 72hpi in comparison with 24hpi. In total, more genes were obtained for these contrasts considering the infection by the adapted isolate than in the non-adapted isolate.

With respect to biological model 3, with the projects in which we evaluated the interaction between time and isolate in the resistant and susceptible genotypes separated, more genes were obtained at both timepoints with the non-adapted isolate for both host genotypes.

Many different genes were expressed commonly in the 6 projects, like the gene “CA.PGAv.1.6.scaffold1155.28”, similar to *RPP13 protein,* a known related gene involved in the defense against *Phytophthora.* This gene expression level varied according to the analyzed project, but was present in all the designed contrasts.

The highlighted genes identified in the six projects are listed in Table 4.

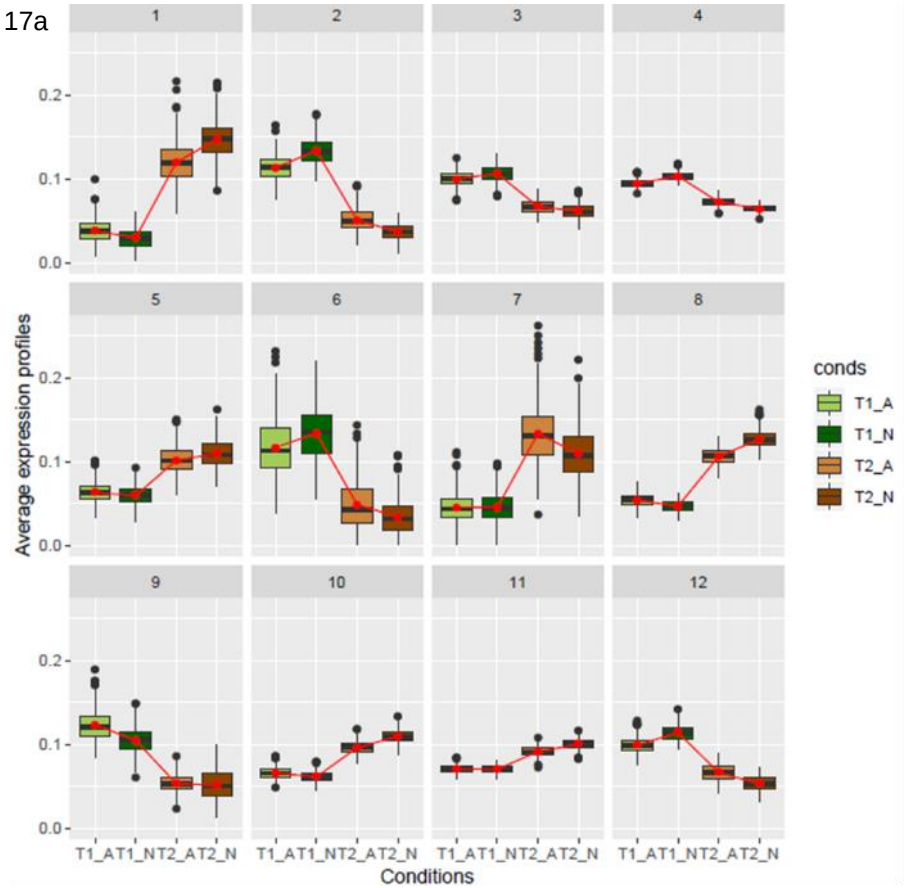
Table 4. List of the selected differentially expressed *Capsicum annuum* genes for each evaluated project.

Gene ID	Annotation	Project
CA.PGAv.1.6.scaffold1155.28	Similar to RPP13L3 Putative disease resistance RPP13-like protein 3	capT1[1]
CA.PGAv.1.6.scaffold981.4	Similar to tlp Thaumatin-like protein	capT1
CA.PGAv.1.6.scaffold149.47	Similar to XA21 Receptor kinase-like protein Xa21	capT1
CA.PGAv.1.6.scaffold388.70	Similar to RLK1 G-type lectin S-receptor-like serine/threonine-protein kinase RLK1	capT1
CA.PGAv.1.6.scaffold787.6	Similar to XA21 Receptor kinase-like protein Xa21	capT1
CA.PGAv.1.6.scaffold1155.28	Similar to RPP13L3 Putative disease resistance RPP13-like protein 3	capT2[2]
CA.PGAv.1.6.scaffold1155.29	Similar to RPP13 Disease resistance protein RPP13 (Arabidopsis thaliana	capT2
CA.PGAv.1.6.scaffold149.47	Similar to XA21 Receptor kinase-like protein Xa21	capT2
CA.PGAv.1.6.scaffold1505.2	Similar to XA21 Receptor kinase-like protein Xa21	capT2
CA.PGAv.1.6.scaffold673.10	Similar to At4g27190 Disease resistance protein At4g27190	capT2
CA.PGAv.1.6.scaffold411.21	Similar to At1g07650 Probable LRR receptor-like serine/threonine-protein kinase At1g07650 (Arabidopsis thaliana OX=3702)	capA[3]
CA.PGAv.1.6.scaffold1155.28	Similar to RPP13L3 Putative disease resistance RPP13-like protein 3 (Arabidopsis thaliana OX=3702) ;;	capA
CA.PGAv.1.6.scaffold1524.13	Similar to RPPL1 Putative disease resistance RPP13-like protein 1 (Arabidopsis thaliana OX=3702)	capA
CA.PGAv.1.6.scaffold1980.2	Similar to RLK7 Receptor-like protein kinase 7 (Arabidopsis thaliana OX=3702) ;;	capA
CA.PGAv.1.6.scaffold818.8	Similar to GH3.5 Jasmonic acid-amido synthetase JAR1 (Oryza sativa subsp. japonica OX=39947)	capA
CA.PGAv.1.6.scaffold608.26	Similar to PR1B1 Pathogenesis-related leaf protein 6 (Solanum lycopersicum OX=4081)	capN[4]
CA.PGAv.1.6.scaffold1155.28	Similar to RPP13L3 Putative disease resistance RPP13-like protein 3 (Arabidopsis thaliana OX=3702)	capN
CA.PGAv.1.6.scaffold1631.6	Similar to KTI2 Kunitz trypsin inhibitor 2 (Arabidopsis thaliana OX=3702)	capN
CA.PGAv.1.6.scaffold818.8	Similar to GH3.5 Jasmonic acid-amido synthetase JAR1 (Oryza sativa subsp. japonica OX=39947)	capN
CA.PGAv.1.6.scaffold322.58	Similar to SABP2 Salicylic acid-binding protein 2 (Nicotiana tabacum OX=4097)	capN
CA.PGAv.1.6.scaffold160.43	Similar to R1B-16 Putative late blight resistance protein homolog R1B-16 (Solanum demissum OX=50514)	capR[5]
CA.PGAv.1.6.scaffold1155.28	Similar to RPP13L3 Putative disease resistance RPP13-like protein 3 (Arabidopsis thaliana OX=3702)	capR
CA.PGAv.1.6.scaffold818.8	Similar to GH3.5 Jasmonic acid-amido synthetase JAR1 (Oryza sativa subsp. japonica OX=39947)	capR
CA.PGAv.1.6.scaffold941.21	Similar to R1A-10 Putative late blight resistance protein homolog R1A-10 (Solanum demissum OX=50514)	capR
CA.PGAv.1.6.scaffold124.1	Similar to At5g63930 Probable leucine-rich repeat receptor-like protein kinase At5g63930	capR
CA.PGAv.1.6.scaffold58.22	Similar to RDR1 RNA-dependent RNA polymerase 1 (Arabidopsis thaliana OX=3702)	capS[6]

Table 4 – Cont.

Gene ID	Annotation	Project
CA.PGAv.1.6.scaffold388.70	Similar to RLK1 G-type lectin S-receptor-like serine/threonine-protein kinase RLK1 (Arabidopsis thaliana OX=3702)	capS
CA.PGAv.1.6.scaffold1155.28	Similar to RPP13L3 Putative disease resistance RPP13-like protein 3 (Arabidopsis thaliana OX=3702)	capS
CA.PGAv.1.6.scaffold65.72	Similar to KTI2 Kunitz trypsin inhibitor 2 (Arabidopsis thaliana OX=3702)	capS
CA.PGAv.1.6.scaffold533.13	Similar to SABP2 Salicylic acid-binding protein 2 (Nicotiana tabacum OX=4097)	capS

[1] Comparing the interaction between host and isolate at 24hpi; [2] Comparing the interaction between host and isolate at 72hpi; [3] Comparing the interaction between host and time in adapted isolate; [4] Comparing the interaction between host and time in non-adapted isolate; [5] Comparing the interaction between time and isolate in resistant host; [6] Comparing the interaction between time and isolate in susceptible host.



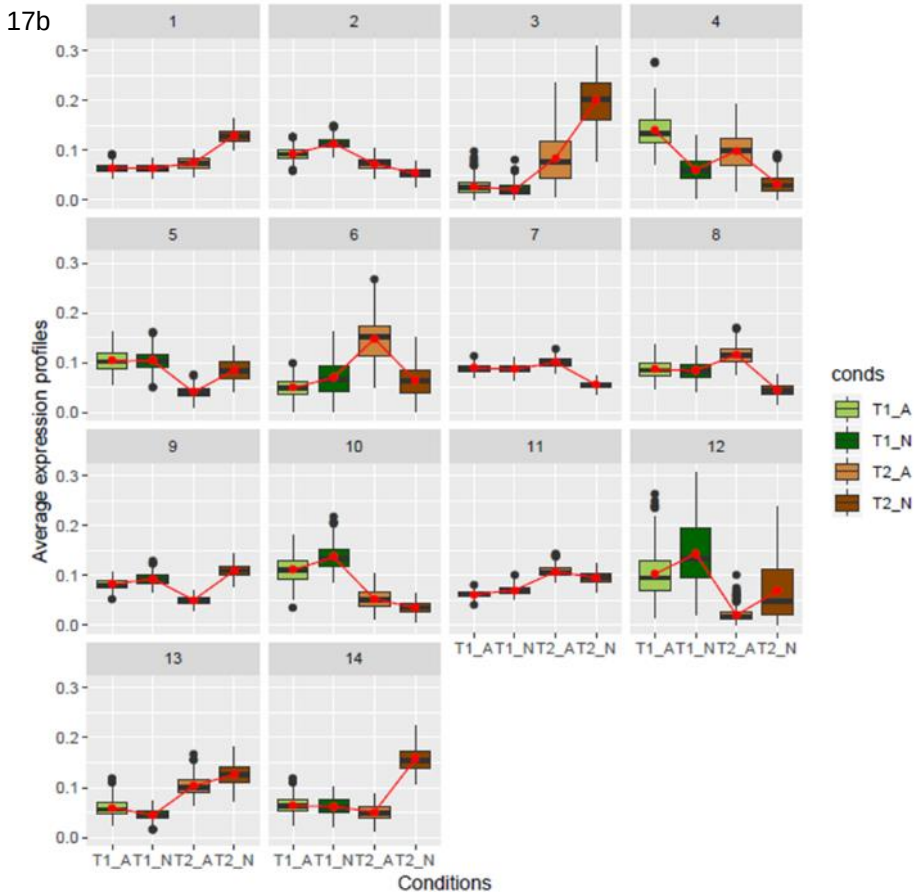


Figure 17. Co-expression of the differentially expressed genes in *Capsicum annuum* in the interaction between time of evaluation and type of isolate, according to host genotype: resistant (Figure 17a) and susceptible (Figure 17b). **T1_A:** 24hpi with adapted isolate; **T1_N:** 24hpi with non-adapted isolate; **T2_A:** 72hpi with adapted isolate; **T2_N:** 72hpi with non-adapted isolate.

3.2.4.6. Gene validation

The expression of four selected differentially expressed genes assigned as similar to R1A-10 putative late blight resistance, At4g03230 G-type lectin S-receptor-like-serine/threonine protein, similar to HSP18.2 class I heat shock protein and similar to At3g47570 probable LRR receptor-like serine/threonine-protein kinase was verified by qRT-PCR (Figure 18). It was possible to confirm through qRT-PCR that the 4 selected DEGs were correlated with the RNA-Seq results for the project capT1, used as model for the genes validation, which indicated the RNA-Seq data in the present study were reliable and could support the transcriptomic analysis presented above.

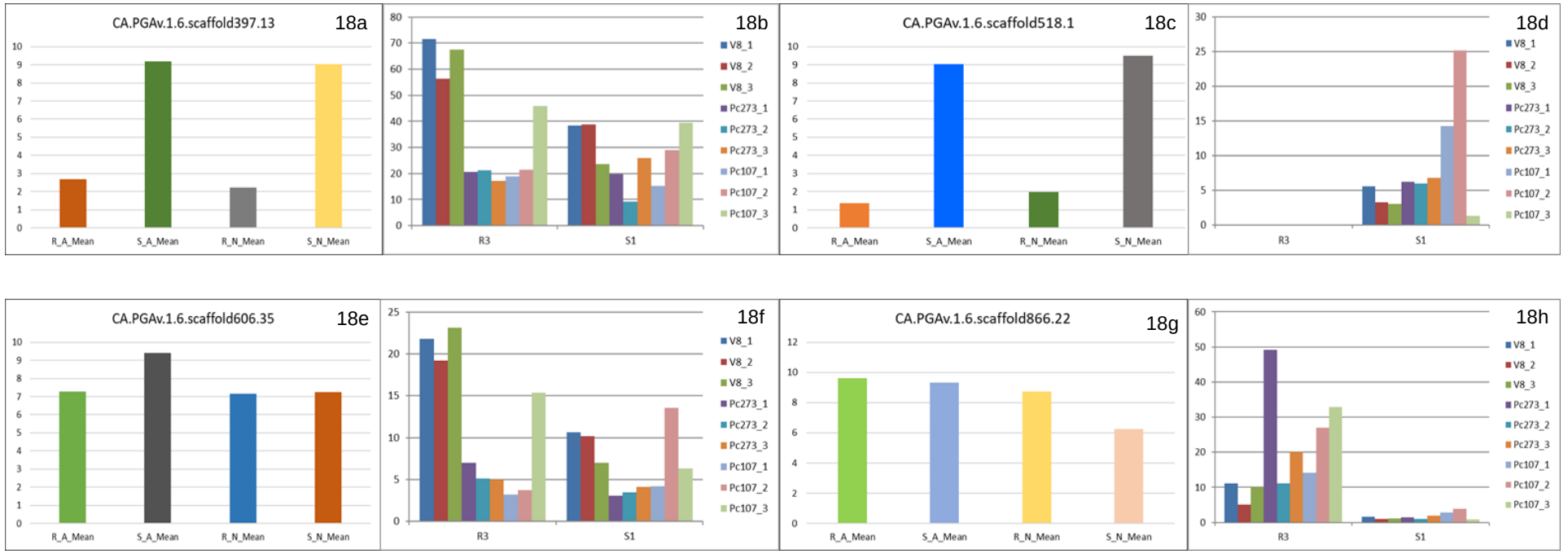


Figure 18. Expression levels of four differentially expressed genes of *Capsicum annuum* by qRT-PCR and RNA-seq analysis. **18a:** RNA-seq results for CA.PGAv.1.6.scaffold397.13; **18b:** qRT-PCR results for the gene in 18a; **18c:** RNA-seq results for CA.PGAv.1.6.scaffold518.1; **18d:** qRT-PCR results for the gene in 18c; **18e:** RNA-seq results for CA.PGAv.1.6.scaffold606.35; **18f:** qRT-PCR results for the gene in 18e; **18g:** RNA-seq results for CA.PGAv.1.6.scaffold866.22; **18h:** qRT-PCR results for the gene in 18g.

3.2.4.7. Top 50 clustering genes

To check for patterns between the up- and downregulated genes, a list of the top 50 genes was produced. Heatmaps were built based on the results obtained in the analysis of Biological Model 2, in which the interaction between genotypes and the evaluation time after inoculation were evaluated according to the isolate, adapted (Figure 19) and non-adapted (Figure 20).

Three different groups were formed for the interactions using the adapted isolate. One group was composed of 19 genes that are upregulated in the resistant and susceptible genotypes at 24hpi; however, in the susceptible genotypes the level of expression is even lower than in the resistant geotypes, with the exception of the gene “CA.PGAv.1.6.scaffold694. 56”, downregulated in susceptible genotypes at 24hpi. These same 19 genes are all downregulated in those resistant to 72hpi and vary between up- and downregulated for susceptible genotypes at the same assessment time.

The second group had a cluster of 16 genes that are upregulated to resistant and susceptible genotypes at 72hpi, with the exception of a few genes like “CA.PGAv.1.6.scaffold631.50”, which is downregulated to resistant genotypes at 72hpi and the gene “CA.PGAv.1.6.scaffold186.76”, downregulated to susceptible genotypes at 72hpi. This set of genes is expressed as downregulated for both genotypes at 24hpi.

Fifteen genes made up the third group, being upregulated for both genotypes at 24hpi. They were downregulated to the resistant genotype at 72hpi. A few were upregulated for susceptible ones at the same time of evaluation, with a low expression level, and other genes presented downregulation for susceptible hosts at 72hpi.

An analysis of the interaction between genotypes and the evaluation time after inoculation using the non-adapted isolates (Figure 20) allows one to observe three groups and one gene contrasting completely in expression level compared with its group.

The first group was composed of 23 genes that presented upregulation for resistant and susceptible genotypes at 24hpi and downregulation for the resistant genotypes at 72hpi. For susceptible genotypes at 72 hpi, different levels of expression were identified, ranging between up- and downregulated responses with low expression levels. The gene “CA.PGAv.1.6.scaffold349.10” presented the

opposite standard, being upregulated at 72hpi and downregulated at 24hpi for both hosts.

Group 2 had a cluster of 20 genes expressed with the same pattern as the previous group, i.e. upregulation at 24hpi and downregulation at 72hpi for both hosts. Seven genes comprised the third group, presenting downregulation for both hosts at 24hpi and upregulation at 72hpi for the resistant and susceptible genotypes.

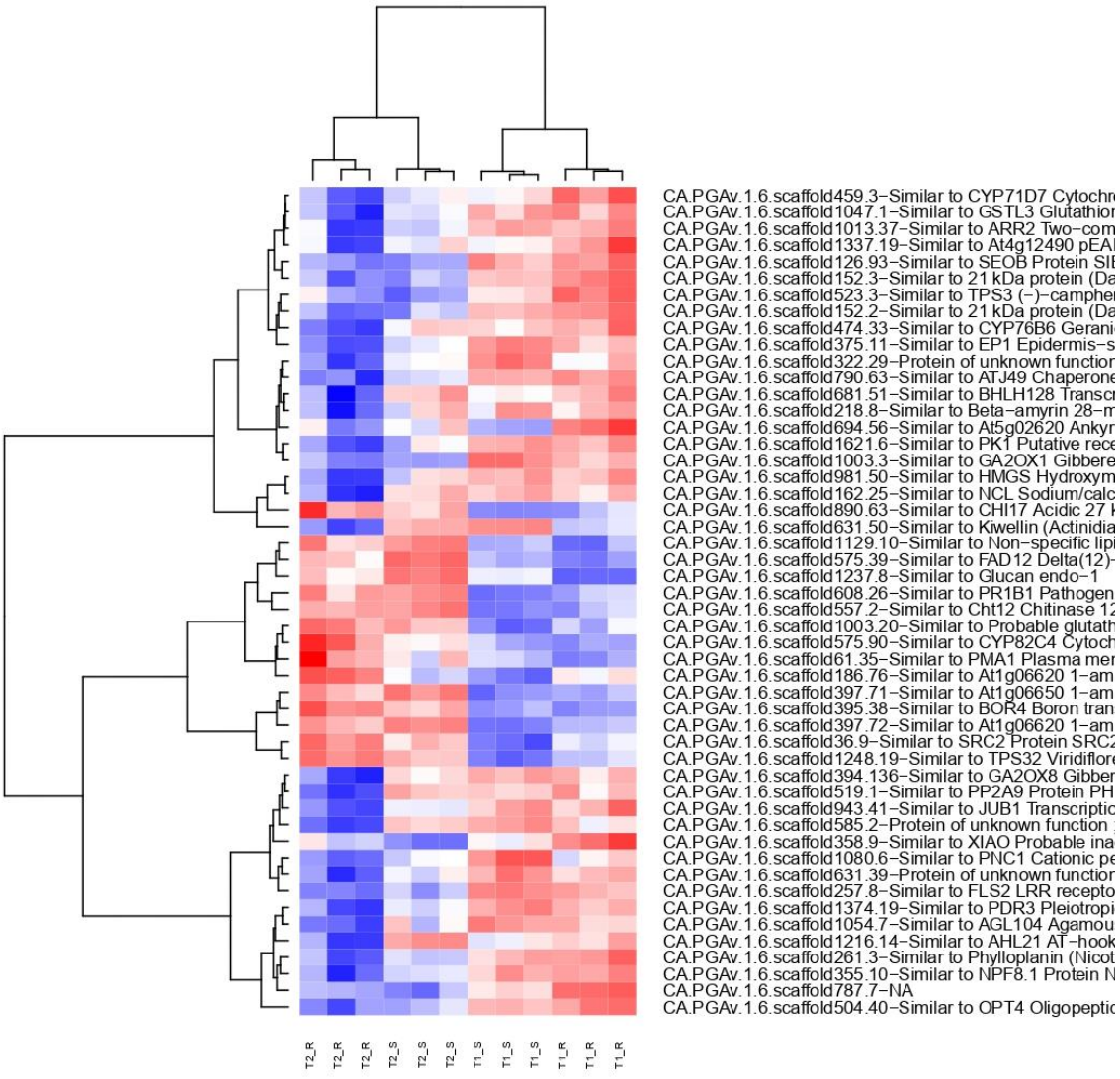


Figure 19. Heatmap analysis of the 50 most variable genes in resistant and susceptile hosts of *Capsicum annuum* at 24 and 72hpi, inoculated with adapted isolate of *Phytophthora capsici*. The lines described the genes and the columns are the conditions: **T1_R:** Resistant genotypes at 24hpi; **T1_S:** Susceptible genotypes at 24hpi; **T2_R:** Resistant genptypes at 72hpi; **T2_S:** Susceptible genotypes at 72hpi. Red color indicates upregulated genes and blue colors indicate downregulated genes.

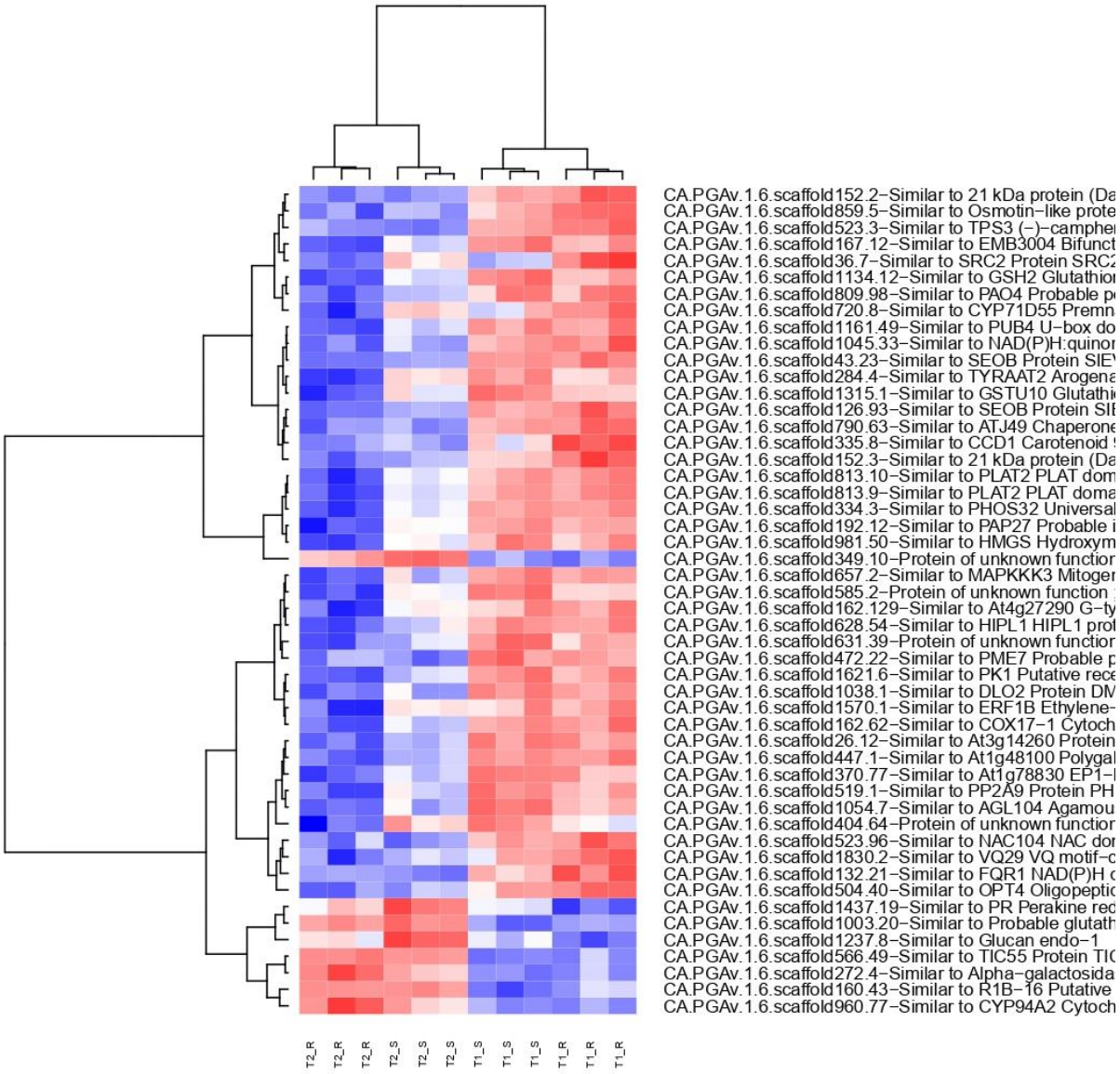


Figure 20. Heatmap analysis of the 50 most variable genes in resistant and susceptile hosts of *Capsicum annuum* at 24 and 72hpi, inoculated with non-adapted isolate of *Phytophthora capsici*. The lines described the genes and the columns are the conditions: **T1_R:** Resistant genotypes at 24hpi; **T1_S:** Susceptible genotypes at 24hpi; **T2_R:** Resistant genptypes at 72hpi; **T2_S:** Susceptible genotypes at 72hpi. Red color indicates upregulated genes and blue colors indicate downregulated genes.

3.2.5. DISCUSSION

The RNA-seq technique provides an exceptional volume of transcriptomic information (Wang et al., 2010). According to Tandonnet and Torres (2017), RNA-

Seq is a powerful strategy for accurately determining gene expression and detecting DEGs at a low cost. However, like all sequencing technologies, it is prone to certain biases, errors and artifacts, necessitating robust and comprehensive quality control. Without an efficient quality control it isn't possible to be certain that these unobserved errors, biases or artifacts do not violate the assumptions of analysis (Hartley and Mulikin, 2015).

Between ~14 to ~18 thousand of genes were evaluated around the six projects (capT1, capT2, capA, capN, capR and capS). When analyzing the expression of key genes involved in the biosynthetic pathway of capsaicin in peppers, Zhang et al. (2016) found a total of 28,434 expressed genes. For their part, when Kang et al. (2020) were evaluating the profiling of abiotic responses to heat, cold, salt and osmotic stress of *Capsicum annuum* L., they found between 70.14% and 90.38% of the mapped preprocessed reads. Finally, in an investigation into the control of pungency in *C. annuum*, Han et al. (2019) found between 56 to 60% of the reads aligned with the genome model.

Considering the three biological models in a general way, more upregulated genes were found between the analyzed contrasts. When they were evaluating genes involved in phenylpropanoid metabolism in response *to Phytophthora capsici* in *Piper nigrum*, Hao et al. (2016) found similarity in the patterns of upregulated and downregulated DEGs. Ali et al. (2018) found more upregulated genes than downregulated ones while studying the Chitin-Binding Proteins in pepper.

The number of DEGs was different in each evaluated project, with a mimimum of 2,104 DEGs in the project capR and a maximum of 7,407 in the project capA. Park et al. (2019), identifying candidate genes for capsaicinoid biosynthesis in the pericarp of *Capsicum chinense*, found 4,513, 6,360, and 2,632 DEGs in different pepper accessions.

Different from the number obtained in this work, Wang et al. (2016), , found 1,220 differentially expressed genes using RNA-seq while studying the expression of candidate genes associated with *P. capsici* in pepper, with many of these DEGs involved in defense responses., Li et al. (2020) found a total of 810 and 1,110 DEGs in CM334 at 12 and 36 hpi, respectively, while evaluating the dynamic transcriptome of *C. annuum* roots infected by *P. capsici,* and identified a total of

291 and 2,465 DGEs in the susceptible NMCA10399 at these two timepoints, respectively.

DEGs were identified in all three of the analyzed biological models, indicating the activation of defense responses to *P. capsici* after infection in all the designed contrasts. Similar results were found by Li et al. (2020), who identified DEGs in the two evaluated timepoints in susceptible and resistant genotypes. Bagheri et al. (2020) related expression in resistant and susceptible genotypes, with major expression levels for resistant ones, after an investigation of the expression of genes involved in pepper defenses against *P. capsici*.

Due to the complex inheritance of *P. capsici* in CM334, breeding for this resistance is quite challenging. Resistance incorporated into commercial pepper lines can be readily overcome by highly virulent *P. capsici* isolates. The identification of genes involved in the interaction between host and isolate is very important for the understanding of this complex interaction and for pepper breeding (Lamour and Hausbeck, 2000; Liu et al., 2014; Wang et al., 2016; Siddique et al., 2019).

Plants are able to recognize pathogen effectors due to plant-pathogen interaction over the course of the years. Many different genes/alleles of resistance are involved in this process. That recognition activates effector-triggered immunity (ETI), followed by a defense response often leading to cell death or a hypersensitive response (HR) (Sekhwal et al., 2015; Zaidi et al., 2018).

According to Kourelis and van der Hoorn (2018), resistance (*R*) genes in plants play a key role in their remarkable immune responses. These genes are usually dominant and provide full or partial resistance to one or more pathogens. Many R genes confer recognition of pathogen-derived effectors and initiate effector-triggered immunity (ETI), which often involves hypersensitive response (HR). However, few mechanisms equipped with R genes involve perceptions related to receptor-like proteins/kinases (RLPs/RLKs), Nod-like receptors (NLRs) and Executor genes.

In this work, many different genes involved in the *RLK* mechanisms were found in the different biological models evaluated, such as the genes “CA.PGAv.1.6.scaffold1980.2”,“CA.PGAv.1.6.scaffold388.70”,“CA.PGAv.1.6.scaffold411.21” and “CA.PGAv.1.6.scaffold124.1”. Yi et al. (2010) concluded that *CaRLK1*, an *RLK* type gene, functions as a negative regulator of plant cell death

via accumulation of superoxide anions in *C. annuum*, protecting the transformed plants against pathogen infection.

Siddique et al. (2019) found three *RLK* genes involved in resistance against *Phytophthora* in pepper. Guan et al. (2018), evaluating the *CaHSL1* gene action as a regulator of pepper thermotolerance, found *RLK* genes involved in plant tolerance against the stress.

Another important *R* family gene found in this research was the *RPP13*, highlighted by the genes "CA.PGAv.1.6.scaffold1155.29" and "CA.PGAv.1.6.scaffold1155.28", this last of which was present in all six of the evaluated projects. Siddique et al. (2019) also found different genes related to *RPP13*, all on chromosome P5. According to Rose et al. (2004), *RPP13* is a CC (coiled-coil)-NBS-LRR (nucleotide-binding site-leucine-rich repeat) domain-containing *R* gene that confers resistance to the oomycete pathogen *Peronospora parasitica* in *Arabidopsis thaliana.*

Few genes similar to Thaumatin-like proteins (TLPs), like the gene "CA.PGAv.1.6.scaffold981.4", were expressed in the projects. This is a highly complex protein family associated with host defense and developmental processes in plants, animals and fungi. These PRs show many different TLP isoforms that may be activated by biotic or abiotic stresses (or both) (Jesus-Pires, et al., 2019).

It is known that genes related to jasmonates (JA) regulate defense during necrotrophic infection, play roles in seed germination, fertility, root growth and pathogen responses. Salicylic acid (SA) is associated with resistance to biotrophs and hemibiotrophs (Santner et al., 2009; Zhao, 2010; Antico et al., 2012; Pandey et al., 2016).

RNA-seq analysis throughout the pepper genome has revealed genes similiar to this pattern, such as "CA.PGAv.1.6.scaffold818.8", a gene similar to *GH3.5 Jasmonic acid-amido synthetase JAR1*. Kong et al. (2019) found a significant *JAR1* gene while studying bell pepper reactions against cold stress. Zhang et al. (2020) also found genes related to *JAR1* involved in pepper defense against *P. capsici*.

Many other relevant genes involved in plant defenses were expressed in the samples. They include genes associated with jasmonic acid expression, ethylene, salicylic acid, genes from the "*SAR*" family, leucine-like receptors (LLR),

argonaute proteins (AGO), dicer-like proteins (DCL) and RNA-dependent proteins. These last 3 proteins are mostly involved in RNA silencing, a control mechanism that efficiently prevents pathogen invasion to protect plants from pathogen attack throughout their life-cycles (Baulcombe, 2004).

These results provide an overview of changes in the gene expression profiles of *C. annuum* under *P. capsici* infection. In general, the analyses reveal the gene expression in different pepper genotypes, susceptible and resistant, under infection by two different isolates of *P. capisici*, one adapted to infect pepper plants and one non-adapted, evaluated at 24 and 72 hpi. Receptor-like-serine related genes, R1A-10 putative late blight resistance genes, among others, were identified to validate their differentially expressed profile using RT-qPCR analysis. In this way, a reliable list of up- and downregulated candidate genes was generated that can be used in future projects to improve knowledge about *C. annuum* × *P. capsici* interactions.

The different responses of these genes was observed around the evaluated projects, with high or low expression for the same gene under different conditions, as well as important differences in the number of genes expressed in each contrast. Resistance to *P. capsici* in pepper hosts is multifactorial and changes according to the biological material used.

3.2.6. CONCLUSIONS

Differences in the number of DEGs were revealed by the interactions between host genotype and pathogen as a function of time; host genotype and time of evaluation as a function of the isolate; and the time of evaluation with isolate as a function of the host genotype;

The analysis reveals distinct gene expression in response to the pathogen in many different pathways, with a predominant presence of *R* family genes.

Gene Ontology analysis is needed in order to perfect biological interpretations of the results and to understand the function and regulation patterns of the differentially expressed genes.

REFERENCES

Ali, A.; Bordoh, P.K.; Singh, A.; SIddiqui, Y.; Droby, S. (2016) Post-harvest development of anthracnose in pepper (Capsicum spp.): Etiology and management strategies. *Crop Protection*, 90(132-141).

Ali, M.; Luo, D.-X.; Khan, A.; Haq, S.U.; Gai, W.-X.; Zhang, H.-X.; Cheng, G.-X.; Muhammad, I.; Gong, Z.-H. (2018) Classification and Genome-Wide Analysis of Chitin-Binding Proteins Gene Family in Pepper (Capsicum annuum L.) and Transcriptional Regulation to Phytophthora capsici, Abiotic Stresses and Hormonal Applications. *Int. J. Mol. Sci*., 19:2216.

Alimi, N.A.; Bink, M.C.A.M.; Dieleman, J.A.; Nicolaı, M.; Wubs, M.; Heuvelink, E.; Magan, J.; Voorrips, R.E.; Jansen, J.; Rodrigues, P.C.; Van der Heijden, G.W.A.M.; Vercauteren, A.; Vuylsteke, M.; Song, Y.; Glasbey, C.; Barocsi, A.; Lefebvre, V.; Palloix, A.; Eeuwijk, A.F. (2013) Genetic and QTL analyses of yield and a set of physiological traits in pepper. *Euphytica*, 190:181–201.

Almeida, C.L.P.; Bento, C.S.; Sudré, C.P.; Pimenta, S.; Gonçalves, L.S.A.; Rodrigues, R. (2020) Genotype-Ideotype distance index and multivariate analysis to select sources of anthracnose resistance in Capsicum spp.. *Eur J Plant Patho*l, 156:223–236.

Andreasson, E.; Ellis, B. (2010) Convergence and specificity in the Arabidopsis MAPK nexus. Trends Plant Sci, 15, 106–113.

Antico, C.J.; Colon, C.; Banks, T; Ramonell, K.M. (2012) Insights into the role of jasmonic acid mediated defenses against necrotrophic and biotrophic fungal pathogens. Front. Biol. 7, 48–56.

Arimboor, R.; Natarajan, R.B.; Menon, K.R.; Chandrasekhar, L.P.; Moorkoth, V. (2015) Red pepper (Capsicum annuum) carotenoids as a source of natural food colors: analysis and stability—a review, *Journal of Food Science and Technology*, 52: 1258-1271.

Arpaci, B.B.; Karatas, K. (2020) Comparison of chili pepper breeding populations for agronomic traits and polygenic resistance to Phytophthora blight. *Horticultura Brasileira*, 38(1), 12-20.

Atkinson, N.J.; Lilley, C.J.; Urwin, P.E. (2013) Identification of Genes Involved in the Response of Arabidopsis to Simultaneous Biotic and Abiotic Stresses. Plant Physiology, 162:2028–2041.

Baba, V.Y.; Powell, A.F.; Ivamoto-Suzuki, S.T.; Pereira, L.F.P., Vanzela, A.L.L.; Giacomin, R.M.; Strickler, S.R.; Mueller, L.A.; Rodrigues, R.; Gonçalves, L.S.A. (2020) Capsidiol-related genes are highly expressed in response to Colletotrichum scovillei during Capsicum annuum fruit development stages. *Sci Rep*, 10:12048.

Baba, V.Y.; Constantino, L.V.; Ivamoto, S.T.; Moreira, A.F.P.; Madeira, T.B.; Nixdorf, S.L.; Rodrigues, R.; Gonçalves, L.S.A. (2019) Capsicum-Colletotrichum interaction: Identification of resistance sources and quantification of secondary metabolites in unripe and ripe fruits in response to anthracnose infection. *Scientia Horticulturae*, 46:469-477.

Babadoost, M. (2005) Phytophthora blight of curcubits. *The Plant Health Instructor*.

Bae, H.G.K.; Jayaprakasha, K.; Crosby, K.S.; Yoo, D.I.; Leskovar, J.; Jifon, B.S. (2014). Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grown peppers. Journal of Food Composition and Analysis, 33(2), p. 195-202.

Bagheri, L.M.; Nasr-Esfahani, M.; Abdossi, V.; Naderi, D. (2020) Analysis of candidate genes expression associated with defense responses to root and

collar rot disease caused by Phytophthora capsici in peppers Capsicum annuum. *Genomics*, 112:2309-2317.

Barchi, L.; Bonnet, J.; Boudet, C.; Signoret, P.; Nagy, I.; Lanteri, S.; Palloix, A.; Lefebvre, V. (2007) A high-resolution, intraspecific linkage map of pepper (Capsicum annuum L.) and selection of reduced recombinant inbred line subsets for fast mapping. *Genome*, 50: 51–60.

Bari, R.; Jones, J.D.G. (2009) Role of plant hormones in plant defence responses. *Plant Mol. Biol*. 69:473–488

Baulcombe, D. (2004) RNA silencing in plants. *Nature*. 431:356–363.

Bazakos, C.; Hanemian, M.; Trontin, C.; Jiménez-Gómez, J.M.; Loudet, O. (2017) New strategies and tools in quantitative genetics: how to go from the phenotype to the genotype. *Annual Review of Plant Biology*, 68:435-455.

Ben-Chaim, A.; Borovsky, Y.; Falise, M.; Mazourek, M.; Kang, B.C.; Paran, I.; Jahn, M. (2006) QTL analysis for capsaicinoid content in Capsicum. *Theoretical and Applied Genetics*, 113:1481.

Ben-Chaim, A.; Paran, I.; Grube, R.C.; Jahn, M. (2001) QTL mapping of fruit-related traits in pepper (Capsicum annuum). *Theor. Appl. Genet*. 102:1016–1028.

Bento, C.S.; de Souza, A.G.; Sudré, C.P.; Pimenta, S.; Rodrigues, R. (2017) Multiple genetic resistances in Capsicum spp. *Genetics and Molecular Research: GMR*, 16(3).

Bernardo, R. (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Scienc*. 48: 1649-1664.

Bhering, L.L.; Cruz, C.D.; Vasconcelos, E.S.; Resende Junior, M.F.R.; Barros, W.S.; Rosado, T.B. (2009) Efficiency of the multilocus analysis for the construction of genetic maps. Crop Breeding and Applied Biotechnology, 9: 308-312.

Bittel, P.; Robatzek, S. (2007) Microbe-associated molecular patterns (MAMPs) probe plant immunity. *Current Opinion in Plant Biology*. 10:335–341.

Boller, T.; Felix, G. (2009) A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annu. Rev. Plant Biol*. 60:379–406.

Bonnet, J.; Danan, S.; Boudet, C.; Barchi, L.; Sage-Palloix, A.M.; Caromel, B.; Palloix, A.; Lefebvre, V. (2007) Are the polygenic architectures of resistance to Phytophthora capsici and P. parasitica independent in pepper? *Theor. Appl. Genet*. 115, 253–264.

Bosland, P.W.; Votava, E.J. (2012) Peppers: vegetable and spice Capsicums, Wallingford: CABI Publishing (Crops Production Science in Horticulture, 22), 224 p.

Boyle, E.A.; Yang, I.L.; Pritchard, J.K. (2017) An expanded view of complex traits: from polygenic to omnigenic. *Cell 169*, 1177–1186.

Bradeen, J.M.; Staub, J.E.; Wye, C.; Antonise, R.; Peleman, J. (2001) Towards an expanded and integrated linkage map of cucumber (Cucumis sativus L.). *Genome*, 44:111-9.

Broman, K.W.; Sen, S.A.A. (2009) Guide to QTL mapping with R/qtl. New York: *Springer*, 396p.

Burdon, J.J; Thrall, P.H. (2009) Coevolution of Plants and Their Pathogens in Natural Habitats. *Science*, 324: 755-756.

Candole, B.L.; Conner, P.J. (2010) Screening Capsicum annuum accessions for resistance to six isolates of Phytophthora capsici. *HortScience*, 45, 254–259.

Carneiro, M.S.; Vieira, M.L.C. (2002) Mapas genéticos em plantas. *Bragantia*, v.61,n.2, p.89-100.

Carvalho, S. I. C.; Bianchetti, L.B. (2007) Sistema de produção de pimentas (Capsicum spp.): botânica, Embrapa Hortaliças, Sistemas de produção, 2 ISSN 1678.

Carvalho, S.I.C.; Bianchetti, L.B.; Lopes, A.C.A.; Cruz, E.M. (2017) Agência de Informação Embrapa, Pimenta. Pré-Produção/ Características. Available in: <http://www.agencia.cnptia.embrapa.br/gestor/pimenta/arvore/CONT000gn05zz5y02wx5ok0liq1mqvd1bet3.html> accessed in January 26, 2021.

Chattopadhyay, A.; Baran Sharangi, A.; Dai, N.; Dutta, S. (2011) Diversity of genetic resources and genetic association analyses of green and dry chillies of Eastern India. *Chilean Journal of Agricultural Research*, 350–356.

Chen, A.L.; Liu, C.Y.; Chen, C.H.; Wang, J.F.; Liao, Y.C.; Chang, C.H.; Tsai, M.H.; Hwu, K.K.; Chen, K.Y. (2014) Reassessment of QTLs for late blight resistance in the tomato accession L3708 using a restriction site associated DNA (RAD) linkage map and highly aggressive isolates of Phytophthora infestans. *PLoS ONE* 9, e96417.

Cheng, J.; Chen, Y.; Hu, Y.; Zhou, Z.; Hu, F.; Dong, J.; Chen, W.; Cui, J.; Wu, Z.; Hu, K. (2020) Fine mapping of restorer-of-fertility gene based on high-density genetic mapping and collinearity analysis in pepper (Capsicum annuum L.). *Theor Appl Genet*, 133, 889–902.

Coelho, A.S.G.; Silva, H.D. (2005) Métodos biométricos aplicados a análise de QTLs. *Anais do SEAGRO*, Universidade Estadual de Londrina.

Collard, B.C.Y.; Jahufer, M.Z.Z.; Brouwer, J.B.; Pang, E.C.K. (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, v. 142, p. 169-196.

Costa-Silva, J.; Domingues, D.; Lopes, F.M. (2017) RNA-Seq differential expression analysis: An extended review and a software tool. *PLoS ONE*. 12(12): e0190152.

Damalas, C.A.; Eleftherohorinos, I.G. (2011). Pesticide Exposure, Safety Issues, and Risk Assessment Indicators. *Int J Environ Res Public Health*, 8(5): 1402–1419.

Diao, Y.Z.; Zhang, C.; Liu, F.; Wang, W.Z.; Liu, L.; Cai, L.; Liu, X.L. (2017) Colletotrichum species causing anthracnose disease of chili in China. *Persoonia*, 38, 20–37.

Dodds, P.; Rathjen, J. (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat Rev Genet*, 11:539–548.

Doyle, J.J.; Doyle, J.L. (1987) A rapid DNA isolation method for small quantities of fresh tissues. Phytochemistry, 19: 11-15.

Eshbaugh, W. H. (1993) Peppers: history and exploitation of a serendipitous new crop discovery. *New Crops*, 132-139.

Eulgem, T.; Somssich, I.E. (2007) Networks of WRKY transcription factors in defense signaling. *Curr. Opin. Plant Biol*. 10:366–371.

Erwin, D.C.; Ribeiro, O.K. (1996) Phytophthora capsici. In *Phytophthora Diseases Worldwide*. p. 262-269.

Ferreira, A.; Silva, M.F.; Silva, L.C.; Cruz, C.D. (2006) Estimating the effects of population size and type on the accuracy of genetic maps. *Genetics Molecular Biology,* v. 29, p.182-192.

Ferreira, M. E.; Grattapaglia, D. (1998) Introducción al Uso de Marcadores moleculares en el Análisis Genético. EMBRAPA-CENARGEN, p.220.

Filgueira F.A.R. (2012). Novo Manual de Olericultura: Agrotecnologia Moderna na Produção e Comercialização de Hortaliças. Editora UFV, 3° ed.

Frederickson Matika, D.E.; Loake, G.J. (2014) Redox Regulation in Plant Immune Function. *Antioxid. Redox Signal*. 21:1373–1388.

Frizzone, J. A.; Gonçalves, A. C. A.; Rezende, R. (2001) Produtividade do pimentão amarelo, Capsicum annuum L., cultivado em ambiente protegido, em função do potencial mátrico de água no solo. Acta Scientiarum, vol. 23, n. 5, 1111 – 1116p.

Fujita, M.; Fijita, Y.; Noutoshi, Y.; Takahashi, F.; Narusaka, Y.; Yamaguchi-Shinozaki, K.; Shinozaki, K. (2006) Crosstalk between abiotic and biotic stress responses: A current view from the points of convergence in the stress signaling networks. *Curr. Opin. Plant Biol*., 9, 436–442.

Gagnot, S.; Tamby, J.P.; Martin-Magniette, M.L.; Bitton, F.; Taconnat, L.; Balzergue, S.; Aubourg, S.; Renou, J. P.; Lecharny, A.; Brunaud, V. (2008). CATdb: a public access to Arabidopsis transcriptome data from the URGV-CATMA platform. Nucleic acids research, 36(Database issue), D986–D990.

Galli, F. (1978) Manual de Fitopatologia vol.2: doenças das plantas cultivadas. 4. Ed. São Paulo: Editora Agronômica Ceres.

Gan, P.; Ikeda, K.; Irieda, H.; Narusaka, M.; O'Connell, R.J.; Narusaka, Y.; Takano, Y.; Kubo, Y.; Shirasu, K. (2013) Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of Colletotrichum fungi. *New Phytol*, 197(4):1236-49.

Garber, M.; Grabherr, M.G.; Guttman, M.; Trapnell, C. (2011) Computational methods for transcriptome annotation and quantification using RNA-seq. Nature Methods. V.8, p.469–477.

Garcia, A.; Kido, E.; Meza, A; et al. (2006) Development of an integrated genetic map of a sugarcane (Saccharum spp.) commercial cross, based on a maximum-likelihood approach for estimation of linkage and linkage phases. *Theor Appl Genet,* 112:298–314.

Geronimo, I.G.C. (2018) Melhoramento de Capsicum annuum visando resistência à antracnose: caracterização de isolados de Colletotrichum spp., herança da resistência e proposta de série diferenciadora. Tese de Doutorado. Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Giacomin, R.M.; Ruas, C.D.F.; Moreira, F.P.; et al. (2020) Inheritance of anthracnose resistance (Colletotrichum scovillei) in ripe and unripe Capsicum annuum fruits. *J Phytopathol*, 00:1–9.

González-Pérez, S.; Garcés-Claver, A.; Mallor, C.; Sáenz De Miera, L.E.S, Fayos, O., Pomar, F.; Merino, F.; Silvar, C. (2014) New insights into Capsicum spp. relatedness and the diversification process of Capsicum annuum in Spain. *PLoS ONE*, 9 (12), 1–23.

Granke, L.L.; Quesada-Ocampo, L.; Hausbeck, M.K. (2012) Advances in Research on Phytophthora capsici on Vegetable Crops in The United States. *Plant Dis*. 95:1588–1600.

Guan, D.; Yang, F.; Xia, X.; Shi, Y.; Yang, S.; Cheng, W.; He, S. (2018) CaHSL1 Acts as a Positive Regulator of Pepper Thermotolerance Under High Humidity and Is Transcriptionally Modulated by CaWRKY40. *Front. Plant Sci*., 9:1802.

Guerra, N. A. (2001) Estudios cromosómicos de cuatro selecciones de Capsicum chinense Jacq. Revista UDO Agrícola, v. 1; n. 1; 34-41 p.

Guttman, M.; Amit, I.; Garber, M.; French, C.; et al. (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature* 458, 223–227.

Hackett, C. A.; Milne, I.; Bradshaw, J.E.; Luo, Z. (2007) Tetraploid Map for Windows: linkage map construction and QTL mapping in autotetraploid species. *The Journal of heredity*, 98:727– 729.

Han, K.; Lee, H.Y.; Ro, N.Y.; Hur, O.S.; Lee, J.H.; Kwon, J.K.; Kang, B.C. (2018) QTL mapping and GWAS reveal candidate genescontrolling capsaicinoid content in Capsicum. *Plant Biotechnology Journal*, 16:1546–1558.

Han, K.; Jang, S.; Lee, JH. et al. (2019) A MYB transcription factor is a candidate to control pungency in Capsicum annuum. *Theor Appl Genet*, 132:1235–1246.

Han, Y.; Gao, S.; Muegge, K.; Zhang, W.; Zhou, B. (2015) Advanced applications of RNA sequencing and challenges. *Bioinform Biol Insights*. 9:29–46.

Hanif, R.; Iqbal, Z.; Iqbal, M.; Hanif, S.; Rasheed, M. (2006) Use of vegetables as nutritional food: Role in human health. *Journal of Agricultural and Biological Science*, 1(1):18-22.

Hao, C.; Xia, Z.; Fan, R.; Tan, L.; Hu, L.; Wu, B.; Wu, H. (2016) De novo transcriptome sequencing of black pepper (Piper nigrum L.) and an analysis of genes involved in phenylpropanoid metabolism in response to Phytophthora capsici. *BMC Genomics*, 17:822.

Hartley, S.W.; Mullikin, J.C. (2015) QoRTs: a comprehensive toolset for quality control and data processing of RNA-Seq experiments. *BMC Bioinformatics*, 16:224.

Hausbeck, M.K; Lamour, K.H. (2004) Research Progress and Management Challenges Phytophthora capsici on Vegetable Crops. *Plant Dis*. 88:1292–1303.

Heath, M.C. (1997) Evolution of plant resistance and susceptibility to fungal parasites. Plant Relationships, Springer, 257 - 276.

Heath, M.C. (2000) Host-pathogen warfare at the plant cell wall. *Current Opinion in Plant Biology*, 12:406 - 416.

Heil, M. (2010) Plastic defence expression in plants. Evolutionary. *Ecology*, 24: 555 -569.

Hill, T.A.; Ashrafi, H.; Wo, R.C.S.; Yao, J.; StoffeL, K.; Truco, J.M.; Kozik, A.; MIchelmore, R.W.; Deynze, A.V. (2013) Characterization of *Capsicum annuum* genetic diversity and population structure based on parallel polymorphism discovery with a 30K unigene pepper genechip*. Plos One*, v. 8, n. 4, p. 1-16.

Hrdlickova, R.; Toloue, M.; Tian, B. (2016) RNA-Seq methods for transcriptome analysis. *WIREs RNA*.

Hurtado-Hernandez, H.; Smith, P.G. (1985) Inheritance of mature fruit color in Capsicum annuum L. J. *Hered.* 76: 211-213.

IBGE, Censo agropecuário de 2017, disponível em: https://sidra,ibge,gov,br/pesquisa/censo-agropecuario/censo-agropecuario-2017,pdf, accessed in January 24, 2021.

Jesus-Pires, C.; Ferreira-Neto, J.R.C.; Bezerra-Neto, J.P.; Kido, E.A.; et al. (2019) Plant Thaumatin-like Proteins: Function, Evolution and Biotechnological Applications. *Current Protein & Peptide Science*, 21:36-51.

Jones, J.D.; Dangl, J.L. (2006) The plant immune system. *Nature*. 444:323-329.

Justino, E.V. (2013) Maturação fisiológica e taxa de cruzamento natural na produção de sementes de Capsicum sp. Dissertação (Mestrado em Agronomia) Brasília – DF - Universidade de Brasília/Faculdade de Agronomia e Medicina Veterinária, 142p.

Kang, B.C.; Nahm, S.H.; Huh, J.H.; Yoo, H.S.; Yu, J.W.; Lee, M.H.; Kim, B.D. (2001) An interspecific (Capsicum annuum × C. chinense) F2 linkage map in pepper using RFLP and AFLP markers. *Theoretical and Applied Genetics*, 102:531–539.

Kanto, T.; Uematsu, S.; Tsukamoto, T.; et al. (2014) Anthracnose of sweet pepper caused by Colletotrichum scovillei in Japan. *J Gen Plant Pathol,* 80:73–78.

Khan, F.A.; Mahmood, T.; Ali, M.; Saeed, A.; Maalik, A. (2014). Pharmacological importance of an ethnobotanical plant: Capsicum annuum L. Natural product research, 28(16). p.1267-1274.

Khan, S. (2015) QTL mapping: A tool for improvement in crops plants. *Research Journal of Recent Sciences*, v. 4, p. 7-12.

Kim, S; Park, M.; Yeom, S-I; Kim, Y-M; *et al*. (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. *Nat Genet* 46, 270–8.

Kim, S.H.; Yoon, J.B.; Do, J.W.; Park, H.G. (2008). A major recessive gene associated with anthracnose resistance to Colletotrichum capsici in chilli pepper (Capsicum annuum L.). *Breeding Science*, 58, 137–141.

Kim, S.; Kim, S-T.; Kim, D-H.; Yang, E-Y.; Cho, M-C.; Arshad, J.; et al (2010) Identification of Quantitative Trait Loci Associated with Anthracnose Resistance in Chili Pepper (Capsicum spp.). Horticultural Science & Technology, 28:1014-1024.

Ko, M.K.; Jeon, W.B.; Kim, K.S.; Lee, H.H.; Seo, H.H.; Kim, Y.S.; Oh, B.J. (2005) A Colletotrichum gloeosporioides-induced esterase gene of nonclimacteric pepper (Capsicum annuum) fruit during ripening plays a role in resistance against fungal infection. *Plant Mol Biol*, 58:529–541

Ko, M.; Cho, J.H.; Seo, HH.; et al. (2016) Constitutive expression of a fungus-inducible carboxylesterase improves disease resistance in transgenic pepper plants. *Planta*, 244:379–392.

Kong, X.; Zhou, Q.; Luo, F.; Wei, B.; Wang, Y.; Sun, H.; et al. (2019). Transcriptome analysis of harvested bell peppers (Capsicum annuum L.) in response to cold stress. *Plant Physiology and Biochemistry*, 139:314–324.

Konishi, A.; Furutani, N.; Minamiyama, Y.; Ohyama, A. (2019) Detection of quantitative trait loci for capsanthin content in pepper (Capsicum annuum L.) at different fruit ripening stages. *Breeding Science Preview*.

Kosambi, D.D. (1944) The estimation of map distance from recombination values. *Annuaire of Eugenetics*, 12:172-175.

Kourelis, J.; van der Hoorn, R.A.L. (2018) Defended to the Nines: 25 Years of Resistance Gene Cloning Identifies Nine Mechanisms for R Protein Function. *The Plant Cell*, 30:285–299.

Kraft, K. H.; Brown, C. H.; Nabhan, G. P.; Luedeling, E.; Luna Ruiz, J. D. J.; Coppens D'eeckenbrugge, G.; Gepts, P. (2014) Multiple lines of evidence for the origin of domesticated chili pepper, Capsicum annuum. *Mexico. Proceedings of the National Academy of Sciences*, 111(17), 6165–6170.

Lahkar, J.; GoswamI, D.; Deka, S.; Ahmed, G. (2018) Novel approaches for application of biosurfactant produced by Pseudomonas aeruginosa for biocontrol of Colletotrichum capsici responsible for anthracnose disease in chilli. European Journal of Plant Pathology, 150(1), 57–71.

Laloi, C.; AppeL, K.; Danon, A. (2004) Reactive oxygen signalling: The latest news. *Curr. Opin. Plant Biol*, 7, 323–328.

Lambert, I.; Roux, C.P-L.; Colella, S.; Martin‐Magniette, M-L. (2020) DiCoExpress: a tool to process multifactorial RNAseq experiments from quality controls to co‐expression analysis through differential analysis based on contrasts inside GLM models. *Plant Methods*, 16:68.

Lamour, K.; Hausbeck, M. (2000) Mefenoxam insensitivity and the sexual stage of Phytophthora capsici in Michigan cucurbit fields. *Phytopathology*, 90:396–400.

Lamour, K.H.; Stam, R.; Jupe, J.; Huitema, E. (2011) The oomycete broad-host-range pathogen Phytophthora capsici. *Molecular Plant Pathology*, 13(4), 329–337.

Lamour, K.H.; Mudge, J.; Gobena, D.; Hurtado-Gonzales, O.P.; Schmutz, J.; Juo, A.; et al. (2012). Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen Phytophthora capsici. *Mol. Plant Microbe Interact*., 10:1350–1360.

Lamour, K.H.; Mudge, J.; Gobena, D.; Hurtado-Gonzales, O. P.; et al. (2012) Genome Sequencing and Mapping Reveal Loss of Heterozygosity as a Mechanism for Rapid Adaptation in the Vegetable Pathogen Phytophthora capsici. *Mol. Plant-Microbe Interact*. 25:1350–1360.

Lander, E.S.; Botstein, D. (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, 1121(1):185-99.

Lander, E.S.; Green, P. ; Abrahamson, J.; Barlow, A.; Daly, M.J.; Lincoln, S.E.; Newberg, L.A. (1987) MAPMAKER: an interactive computer package for

constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, 1:174-181.

Lannou, C. (2012) Variation and selection of quantitative traits in plant pathogens. *Annual Review of Phytopathology*, Palo Alto, v. 50, p. 319-338.

Lee, S.B.; Kim, J.E.; Kim, H.T.; Lee, G.M.; Kim, B.S.; Lee, J.M. (2020) Genetic mapping of the c1 locus by GBS-based BSA-seq revealed Pseudo-Response Regulator 2 as a candidate gene controlling pepper fruit color. *Theor Appl Genet* 133, 1897–1910.

Lee, H.R.; Bae, I.H.; Park, S.W.; Kim, H.J.; Min, W.K.; Han, J.H.; Kim, K.T.; Kim, B.D. (2009) Construction of an integrated pepper map using RFLP, SSR, CAPS, AFLP, WRKY, rRAMP, and BAC end sequences. *Mol. Cells,* 27: 21–37.

Lee, H.R., Kim, K.T., Kim, H.J., Han, J.H., Kim, J.H., Yeom, S.I., Kim, H.J., Kang, W.H., Shi, J., Park, S.W., Bae, I.H., Lee, S., Cho, J., Oh, D., Kim, B.D. (2011) QTL analysis of fruit length using rRAMP, WRKY, and AFLP markers in chili pepper. *Horticulture, Environment, and Biotechnology*, 52(6):602–613.

Lee, J.; Hong, J-W.; Do, J.W.; Yoon, J.B. (2010) Identification of QTLs for Resistance to Anthracnose to Two Colletotrichum Species in Pepper. J. Crop Sci. Biotech, 13:227-233.

Lefebvre, V.; Kuntz, M.; Camara, B.; Palloix, A. (1998) The capsanthin-capsorubin synthase gene; a candidate gene for the y locus controlling the red fruit colour in pepper. *Plant Mol. Biol.* 36: 785-789.

Lefebvre, V.; Palloix, A.; Caranta, C.; Pochard, E. (1995) Construction of an intraspecific integrated linkage map of pepper using molecular markers and doubled-haploid progenies. *Genome,* 38: 112–121.

Lefebvre, V.; Palloix, A. (1996) Both epistatic and additive effects of QTLs are involved in polygenic induced resistance to disease: a case study, the interaction pepper- Phytophthora capsici Leonian. *Theor Appl Genet*, 93:503-511.

Li, N.; Yin, Y; Wang, F.; Yao, M. (2018) Construction of a high-density genetic map and identification of QTLs for cucumber mosaic virus resistance in pepper

(Capsicum annuum L.) using specific length amplified fragment sequencing (SLAF-seq). *Breeding Science,* 68: 233–241.

Li, P.; Piao, Y.; Shon, H.S.; Ryu, K.H. (2015) Comparing the normalization methods for the differential analysis of Illumina high-throughput RNA-Seq data. *BMC bioinformatics*. 16(1):347.

Li, W.; Cheng, J.; Wu, Z.; Qin, C.; Tan, S.; Tang, X.; Cui, J.; Zhang, L.; Hu, K. (2015) An InDel-based linkage map of hot pepper (Capsicum annuum). *Molecular Breeding*, v.35, Article number: 32.

Li, Y.; Yu, T.; Wu, T.; Wang, R.; Wang, H.; Du, H.; et al. (2020). The dynamic transcriptome of pepper (Capsicum annuum) whole roots reveals an important role for the phenylpropanoid biosynthesis pathway in root resistance to Phytophthora capsici. *Gene*, 144288.

Lin, Q.; Kanchana-Udomkan, C.; Jaunet, T.; Mongkolporn, O. (2002) Análise genética da resistência à antracnose de pimenta causada por Colletotrichum capsici. *Tailândia Journal of Agricultural Science*, 35:259-264.

Liu, B.H. (1998) Statistical genomics: linkage, mapping, and QTL analysis. *Boca Raton: CRC Press*, p.611.

Liu, F.; Yu, H.; Deng, Y.; Zheng, J.; Liu, M. (2017) PepperHub, a Pepper Informatics Hub for thechili pepper research community. *Mol. Plant*, 10, 1129−1132.

Liu, W.Y.; Kang, J.H.; Jeong, H.S.; Choi, H.J.; Yang, H.B.; Kim, K.T.; Choi, D.; Choi, G.J.; Jahn, M.; Kang, B.C. (2014) Combined use of bulked segregant analysis and microarrays reveals SNP markers pinpointing a major QTL for resistance to Phytophthora capsici in pepper. *Theor. Appl. Genet*. 127, 2503–2513.

Liu, Z.; L, J.; Zhang, Z.; Li, H.; Yang, B. (2019) Integrative Transcriptome and Proteome Analysis Identifies MajorMetabolic Pathways Involved in Pepper Fruit Development. *J.ProteomeRes*, 18:982−994.

Livingstone, K.D.; Lackney, V,K.; Blauth, J.R.; van Wijk, R.; Jahn, M.K. (1999) Genome mapping in capsicum and the evolution of genome structure in the solanaceae. *Genetics,* 152:1183-202.

Loake, G.; Grant, M. (2007) Salicylic acid in plant defence-the players and protagonists. *Curr. Opin. Plant Biol*. 10:466–472.

Lobo, Jr, M.; Silva-Lobo, V. L; Lopes, C. A. (2001) Reação de genótipos de Capsicum spp. (pimentas e pimentão) à antracnose (Colletotrichum gloeosporioides). *Fitopatologia Brasileira*, 26:373.

Lobo, V.L. da S.; Giordano, L.; Lopes, C.A. (2005) Herança da Resistência à Mancha-Bacteriana em Tomateiro. Fitopatologia Braileira. 30(4): 343 – 349.

Lopes, C.A., Ávila, A.C. (2002) Informações inadequadas sobre a resistência a doenças em catálogos de cultivares de hortaliças: um exemplo para tomate e pimentão. *Horticultura Brasileira*, Brasília, 20(2):130-132

Lorieux, M.; Ndjiondjop, M-N; Ghesquiere, A. (2000) A first interspecific Oryza sativa x Oryza glaberrima microsatellite-based genetic map. *Theor. Appl. Genet*. V. 100, p. 591-601.

Lu, J.; Guo, M.; Zhai, Y.; Gong, Z.; Lu, M. (2017) Differential Responses to the Combined Stress of Heat and Phytophthora capsici Infection Between Resistant and Susceptible Germplasms of Pepper (Capsicum annuum L.). *Journal of Plant Growth Regulation*. V.36, 161–173.

Lynch, M.; Walsh, B. (1998) Genetics and analysis of quantitative traits. *Sunderland: Sinauer*, p. 980.

Mackey, D.; Mcfall, A.J. (2006) MAMPs and MIMPs: proposed classifications for inducers of innate immunity. *Mol Microbiol*. 61:1365-1371. 2.

Mahasuk, P.; Khumpeng, N.; Wasee, S.; Taylor, P.W.J.; Mongkolporn, O. (2009) Inheritance of resistance to anthracnose (Colletotrichum capsici) at seedling and fruiting stages in chili pepper (Capsicum spp.). *Plant Breed,* 128:701–706.

Mahasuk, P.; Struss, D.; Mongkolporn, O. (2016) QTLs for resistance to anthracnose identified in two Capsicum sources. *Mol Breeding*, 36:10.

Maillot, G. (2018) Étude d'effecteurs de l'oomycète pathogène Phytophthora capsici exprimés au cours de l'interaction avec le piment et la tomate. Sciences agricoles. Université d'Avignon, France.

Majid, M.U.; Awan, M.F.; Fatima, K.; Tahir, M.S.; Ali, Q.; Rashid, B.; Rao, A.Q.; Nasir, I.A.; Husnain, T. (2016) Phytophthora capsici on chilli pepper (Capsicum annuum L.) and its management through genetic and bio-control: a review. Zemdirbyste-Agriculture, 103:419-430.

Mallard, S.; Cantet, M.; Massire, A.; Bachellez, A.; Ewert, S.; Lefebvre, V. (2013) A. key QTL cluster is conserved among accessions and exhibits broad-spectrum resistance to Phytophthora capsici: a valuable locus for pepper breeding. *Mol. Breeding*, 32, 349–364.

Mansfeld, B.N.; Colle, M.; Kang, Y.; Jones, A.D.; Grumet, R. (2017). Transcriptomic and metabolomic analyses of cucumber fruit peels reveal a developmental increase in terpenoid glycosides associated with age-related resistance to Phytophthora capsici. *Hortic. Res*, 4:17022.

Margarido, G.R.A.; Grcia, A.A.F.; Souza, A.P. (2007) OneMap: software for genetic mapping in outcrossing species. *Hereditas,* Lund, v. 144, p. 78-79.

Marra, M.; Hillier, L.; Allen, M.; Kucaba, T. (1999) An encyclopedia of mouse genes. *Nat. Genet*. 21, 191–194.

Marto, F.; Viana, P.; Parente, G.B. (2007) Controle das principais doenças do pimentão cultivado nas regiões serranas do Estado do Ceará.

Matiello, R.R.; Barbieri, R.L.; Carvalho, F.I.F. (1997). Resistência das plantas a moléstias fúngicas. *Ciência Rural*, 27(1), 161-168.

Minamiyama, Y., Tsuro, M., Hirai, M. (2006) An SSR-based linkage map of Capsicum annuum. *Molecular Breeding*, 18:157–169.

Minamiyama, Y.; Tsuro, M.; Kubo, T.; Hirai, M. (2007) QTL analysis for resistance to Phytophthora capsici in pepper using a high density SSR-based map. Breeding Science, 57:129–134.

Mimura, Y.; Inoue, T.; Minamiyama, Y.; Kubo, N. (2012) An SSRbased genetic map of pepper (Capsicum annuum L.) serves as na anchor for the alignment of major pepper maps. *Breed. Sci*., 62:93–98.

Mollinarii, M. (2009) Comparação de algoritmos usados na construção de mapas genéticos. Dissertação de mestrado em Genética e Melhoramento de Plantas,

Escola Superior de Agricultura “Luiz de Queiroz”, *Universidade de São Paulo*, Piracicaba, p. 73.

Mongkolporn, O.; Taylor, P.W.J. (2018) Chili anthracnose: Colletotrichum taxonomy and pathogenicity. *Plant Pathology*, 67, 1255–1126.

Montri, P.; Taylor, P.W.J.; Mongkolporn, O (2009). Pathotypes of Colletotrichum capsici, the Causal Agent of Chili Anthracnose, in Thailand. Plant Disease, n. 93, 17-20 p.

Moore, J.W.; Loake, G.J.; Spoel, S.H. (2011) Transcription Dynamics in Plant Immunity. 23:2809–2820.

Moscone, E.A.; Baranyi, M.; Ebert, I.; Greilhuber, J.; Ehrendorfer, F. (2003) Analysis of nuclear DNA content in Capsicum (Solanaceae) by flow cytometry and feulgen densitometry. *Annals of Botany*, 92:21–29.

Moscone, E.A.; Scaldaferro, M.A.; Gabriele, M.; Cecchini, N.M.; García, Y.S.; Jarret, R.; Daviña, J.R.; Ducasse, D.A.; Barboza, G.E.; Ehrendorfer, F. (2007) The Evolution of Chili Peppers (Capsicum – Solanaceae): a Cytogenetic Perspective. *Acta Horticulture* 745. 137- 170.

Moulin, M.M. (2013) Construção de mapa genético, identificação de QTLs associados a caracteres agronômicos e detecção de inibidores de protease em Capsicum baccatum var. pendulum. (Tese de doutorado) 129p. Universidade Estadual do Norte Fluminense (UENF). Campos dos Goytacazes-RJ.

Moulin, M.M.; Rodrigues, R.; Bento, C.S.; Gonçalves, L.S.A.; Santos, J.O.; Sudré, C.P.; Viana, A.P. (2015) Genetic dissection of agronomic traits in Capsicum baccatum var. pendulum. Genetics and Molecular Research 14 (1): 2122-2132.

O'Connell, R.J.; Thon, M.R.; Hacquard, S.; Amyotte, S.G.; Kleemann, J.; Torres, M.F.; Damm, U.; Buiate, E.A.; Epstein, L.; et al. (2012) Lifestyle transitions in plant pathogenic Colletotrichum fungi deciphered by genome and transcriptome analyses. *Nat Genet*., 44(9):1060-5.

Oo, M,M.; Lim, G.; Jang, H.A.; Oh, S.K. (2018) Characterization and Pathogenicity of New Record of Anthracnose on Various Chili Varieties Caused by Colletotrichumscovillei in Korea. *Mycobiology*, 45:3, 184-191.

Oshlack, A.; Robinson, M.D.; Young, M.D. (2010) From RNA-seq reads to differential expression results. *Genome biology*. 11(12):1.

Pakdeevaraporn, P.; Wasee, S.; Taylor, P.W.J.; Mongkolporn, O. (2005) Inheritance of resistance to anthracnose caused by Colletotrichum capsici in Capsicum. *Plant Breeding*, 124(2):206–208.

Pandey, D.; Rajendran, S.R.C.K.; Gaur, M.; Sajeesh, P.K.; Kumar, A. (2016) Plant defense signaling and responses against necrotrophic fungal pathogens. J. Plant Growth Regul. 35(4), 1159–1174.

Paran, I,; Voort, J.R.V.; Lefebvre, V.; Jahn, M.; Landry, L.; Schriek, M.; Tanyolac, B.; Caranta, C,; Chaim, A.B.; Livingstone, K.; Palloix, A.; Peleman, J. (2004) An integrated genetic linkage map of pepper (Capsicum spp.). *Molecular Breeding*, 13:251–261.

Parlevliet, J.E. (2002) Durability of resistance against fungal, bacterial and viral pathogens; present situation. Euphytica, 124:147–156.

Park, H.K.; Kim, B.S.; Lee, W.S. (1990) Herança de resistência à antracnose 15 (Colletotrichum spp.) em pimenta (Capsicum annuum L.). I. Análise genética da resistência à antracnose por cruzamentos dialélicos. *Journal of the Korean Society for Horticultural Science,* 31: 91-105.

Park, M.; Lee, JH.; Han, K.; et al. (2019) A major QTL and candidate genes for capsaicinoid biosynthesis in the pericarp of Capsicum chinense revealed using QTL-seq and RNA-seq. *Theor Appl Genet*, 132:515–529.

Park, S.; Jeong, W.Y.; Lee, J.H.; Kim, Y.H.; Jeong, S.W.; Kim, G.S.; shin, S.C. (2012) Determination of polyphenol levels variation in Capsicum annuum cv. Chelsea (yellow bell pepper) infected by anthracnose (Colletotrichum gloeosporioides) using liquid chromatography–tandem mass spectrometry. *Food Chemistry*, 130:981–985.

Paterson, A.H.; Tanksley, S.D.; Sorrels, M.E. (1991) DNA markers in plant improvement. *Advances in Agronomy*, v.46, p.39-89.

Paterson, A.H.; Bowers, J.E.; Burow, M.D.; Draye, X.; Elsik, C.G.; Jiang, C.X.; et al (2000). Comparativegenomics of plant chromosomes. Plant Cell, 12:1523-1539.

Pavan, M.A.; Krause-Sakate, R.; Moura, M.F.; Kurozawa, C. (2016) Doenças das Solanáceas. In: Manual de fitopatologia: Doenças das plantas cultivadas. CERES.

Pereira, L.S.; Souza, T.A.M.; Walter, R.; Sudré, C.P.; Santos, L.A.; *et al.* (2021) Identification of enzyme inhibitors and antimicrobial activities from *Capsicum annuum* L. protein extracts against *Colletotrichum scovillei*. *Hortic. Environ. Biotechnol.*

Perry, L.; Dickau, R.; Zarrillo, S.; Holst, I.; Pearsall, D. M.; Piperno, D. R.; Zeidler, J. A. (2007) Starch fossils and the domestication and dispersal of chili peppers (Capsicum spp. L.). *Americas. Science*, 315(5814), 986–988.

Pickersgill, B. (1986) Capsicum spp. In: León, J. (Org.). Guidelines for seed exchange and plant introduction in tropical crops. FAO, 73-78.

Pickersgill, B. (1991) Cytogenetics and evolution of Capsicum L. In: Tsuchiya, T., Gupta, P.K. (eds.) Chromosome engineering in plants: genetics, breeding, evolution, part B, Amsterdam: *Elsevier*. p.139-160.

Popovsky, S.; Paran, I. (2000) Molecular genetics of the y locus in pepper; its relation to capsanthin-capsorubin synthase and to fruit color. *Theor. Appl. Genet.* 101: 86-89.

Prince, J.; Pochard, E.; Tanksley, S. (1993) Construction of a molecular linkage map of pepper and a comparison of synteny with tomato. *Genome*, 36:404–417.

Quesada-Ocampo, L.M.; Hausbeck, M.K. (2010) Resistance in tomato and wild relatives to crown and root rot caused by Phytophthora capsici. *Phytopathology*, 100, 619– 627.

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramalho, M.A.P.; Santos, J.B.; Zimmermann, M.J. (1993) Genética quantitativa em plantas autógamas: aplicações ao melhoramento do feijoeiro. Editora UFG, Goiânia, 271p.

Rehrig, W.Z.; Ashrafi, H.; Hill, T.; Prince, J.; Van Deynze, A. (2014) CaDMR1 cosegregates with QTL Pc5.1 for resistance to Phytophthora capsici in pepper (Capsicum annuum). *Plant Genome* 7.

Reifschneider, F.J.B. (2000), Capsicum: pimentas e pimentões no Brasil. EMBRAPA Hortaliças, Brasília. p. 11.

Reis, A.; Boiteux, L.S.; Heins, G.P. (2009) Antracnose em Hortaliças da Família Solanacea. Circular Técnica 79, EMBRAPA Hortaliças.

Ribeiro, C.S.C.; Reifschneider, F.J B. (2008) Genética e melhoramento. In: Ribeiro, C.S.C.; Lopes, C.A.; Carvalho, S.I.C.; Henz, G.P.; Reifschneider, F.J.B. (org). Pimentas Capsicum. Brasilia: Embrapa Hortaliças, 2008. p. 55-69.

Ridzuan, R.; Rafii, M.Y.; Ismail, S.I.; Yusoff, M.M.; Miah, G.; Usman, M. (2018) Breeding for Anthracnose Disease Resistance in Chili: Progress and Prospects. *Int. J. Mol*. Sci. 2018, 19(10), 3122.

Roberts, P.D.; Pernezny, K.L.; Kucharek, T.A. (2001) Anthracnose caused by Colletotrichum sp. on pepper [Internet]. Gainesville (FL): University of Florida.

Rosa, A.; Deiana, M.; Casu, V.; Paccagnini, S.; Appendino, G.; Ballero, M.; Dessí, A.M. (2002). Antioxidant activity of capsinoids. *Journal of agricultural and food chemistry,* 50:7396-7401.

Rose, L.E.; Bittner-Eddy, P.D.; Langley, C.H.; Holub, E.B.; Michelmore, R.W.; Beynon, J.L. (2004) The Maintenance of Extreme Amino Acid Diversity at the Disease Resistance Gene, RPP13, in Arabidopsis thaliana. *Genetics*, 166:1517–1527.

Rotblat, B.; Enshell-Seijffers, D.; Gershoni, J.M.; Schuster, S.; Avni, A. (2002) Identification of an essential component of the elicitation active site of the EIX protein elicitor. *Plant J.* 32:1049-1055.

Rufino, J.L.S.; Penteado, D.C.S. (2006) Importância econômica, perspectivas e potencialidades do mercado para pimenta. Informe Agropecuário, 27:7-15.

Sahitya, U.; Sri, D.R.; Pedda, K.D.; Suneetha, P.; Krishna, M.S.R. (2014) Anthracnose, a prevalent disease in capsicum. Res. J of Pharma. *Biol. and Chemical Sci*, 5(3):1583-1604.

Santner, A.; Calderon-Villalobos, L.I.A.; Estelle, M. (2009) Plant hormones are versatile chemical regulators of plant growth. *Nature Chem Biol*, 5:301–307.

Santos, H.S.; Goto, R. (2004) Enxertia em plantas de pimentão no controle da murcha de fitóftora em ambiente protegido. *Hortic. Brasileira*: 22:45-49.

Schuster, I.; Cruz, C.D. (2004) Estatística genômica aplicada a populações derivadas de cruzamentos controlados. Editora UFV, Viçosa, 568p.

Sekhwal, M.K.; Li, P.; Lam, I.; Wang, X.; Cloutier, S.; You, F.M. (2015) Disease resistance gene analogs (RGAs) in plants. *International Journal of Molecular Sciences,* 16:19248–19290.

Semagn, K.; Bjørnstad, Å.; Ndjiondjop, M.N. (2006) An overview of molecularmarker methods for plant. *Afr. J. Biotechnol*, v. 25, p. 2540-2569

Seybold, H.; Trempel, F.; Ranf, S.; Scheel, D.; Romeis, T.; Lee, J. (2014) Ca2+ signalling in plant immune response: From pattern recognition receptors to Ca2+ decoding mechanisms. *New Phytol*. 204:782–790.

Shirasawa, K.; Ishii, K.; Kim, C.; Ban, T.; Suzuki, M.; Ito, T.; Muranaka, T.; Kobayashi, M.; Nagata, N.; Isobe, S.; Tabata, S. (2012) Development of Capsicum EST–SSR markers for species identification and in silico mapping onto the tomato genome sequence. *Molecular Breeding*, 4:201-1210.

Siddique, M.I.; Lee, H.Y.; Ro, N.Y; Han, K.; venkatesh, J.; Solomon, A.M.; Patil, A.S.; Changkwian, A.; Kwon, J.K.; Kang, B.C. (2019) Identifying candidate genes for Phytophthora capsici resistance in pepper (Capsicum annuum) via genotyping-by-sequencing-based QTL mapping and genome-wide association study. *Scientific Reports*., 9:9962.

Silva, L.C. (2005) Simulação do tamanho da população e da saturação do genoma para mapeamento genético de RILs. Tese de Doutorado. Universidade Federal de Viçosa.

Silva, L.R.A.; Rodrigues, R.; Pimenta, S.; Correa, J.W.S.; Araújo, M.S.B.; Bento, C.S.; Sudré, C.P. (2017) Inheritance of bacterial spot resistance in Capsicum annuum var. annuum. *Genetics and Molecular Research*, 16 (2): gmr16029631.

Silva, L.R.A. (2018) Genetic resistance to xanthomonas euvesicatoria and X. gardneri IN Capsicum annuum: inheritance studies and the proposal of a new gene involved in the hypersensitive response. Tese de Doutorado. Universidade Estadual do Norte Fluminense Darcy Ribeiro.

Silva, S.A.M.; Rodrigues, R.; Gonçalves, L.S.A.; Sudré, C.P.; Bento, C.S.; Carmo, M.G.F.; Medeiros, A.M. (2014) Resistance in Capsicum spp. to anthracnose affected by different stages of fruit development during pre- and post-harvest. *Trop. plant pathol*., 39:335-341.

Slate, J. (2005) Quantitative trait locus mapping in natural populations: progress, caveats and future directions. *Molecular ecology*, v. 14, n. 2, p. 363–79.

Souza, S.J.; Camargo, A.A.; Briones, M.R.; Costa, F.F.; et al. (2000) Identification of human chromosome 22 transcribed sequences with ORF expressed sequence tags. *Proc. Natl. Acad. Sci*. USA. 97, 12690–12693.

Souza, C.S.; Paulsen, B.S.; Devalle, S.; Costa, L.S.; Borges, H.L.; Rehen, S.T. (2015) Commitment of human pluripotent stem cells to a neural lineage is induced by the pro-estrogenic flavonoid apigenin. *Advances in Regenerative Biology*, 2: 29244.

Spoel, S.H.; Dong, X. (2008) Making sense of hormone crosstalk during plant immune response. *Cell Host Microbe*, 3, 348–351.

Sudré, C.P.; Gonçalves, L.S.A.; Rodrigues, R.; Amaral Júnior, A.T.; Riva-Souza, E.M.; Bento, C.S. (2010) Genetic variability in domesticated Capsicum spp as assessed by morphological and agronomic data in mixed statistical analysis. *Genetics and Molecular Research*, 9(1):283-294.

Sugita, T.; Kinoshita, T.; Kawano, T.; Yuji, Y.K.; Yamaguchi, K.; Nagata, R. (2005) Rapid construction of a linkage map using hight-efficiency genome scanning/ AFLP and RAPD, based on an Intraspecific doubled-haploid population of Capsicum annuum. *Breeding Science*, 55:287-295.

Sun, C.Y.; Mao, S.L.; Zhang, Z.H.; Palloix, A.; Wang, L. H.; Zhang, B. X. (2015) Resistances to anthracnose (Colletotrichum acutatum) of Capsicum mature green and ripe fruit are controlled by a major dominant cluster of QTLs on chromosome P5. *Scientia Horticulturae*, 181, 81–85.

Tandonnet, S; Torres, T.T. (2017) Traditional versus 3' RNA-seq in a non-model species. Genom. Data 18(11), 9–16.

Tanksley, S.D. (1993) Mapping polygenes. *Annual Review Genetics*, Palo Alto, v.27, p.205-33.

Tanksley, S.D.; Bernatzky, R.; Lapitan, N.L.; Prince, J.P. (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proceedings of the National Academy of Sciences of the United States of America, 85:6419–6423.

Thabuis, A.; Palloix, A.; Pflieger, S.; Daubeze, A.M.; Caranta, C.; Lefebvre, V. (2003) Comparative mapping of Phytophthora resistance loci in pepper germplasm: evidence for conserved resistance loci across Solanaceae and for a large genetic diversity. *Theor. Appl. Genet*. 106, 1473– 1485.

Thabuis, A.; Lefebvre, V.; Bernard, G.; Daubèze, A.M.; Phaly, T.; Pochard, E.; Palloix, A. (2004) Phenotypic and molecular evaluation of a recurrent selection program for a polygenic resistance to Phytophthora capsici in pepper. *Theor. Appl. Genet*. 109, 342–351.

Thakur, R.P.; Rao, V.P.; Wu, S.M.; Subbarao, K.V.; Mathur, K.; Tailor, H.C.; Kushwaha, U.S.; Dwivedi, R.R.; Krisnaswamy, R.; Hiremath, R.V.; Indira, S. (2007) Genetic resistance to foliar anthracnose in sorghum and pathogenic variability in Colletotrichum graminicola. *Indian Phytopath,* 60:13–23

Than, P.P.; Jeewon, R.; Hyde, K.D.; Pongsupasamit, S.; Mongkolporn, O.; Taylor, P.W.J. (2008) Characterization and pathogenicity of Colletotrichum species

associated with anthracnose on chilli (Capsicum spp.) in Thailand *Plant Patology*; n. 57; 562-572 p.

The Plant List. Available in: http://www,theplantlist,org/1,1/browse/A/Solanaceae, accessed in December 20, 2020.

Toledo, E.R.; Leandro, R.A.; Souza Júnior, C.L.; Souza, A.P. (2008) Mapeamento de QTLs: uma abordagem bayesiana. *Revista Brasileira de Biometria*, 26:107-114.

Torres-Calzada, C.; Tapia-Tussel, R. (2015) Sensitivity of Colletotrichum truncatum to four fungicides and characterization of thiabendazole-resistant isolates. Plant Disease, 99:1590-1595.

Tozze Júnior, H.J.; Firmino, A.C.; Fischer, I.H.; Furtado, E.L.; Massola Júnior, N.S. (2015). Caracterização de isolados de Colletotrichum spp. associados às frutíferas no Estado de São Paulo. Summa Phytopathologica, 41(4), 270-280.

Truong, H.T.H.; Kim, K.T.; Kim, D.W.; Kim, S.; Chae, Y.; Park, J.H.; Oh, D.G.; Cho, M.C. (2012) Identification of isolate-specific resistance QTLs to phytophthora root rot using an intraspecific recombinant inbred line population of pepper (Capsicum annuum). *Plant Pathol*. 61, 48–56.

Untergasser, A.; Cutcutache, I.; KOressaar, T.; Ye, J.; Faircloth, B.C.; Remm, M.; Rozen, S.G. (2012) Primer3 - new capabilities and interfaces. Nucleic Acids Research, 40(15):e115.

Van Dijk, E.L.; Jaszczyszyn, Y.; Thermes, C. (2014) Library preparation methods for next-generation sequencing: tone down the bias. *Exp Cell Res*. 322:12–20.

Van Loon, L.C.; Rep, M.; Pieterse, C.M.J. (2006) Significance of inducible defense- related proteins ininfected plantas. *Annual Revisiw Phytopathol*, 44: 135-162.

Van Ooijen, J.W.; Voorrips, R.E. (2001) JoinMap 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands.

Van Ooijen, J.W. (2006) JoinMap version 4.0: software for the calculation of genetic linkage maps. Kyazma BV, Wageningen, The Netherlands, 23p.

Van Zonneveld, M.; Ramirez, M.; Williams; D. E.; Petz, M.; Meckelmann, S.; Avila; T.; Scheldeman, X. (2015) Screening genetic resources of Capsicum peppers in their primary center of diversity in Bolivia and Peru. *PLoS ONE*.

Vleeshouwers, V.G.A.A.; Rafaelle, S.; Vossen, J.H.; Champouret, N.; Oliva, R.; et al. (2011) Understanding and exploiting late blight resistance in the age of effectors. Annu. *Rev. Phytopathol*. 49, 507–31.

Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Lee, T.; Hornes, M.; Friters, A.; Pot, J.; Paleman, J.; Kuiper, M.; Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting, *Nucleic Acids Research*, 23:4407–4414.

Walker, S.J.; Bosland, P.W. (1999). Inheritance of Phytophthora root rot and foliar blight resistance in pepper. *J. Am. Soc. Hortic. Sci*., 124:14–18.

Wang, D.; Bosland, P. W. (2006) The genes of Capsicum. HortScience, 41(5), 1169–1187.

Wang, E.T.; Sandberg, R.; Luo, S.; Khrebtukova, I.; et al. (2008) Alternative isoform regulation in human tissue transcriptomes. *Nature*. 456, 470–476.

Wang, P.; Wang, L.; Guo, J.; Yang, W.; Shen, H. (2016) Molecular mapping of a gene conferring resistance to Phytophthora capsici Leonian race 2 in pepper line PI201234 (Capsicum annuum L.). Mol. Breeding 36, 66.

Wang, Z.; Gerstein, M.; Snyder, M. (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*. 10(1):57–63.

Wan, J.S.H.; Liew, E.C.Y. (2020) Efficacy of chemical and biological agents against pepper blight (Phytophthora capsici Leonion) in East Asia: a meta-analysis of laboratory and field trial data. *Journal of Plant Pathology*. 102, 835–842 (2020).

Wasternack, C.; Hause, B. (2013) Jasmonates: Biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Ann. Bot*. 111:1021–1058.

Welbaum, G.E. (2015). Family solanaceae in vegetable production and practices (p. 486). *Davis, CA: CAB*.

Wen, J; Zhang, Y.-M. (2013) Multi-QTL mapping for quantitative traits using distorted markers. *Mol. Breeding*, 31, 395–404.

Wit, P.J.G.M. (2007) How plants recognize pathogens and defend them selves cellular and molecular. *Life Sciences Swtzerrland*, 64: 2726 - 2732.

Wu, C.; Chao, G.; Lu, W. (2007) Genetic Analysis of Grain filling rate in different growth stages of superior and inferior grains in rice. *Scientia Agricultura Sinica,* 40:1135-1141.

Wu, F.; Eannetta, N.T.; Xu, Y.; Durrett, R.; Mazourek, M.; Jahn, M.M.; Tanksley, S.D. (2009) A COSII genetic map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus Capsicum. *Theor. Appl. Genet*., 118: 1279–1293.

Wu, Y.; Zhang, D.; Chu, J.Y.; Boyle, P.; Wang, Y.; Brindle, I.D.; De Luca, V.; Després, C. (2012) The Arabidopsis NPR1 Protein Is a Receptor for the Plant Defense Hormone Salicylic Acid. Cell Rep. 1:639–647.

Xu, S. (2008) Quantitative Trait Locus Mapping Can Benefit From Segregation Distortion. *Genetics*, 180:2201-2208.

Xu, X.; Chao, J.; Cheng, X.; Wang, R.; Sun, B.; Wang, H.; Luo, S.; Xu, X.; Wu, T.; Li, Y. (2016) Mapping of a novel race specific resistance gene to phytophthora root rot of pepper (Capsicum annuum) using bulked segregant analysis combined with specific length amplified fragment sequencing strategy. *PLoS ONE 11*, e0151401.

Yang, J.; Benyamin, B.; Mcevoy, B. P.; Gordon, S.; Henders, A. K.; Nyholt, D. R.; et al. (2010) Common SNPs explain a large proportion of the heritability for human height. *Nat. Genet*. 42, 565–569.

Yarnes, S.C.; Ashrafi, H.; Reyes-Chin-Wo, S.; Hill, T.A.; Stoffel, K.M.; Deynze, A.V. (2013) Identification of QTLs for capsaicinoids, fruit quality, and plant architecture-related traits in an interspecific Capsicum RIL population. *Genome*, v.56, Number 1.

Yi, G.; Lee, J.M.; Lee, S.; Choi, D.; Kim, B.D. (2006) Exploitation of pepper EST–SSRs and an SSR-based linkage map. *Theoretical and Applied Genetics*, 114:113–130.

Yi, Y.S.; Lee, D.J.; Yeom, S-I.; Yoon, J.; Kim, Y-K.; Kwon, S-Y.; Choi, D. (2020) A novel pepper (Capsicum annuum) receptor-like kinase functions as a negative regulator of plant cell death via accumulation of superoxide anions. *New Phytologist*, 185: 701-715.

Zaidi, S.S.; Mukhtar, M.S.; Mansoor, S. (2018) Genome Editing: Targeting susceptibility genes for plant disease resistance. *Trends in Biotechnology*, 36:898–906.

Zimmer, A.R.; Leonardi, B.; Miron, D.; Schapoval, E.; Oliveira, J.R.; Gosmann, G. (2012) Antioxidant and anti-inflammatory properties of Capsicum baccatum: from traditional use to scientific approach. *Journal of Ethnopharmacology*. 139:228- 2.

Zhang, H-X.; Feng, X-H.; Jin, J-H.; Khan, A.; Guo, W-L.; Du, X-H.; Gong, Z-H. (2020) CaSBP11 Participates in the Defense Response of Pepper to Phytophthora capsici through Regulating the Expression of Defense-Related Genes. *Int. J. Mol. Sci*., 21:9065.

Zhang, X.F.; Wang, G.Y.; Dong, T.T.; Chen, B.; Du, H.S.; Li, C.B.; et al. (2019) High-density genetic map construction and QTL mapping of first flower node in pepper (Capsicum annuum L.). *BMC Plant Biol*, 19, 167.

Zhang, Z.H.; Jhaveri, D.J.; Marshall, V.M,; Bauer, D.C.; Edson, J.; Narayanan, R.K.; et al. (2014) A comparative study of techniques for differential expression analysis on RNA-Seq data. *PloS one*. 9(8):e103207.

Zhang, Z.X.; Zhao, S.N.; Liu, G.F.; et al. (2016) Discovery of putative capsaicin biosynthetic genes by RNA-Seq and digital gene expression analysis of pepper. *Sci Rep* 6, 34121.

Zhao, Y. (2010) Auxin biosynthesis and its role in plant development. *Annu Rev Plant Biol*, 61:49–64.

APPENDIX

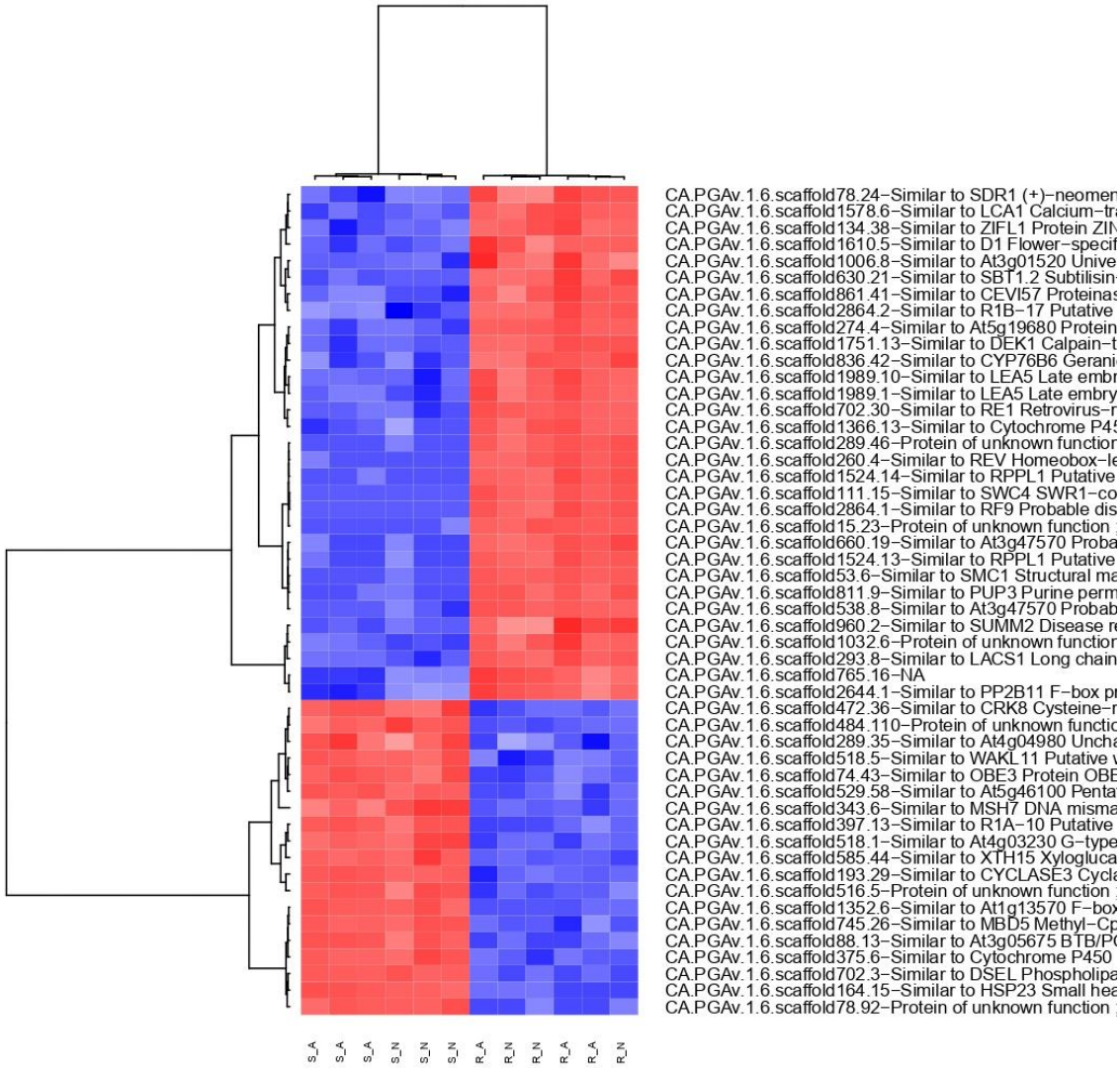


Figure 1. Heatmap analysis of the 50 most variable genes in resistant and susceptile hosts of *Capsicum annuum* inoculated with adapted and non-adapted isolate of *Phytophthora capsici* evaluated at 24 hpi,. The lines described the genes and the columns are the conditions: **S_A:** Susceptible genotype infected by the adapted isolate; **R_A:** Resistant genotype infected by the adapted isolate; **S_N:** Susceptible genotype infected by the non-adapted isolate; **R_N:** Resistant genotype infected by the non-adapted isolate; Red color indicates up-regulated genes and blue colors indicate down-regulated genes.

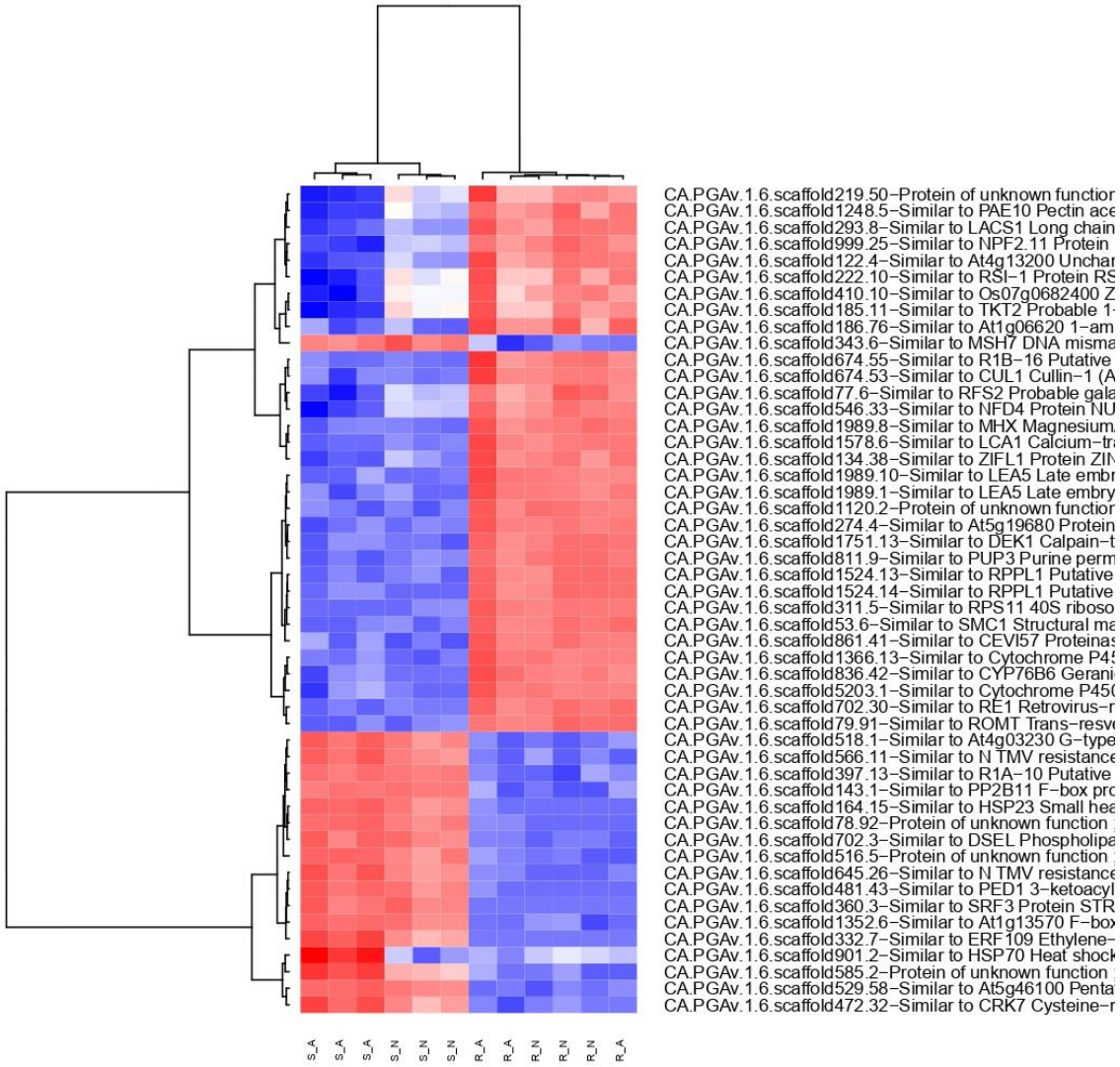


Figure 2. Heatmap analysis of the 50 most variable genes in resistant and susceptile hosts of *Capsicum annuum* inoculated with adapted and non-adapted isolate of *Phytophthora capsici* evaluated at 72 hpi,. The lines described the genes and the columns are the conditions: **S_A:** Susceptible genotype infected by the adapted isolate; **R_A:** Resistant genotype infected by the adapted isolate; **S_N:** Susceptible genotype infected by the non-adapted isolate; **R_N:** Resistant genotype infected by the non-adapted isolate; Red color indicates up-regulated genes and blue colors indicate down-regulated genes.

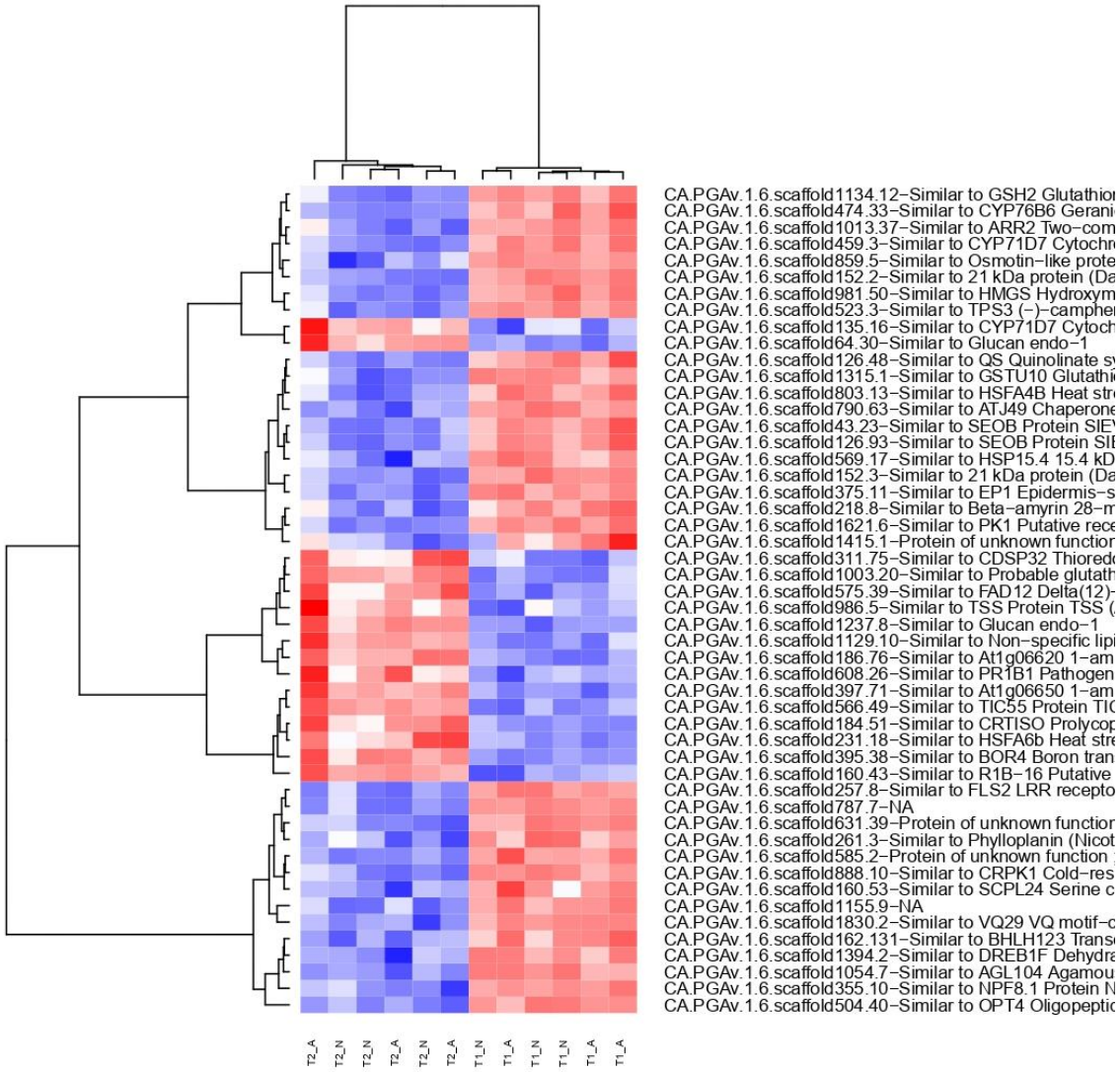


Figure 3. Heatmap analysis of the 50 most variable genes in resistant *Capsicum annuum* host, inoculated with adapted and non-adapted isolate of *Phytophthora capsici* and evaluated at 24 and 72 hpi,. The lines described the genes and the columns are the conditions: **T1_A:** Adapted isolate at 24hpi; **T1_N:** Non-adapted isolate at 24hpi; **T2_A:** Adapted isolate at 72hpi; **T2_N:** Non-adapted isolate at 72hpi.

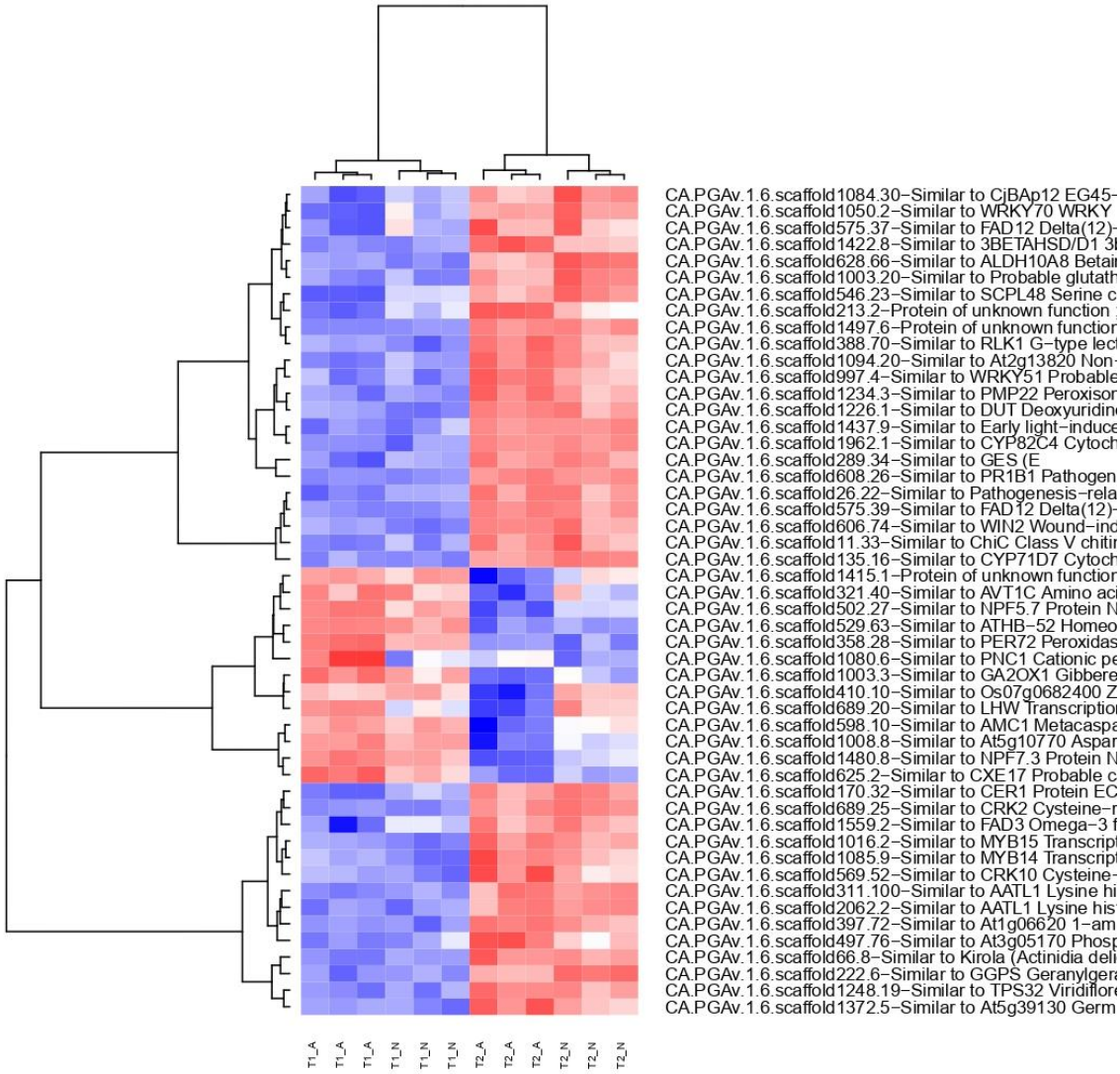


Figure 4. Heatmap analysis of the 50 most variable genes in susceptible *Capsicum annuum* host, inoculated with adapted and non-adapted isolate of *Phytophthora capsici* and evaluated at 24 and 72 hpi,. The lines described the genes and the columns are the conditions: **T1_A:** Adapted isolate at 24hpi; **T1_N:** Non-adapted isolate at 24hpi; **T2_A:** Adapted isolate at 72hpi; **T2_N:** Non-adapted isolate at 72hpi.