UNIVERSITY OF BIRMINGHAM

Complex trait analysis in the autotetraploid potato and the response of the potato cell wall to abiotic and biotic stress.

by

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ABSTRACT

Potato is important for food security due to its nutritional value and ability to grow in diverse environments around the world. However, its productivity is highly affected by two principal stressors: late blight caused by *P. infestans* and drought. Previous analyses described the dynamic role of the plant cell wall in growth and development, and its response to various external factors, including biotic or abiotic stresses. Indeed, it is the first barrier that pathogens must cross to successfully infect the plant, while modulation of components to fortify the cell wall is important for stress tolerance. Nevertheless, there is a need to understand in more detail how the dynamic response of individual cell wall components influences stress tolerance in potato varieties.

This thesis employs glycome and transcriptomic analyses in varieties with contrasting tolerance phenotypes to identify genes and cell wall components associated with tolerance or resistance of potato varieties to *P. infestans* and drought stress. For both types of stress, there were tolerant-specific responses, but also many commonalities in the response of tolerant and susceptible varieties, although tolerant varieties tended to mount a stronger and faster response, which is likely crucial. Pre-existing differences in gene expression and cell wall composition were also widespread and likely prepare the tolerant varieties to enable a more effective response when the stressor arises. Key differences relating to the pectin component of the cell wall included higher levels of pectin methylesterification in tolerant varieties before stress and its effective modification in response to stress to strengthen cell wall structure or release cell wall fragments to stimulate an immune response. Key commonalities in the response of tolerant varieties to both types of stress included upregulation of an expansin gene and stronger upregulation of genes involved in lignin synthesis. An important goal in future work will be to relate such changes to guard cell development and the regulation of stomatal opening/closure, a key component of the response to both types of stress.

Ultimately, an improved understanding of the response to stress paves the way for the development of cultivars with improved stress resistance and yield stability under stress. However, since its genome is autotetraploid, there remain many challenges for genetic analysis and/or manipulation of complex traits in potato. Therefore, the final part of this project presented in “Appendix - Linkage disequilibrium” provides a theoretical exploration of one of the key concepts for genome-wide association analyses (linkage disequilibrium) in autotetraploid genomes, addressing its symmetry and dependence on key features of an autotetraploid meiosis.

I dedicate this thesis to my parents and siblings for all the love and support that, even being far away, I have received so warmly. To my beloved husband, Jimmy, who throughout this period has always been by my side, encouraging and advising me, always being my great companion. And especially to my baby inside me, whom I look forward to while I write this thesis.

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AIR: alcohol insoluble residual

CoMPP: Comprehensive microarray polymer profiling

CWDE: cell wall degrading enzymes

DAMP: damage-associated molecular pattern

DEG: Differentially expressed genes

ETI: effector-triggered immunity

GWAS: Genome-wide association mapping

HG: homogalacturonan

H.P.I: Hours post infection

D: Linkage disequilibrium

OGAs: oligogalacturonides

PCA: Principal component analysis

PG: polygalacturonases

PL: pectate lyases

PME: pectin methyl esterase

PMEI: pectin methyl esterase inhibitor

PRR: pattern-recognition receptor

PTI: pattern-triggered immunity

QTL: quantitative trait locus

RLKs: Receptor Like Kinases

RLPs: Receptor Like Proteins

RG-I: rhamnogalacturonan I

RG-II: rhamnogalacturonan II

WAK: wall-associated kinase

XTH: xyloglucan endotransglucosylase/hydrolase

CHAPTER 1: General Introduction

1.1. Importance of the potato crop

Potato is one of the most important crops worldwide, ranking fourth with respect to total global production and third based on global consumption (FAOSTAT, 2020; Devaux *et al*., 2021). This is mainly due to its high nutritional value and ability to grow in diverse cultivation environments. Potato tubers are rich in nutrients. In addition to their high carbohydrate, potato tubers also contain protein, minerals, vitamins A and C, and a moderate amount of fat. The ecological plasticity of cultivated potato varieties allows them to be cultivated in different regions around the world with different altitudes, from sea level to 5,000 m above sea level, and different latitudes, from countries with a day length like Ecuador to day length like Finland (Campos and Ortiz, 2020). These characteristics allow this crop to reach a global annual production of potato of 359 million tons in 2020 (FAOSTAT, 2020). For all these reasons, potato is considered a vital crop for global food security (Devaux, Kromann, and Ortiz, 2014).

Potato is the crop with a highly abundant genetic diversity (Hardigan *et al*., 2017). Both cultivated and wild potatoes present various levels of ploidy, ranging from monoploid (n = 12) to pentaploid (5n = 60), reaching up to hexaploids in the case of wild varieties. The cultivated varieties are grouped into 4 species. One of them is *Solanum tuberosum*, which is grouped into two subspecies: subsp. *Andigena*, of variable ploidy, adapted to short-day conditions and grown mainly in the Andes; and subsp. *Tuberosum* or also called *Chilotanum*, is a tetraploid adapted to long-day conditions and the most widely cultivated variety in the rest of the world.

The tetraploid condition of the cultivated potato and its highly heterozygous genome complicates breeding programs. Due to the complicated pattern of chromosomal segregation, it is challenging to determine the genetic basis of some important traits. Also, it is difficult to obtain the combination of

favourable alleles in more than two loci to obtain a desirable phenotype (Muthoni *et al*., 2019). For this reason, breeders often prefer to use diploid varieties, although tetraploid varieties may yield more than their diploid counterparts (Muthoni *et al*., 2019). Recently, new methodologies and tools have been adapted for the analysis of tetraploid populations and have helped in determining the genetic bases of traits (Rosyara *et al*., 2016; Chen *et al*., 2018). However, there are still some concepts that need to be refined in relation to tetraploid inheritance. One of these is linkage disequilibrium, the non-random association of two alleles at different loci. Linkage disequilibrium is important because the estimate of linkage decay allows us to determine the number of molecular genetic markers that need to be employed in genetic association studies for a particular species (Bourke *et al*., 2018).

The potential or expected yield of the potato crop is usually not the actual yield produced in the field. This gap exists mainly because of abiotic or biotic stresses that affect the development of the plant and, particularly, the tubers (Koch *et al*., 2020) and will be expected to increase due to climate change (Savary *et al*., 2019). Due to biotic stresses, a global loss of 17% in the potato crop occurred between 2010 and 2014, with the main cause being late blight disease (Savary *et al*., 2019). Potato cultivation is also negatively affected by several environmental factors that include long periods of drought, extreme temperatures, and ion toxicity, such as salinity stress. Potato plants are highly sensitive to these environmental factors, and the combination of heat, drought, and salinity stress will increase potato crop losses. The quality of the crop is also affected; for example, under drought, the potato produces tubers with cracks or internal brown spots, in addition to a large amount of toxic glycoalkaloids (Dahal *et al*., 2019). It is predicted that the annual loss of potato production caused only by drought will be 74-95% by 2050s in England and Wales (Daccache *et al*., 2012). Even if improved varieties are planted in the fields, 9 to 18% of the potato production is estimated to decrease due to climate change. However, in the absence of improved potato varieties, the decline will be accentuated to 18 to 32% (Hijmans, 2003).

1.2. Plant responses to abiotic and biotic stresses.

Plants are sessile organisms that must withstand different environmental conditions as well as interact with different microorganisms growing in the same environment. To survive against negative impacts from their environment, plants possess special structures or molecular mechanism that can be constitutively present or activated under specific negative conditions (abiotic stress) or microbial attacks (biotic stress).

1.2.1. Plant responses to abiotic stress

Depending on duration and intensity, different stresses can produce detrimental changes in plants, reducing their survival, final yield, and quality. Depending on the crop variety, various stresses can also generate an adaptative response. A multilevel response will determine these final consequences, first by sensing the stress, second by emitting signals into the cell, third by activating downstream pathways, and fourth by changes at the transcription, translation, or post-translational levels (Zhu *et al*., 2016, Zhang *et al*., 2022).

Plants can sense environmental stresses through their cellular structures like membrane receptors or by the changes in the biochemical state of their cells (Zhu *et al*., 2016, Zhang *et al*., 2022). An example of a membrane receptor is COLD1, which is involved in cold perception and chilling tolerance in rice (Ma *et al*., 2015). Change in the cellular state can be sensed by alteration in the composition or fluidity of the plasma membrane, in response to temperature shifts (Cano-Ramirez *et al*., 2021). After sensing the stress, common intracellular stress signals, as observed in osmotic, salt, cold, and heat stress, include the increment of intracellular calcium and reactive oxygen species (ROS), and the activation of serine and threonine protein kinases called mitogen-activated protein kinases (MAPKs) (Zhu, 2016). The Ca^{2+} and ROS waves can be rapidly transmitted over long distances from cell to cell and their effect in distal cells can be observed at the transcriptional level, giving rise to a systemic acquired acclimation (SAA) (Szechyńska-Hebda *et al*., 2017).

1.2.1.1. Plant responses to drought.

Plants have developed different strategies to respond to drought, such as escape, avoidance, tolerance, and recovery, which are not mutually exclusive (Manavalan *et al*., 2009). Physiologically, the reduced availability of water under drought negatively affects the process of photosynthesis, limiting the amount of photosynthates that are transported to sink organs. This water loss can be controlled by stomatal opening/closure and density, as observed in woody species (Yin *et al*., 2020). Under normal conditions, the first mechanism that regulates the opening of the stomata is the pathway related to the light signal transduction of the guard cells, while under stress conditions, the mechanism is influenced by the phytohormone abscisic acid (ABA) (Roelfsema and Hedrich, 2005, Jia and Zang, 2008).

ABA is an important phytohormone that mediates drought responses and tolerance. It is synthesized in the vascular tissue from where is delivered to guard cells (Endo *et al*. 2008; Merilo *et al*., 2015). Two ABA precursors are synthesized in the chloroplast by the carotenoid biosynthetic pathway. These two precursors, 9-*cis*-neoxanthin and 9-*cis*-violaxanthin, are converted to xanthoxin by the action of 9-*cis*-epoxycarotenioid dioxygenase (NCED), the rate-limiting enzyme in ABA synthesis. After two reactions in the cytosol, by the action of SDR/ABA2 plus ABA3 and AAO enzymes, newly synthesized ABA is imported into guard cells by the ABCG40 transporter (Ali *et al*., 2020). Inside, ABA binds the PYR/PYL/RCAR complex, which in turn binds to PP2C. In the absence of ABA, PP2C is bound to and inactivates the subclass III SnRK2, but when ABA is present, PP2C binds the complex of ABA-PYR/PYL/RCAR and liberates the SnRK2. Then, the subclass III SnRK2 activates itself by autophosphorylation, and can then phosphorylates transcription factors (TFs) (Ali *et al*., 2020). Phosphorylation can either activate TFs, such as AREB/ABF, or inactivate TFs, such as AKS1, which is a bHLH transcription factor that promote the transcription of the KAT1 transporter (Takahashi *et al*., 2017) (**Figure 1.1**).

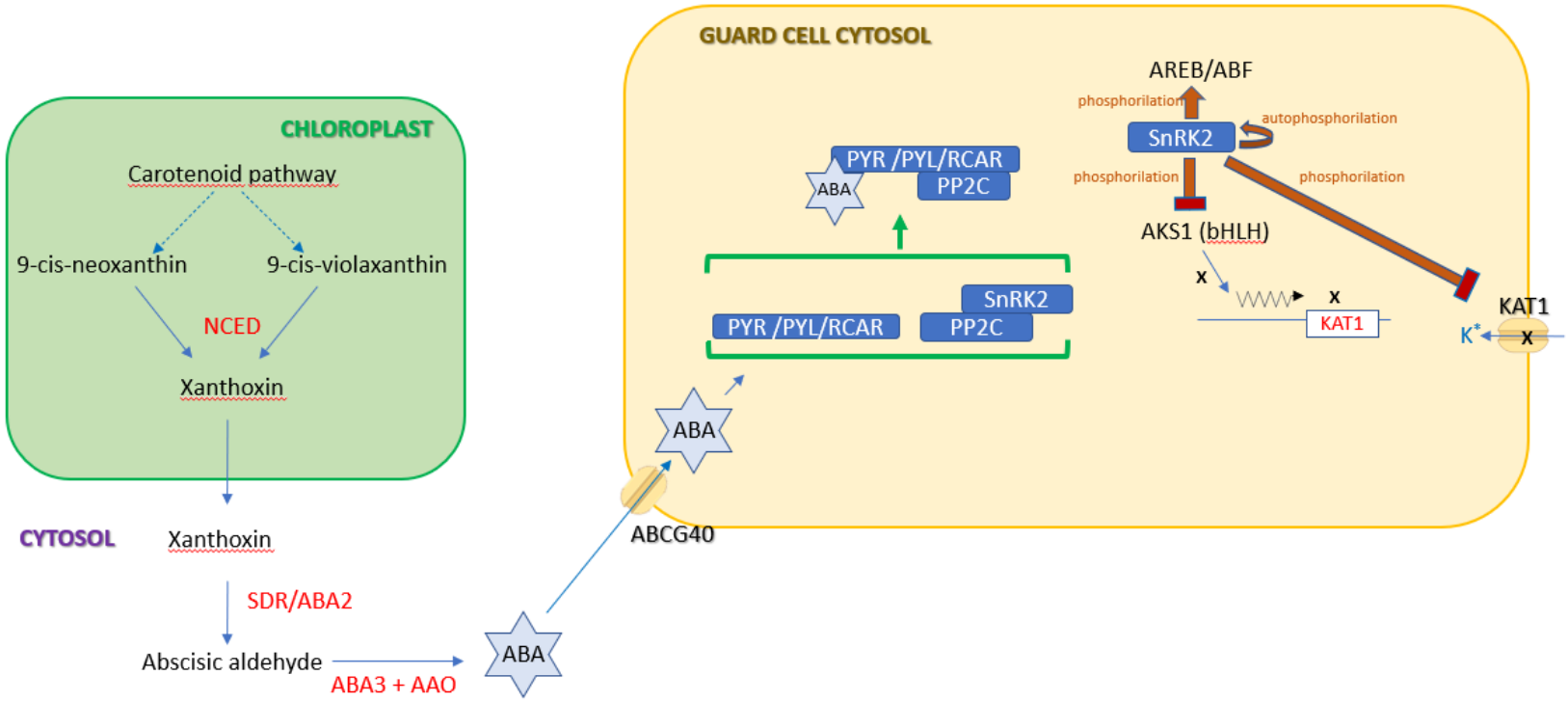


Figure 1.1 Synthesis and response to ABA under drought. Precursors of ABA, 9-*cis*-neoxanthin and 9-*cis*-violaxanthin, are synthesized in the chloroplast by a carotenoid pathway. They are later transformed into xanthonxin by the NCED (9-*cis*-epoxycarotenioid dioxygenase) enzyme. Xanthonxin is exported to the cytosol and converted into abscisic aldehyde by the SDR/ABA2 (short-chain dehydrogenase/reductase/ABA2). Then, by the action of ABA3 and AAO (Arabidopsis aldehyde oxidase 3), abscisic aldehyde is converted to ABA. ABA is imported into the guard cell cytosol by the ABCG40 (ATP-binding cassette G-40 transporter) transporter. In the guard cell, ABA binds to PYR/PYL, which then binds to PP2C (protein phosphatase 2), releasing SnRK2 (sucrose nonfermenting-1-related protein kinase 2). Free SnRK2 is activated by autophosphorylation and by phosphorylation it can activate AREB/ABF transcription factors and inactivate AKS1, a bHLH transcription factor, which represses the transcription of KAT1 (ABA-responsive kinase 1) transporter. SnRK2 can also inactivate the KAT1 transporter by phosphorylation and restrict the import of K^+.

SnRK2 also inactivates the KAT1 transporter by phosphorylation. KAT1 is involved in K^+ influx, therefore inactivation or a low concentration of this transporter decreases K^+ concentration in the guard cell, which along with its release, is necessary for stomatal closure under drought stress (Ali *et al*., 2020). SnRK2 also phosphorylates and activates other transporters such as the slow anion channel associated 1 (SLAC1), which is involved in anion exchange for stomatal closure. ABA also induces the influx of Ca^{2+} into the guard cells, producing a reduction in the stomatal opening. (Ali *et al*., 2020). Stomatal closure generates a decrease in CO_2 assimilation, which in turn reduces the regeneration of $NADP^+$ in the Calvin cycle and the carboxylation function of RuBisCo, both contributing to the generation of ROS. In response to oxidative stress, various antioxidant enzymes are produced to attenuate oxidative damage (Legay *et al*., 2011).

Under drought stress, the level of cellular metabolites is also altered. Sugars, like glucose, fructose, and the raffinose family oligosaccharides (RFOs), including raffinose and galactinol, accumulate to maintain osmotic balance, during the early stress response. This is followed by an increase in the concentration of amino acids, such as proline and GABA. Changes in sugar and amino acid concentrations are therefore considered to be responses related to moderate stress and severe stress, respectively. Other types of amino acids whose levels increase under severe stress are the branched-chain amino acids, such as valine, leucine, and isoleucine (Fàbregas *et al*., 2019). Aromatic acids, like phenylalanine and tryptophan, also accumulate under osmotic stress (Fàbregas *et al*., 2019). Phenylalanine is an important precursor for the synthesis of phenols and functions as a scavenger of ROS, conferring tolerance to various abiotic stresses (Sharma *et al*., 2019). When ROS balance is lost, protein structures are damaged, and to stabilize their conformations, the chaperones such as heat shock proteins (HSPs) are induced (Legay *et al*., 2011).

1.2.2. Plant responses to biotic stress

Plants possess mechanisms to protect themselves and recognize invading pathogens at the early stages of infection. Pathogens, however, continually develop new strategies to evade plant defences. This creates a competition in which molecular interactions between plants and pathogens are continuously evolving, a process often referred to as an "arms race". The first line of defence in plants is the Pattern-Triggered Immunity (PTI). It is activated by the detection of highly conserved structures on the pathogens, called pathogen-associated molecular patterns (PAMPs), or by detecting damage associate molecular patterns (DAMPs) of the plant cell. PAMPs include flagellins, lipopolysaccharides, peptidoglycans, and fungal chitin, all of which are essential molecules for pathogen survival. DAMPs are components from the cell host released from damaged or infected cells, including cell wall fragments, extracellular protein fragments, peptides, nucleotides, amino acids, etc. (Malinovsky, Fangel, and Willats, 2014). PAMPs and DAMPS are recognized by plant pattern-recognition receptors (PRRs) that are localized in the plasma membrane of each cell. PRRs include Receptor-Like Kinases (RLKs) or Receptors-Like Proteins (RLPs). The extracellular recognition given by PRRs generates a pattern-triggered immunity (PTI). This PTI response involves an increase in the concentration of Ca^{2+} and H^{+} influx in the cytosol, accumulation of ROS, hormones such as salicylic acid (SA), callose deposition, and widespread transcriptional changes (Li *et al.*, 2016).

Pathogens can overcome plant defences by secreting proteins called effectors that can modulate and inhibit PTI. In turn, plants possess intracellular receptors that can recognize these effectors and activate their second line of defence, called Effector Trigger Immunity (ETI). This ETI response is generally associated with a hypersensitive response, which is a localized cell death in the site where infection occurs, to prevent pathogen spread. ETI is activated directly by the detection of pathogen effectors or by the identification of changes in host protein structures modified by the pathogen effectors. This detection is facilitated by monitoring proteins that function as molecular guards and are called resistance (R) proteins encoded by R genes (Miller *et al*., 2017) (**Figure 1.2**).

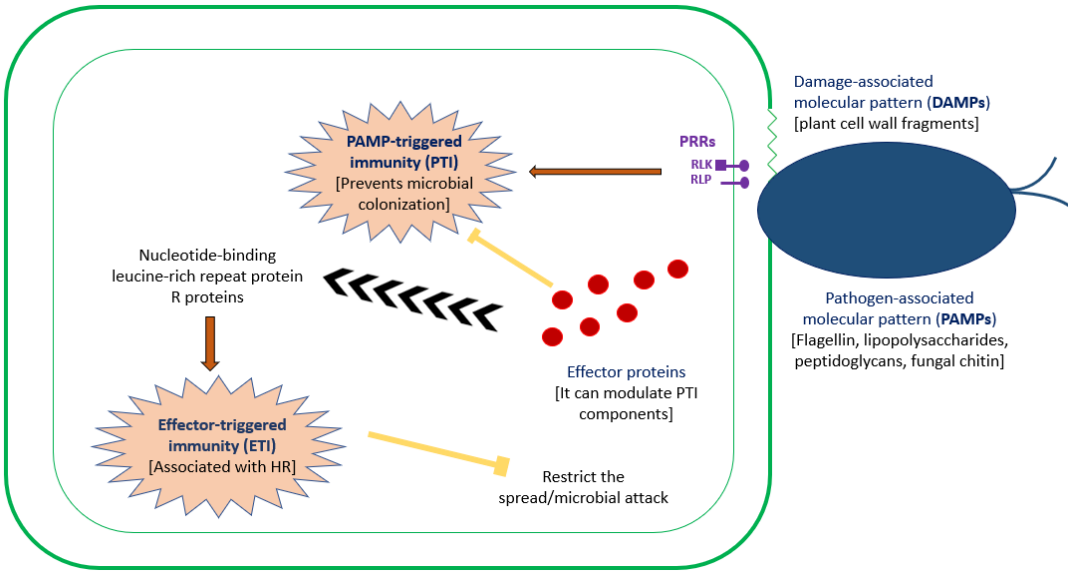


Figure 1.2. Overview of plant-pathogen interaction. Plants can sense the infection by recognizing either pathogen associated- or damage associated- molecular patterns (PAMPs, DAMPs). DAMPs and PAMPs can be recognized by transmembrane receptors called pattern-recognition receptors (PRRs). PRRs can either possess or not possess an intracellular kinase domain and are called receptor like kinase (RLK) or receptor like proteins (RLP), respectively. After DAMP or PAMP recognition, the host plant triggers a first line of immune response called PAMP-triggered immunity (PTI). PTI can be suppressed by pathogen effectors secreted by the pathogen. In turn, these effectors can be recognized by intracellular plant receptors called R proteins, which trigger a second line of immune response called effector-triggered immunity (ETI), associated with a hypersensitive response (HR) and cell death.

PRRs involved in cell surface immunity possess an extracellular domain that interacts with extracellular ligands, and depending on this domain, they are classified into subfamilies. These subfamilies include leucine-rich repeat (LRR), lysin motif (LysM), lectin, epidermal growth factor (EGF)-RLK, or RLP receptors. This diversity is important because different extracellular regions recognize different structures (Bentham *et al*., 2020). For example, receptors with an extracellular LRR domain recognize protein ligands, while receptors with the extracellular EGF domain, like the wall-associated kinase (WAK) receptor, can recognize pectin fragments derived from the host cell wall and emitted as a DAMP signal. After ligand recognition, PRR receptors can form homodimers or interact with co-receptors to form heterodimers, to produce an intracellular response (Bentham *et al*., 2020) (**Figure 1.3**).

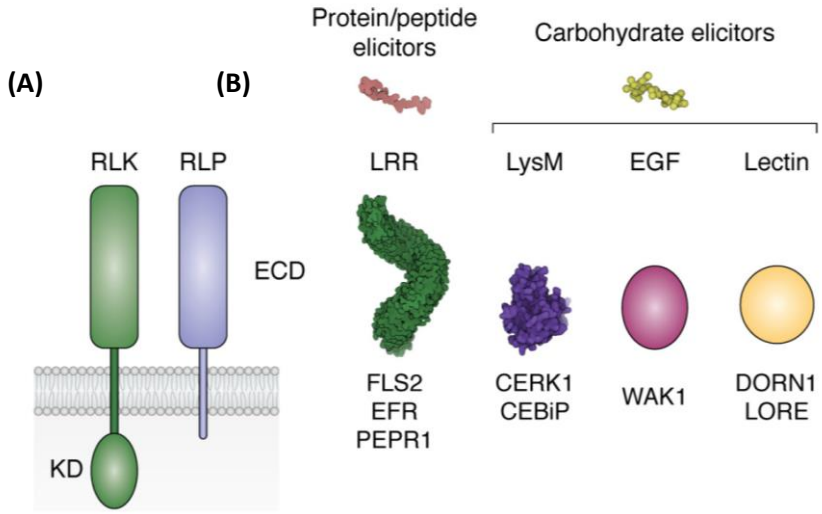


Figure 1.3 PRRs and their extracellular domains. PRRs are divided into two groups depending on their intracellular domain. If it has a kinase domain (KD), it is named as a receptor like kinase (RLK); if not, it is named as a receptor like protein (RLP) **(A)**. Depending on their extracellular domain, the receptors recognize different elicitors **(B)**. Reproduced from Bentham *et al*., 2020.

The majority of plant R genes encode for intracellular receptors classified as NLR immune receptors, with a structure containing a nucleotide binding site (NBS) domain and a leucine-rich repeat (LRR) domain. The NBS domain, also known as the NB-ARC domain (nucleotide-binding (NB) adaptor shared by ARC (APAF1 (apoptotic protease activating factor 1), R proteins and CED-4 (cell death 4)) domain) is a highly conserved central domain involved in activation of the protein. This region can bind to ADP or ATP and change its conformation from an inactive (bound to ADP) to an active (bound to ATP) form. Indeed, after effector recognition, ATP binding is observed to cause a conformational change that results in activation (de Araújo *et al*., 2019). The LRR domain, located in the C-terminal region, is composed of 10-40 tandem LRRs and can recognize pathogen effectors. The N-terminal is a variable structure that can contain either a Toll/interleukin-1 (TIR) domain, called TNL, or a non-TIR domain. The non-TIR domain can be a coil-coil (CC) or an RPW8 domain, called CNL and RNL, respectively. This region plays a role in activating the pathway for a hypersensitive response (de Araújo *et al*., 2019) (**Figure 1.4**).

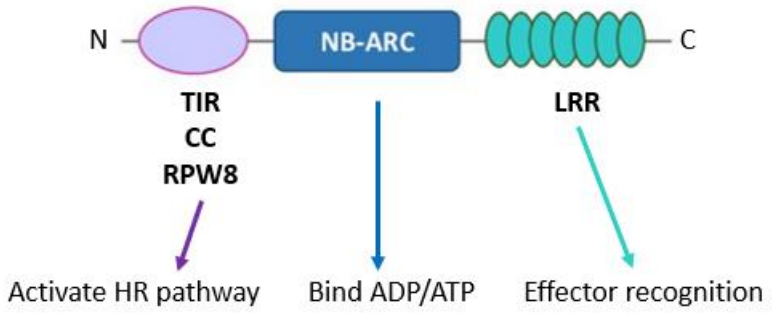


Figure 1.4 Structure of R proteins. R proteins carry a central NB-ARC (Nucleotide-Binding adaptor shared by Apoptotic protease activating factor 1 (APAF1), R proteins and Cell death 4(CED-4)) domain capable of interacting with ADP or ATP to transform into an activated form. The C-terminal region has an LRR (leucine-rich repeat) domain involved in effector recognition. In the N-terminal region different domains can be found, such as TIR (or Toll/Interleucina-1 receptor), CC (coil-coil) or RPW8 (Resistance to Powdery Mildew 8). These domains can activate the pathway that generates a hypersensitive response (HR). Adapted from Gottin *et al*., 2021.

R proteins can interact directly or indirectly with pathogen effectors to trigger an immune response, with direct recognition of the effectors mediated by the LRR domain. Indirect interaction can be observed by an intermediate protein that after interacting with the effector undergoes a conformational change that is recognized by the R proteins (van der Hoorn and Kamoun, 2008).

After ligand perception by PRRs or by the R proteins, several mechanisms are activated in the cell, some of which are common between the PTI and ETI responses. After perception of PAMPs or DAMPs by PRRs, an increase in intracellular Ca^{2+} and an oxidative burst is observed, as well as callose deposition, activation of MAPKs, and an increase in phytohormones, including SA, jasmonic acid (JA), and ethylene (Liu *et al*., 2013, Lal, *et al*., 2018, Yuan, *et al*., 2021). All of these responses have been observed after the activation of the cytosolic kinase BIK protein that binds a PRR with its co-receptor (Liu *et al*., 2013, Lal *et al*., 2018). In addition, Ca^{+2} influx generated in the PTI response can produce stomatal closure (Thor *et al*., 2020). In *Arabidopsis*, after the perception of the bacterial elicitor flg22, BIK phosphorylates the N-terminal region of the OSCA1.3 channel, thus activating it and allowing the entry of Ca^{+2} into the guard cells and inducing stomatal closure (Thor *et al*., 2020).

The recognition of effectors by R genes also triggers most of the responses observed in the PTI response, but also transcriptional activation of defence genes, like the pathogen-related (PR)-genes, a hypersensitive response (HR), and the induction of local and systemic resistance. HR was extensively associated with the ETI response after R gene recognition. However, there are reports indicating that PRRs are also an important component to generate HR in infected cells since mutants not expressing them are impaired in the HR response (Ma *et al*., 2012).

Plants cannot constitutively express all the mechanisms generated by PTI and ETI, because it demands energy that must be obtained at the cost of reducing other physiological processes, affecting some traits like biomass or seed production. For this reason, the evolution of the cell-autonomous monitoring system, like the one present in the cell wall, is an important factor in plants to detect, react and overcome different types of stress (Bacete *et al.*, 2018).

1.2.3. Crosstalk between abiotic and biotic stress responses in plants

In nature, plants are not only faced with a single type of stress. Instead, at the same time, plants can be affected by several pathogens and/or environmental stresses. Both types of stress can influence the overall response. For example, abiotic stress can increase plant susceptibility to pathogens. Increased temperatures reduced the resistance of *A. thaliana* against *Pseudomonas syringae* and two viral effectors (Wang *et al*., 2009a); disease in *Phaseolus vulgaris* caused by the fungus *Macrophomina phaseolina* is more severe under salinity stress than in normal conditions (You *et al*., 2011). The opposite relationship also occurs. For example, the increment in Ca^{2+} caused by salinity stress is toxic for fungi because it affects their development (Fones *et al*., 2010). On the other hand, pathogen infection can also impact the resistance of plants to abiotic stress. It was also observed that exposure to viruses and the *Verticillium* fungi can increase tolerance to drought (Xu *et al*., 2008; Reusche *et al*., 2012). Therefore, the effect of combined stress depends on the type of stress and the particular plant species and pathosystem (Kissoudis, *et al*., 2014).

The response of plants to both types of stress is connected by molecular pathways that are activated under both stresses. These include the regulation of phytohormone production (SA, JA, ET, ABA), changes in intracellular Ca^{2+} concentration, HSP and ROS production, activation of the MAPK phosphorylation cascade, and transcriptional reprogramming (Kissoudis *et al*., 2014, Nejat and Mantri, 2017). Transcription factors, including NAC and MYB, have been involved in the tolerance to drought and salt stress, and in the resistance to pathogens (Nejat and Mantri, 2017). Cross-talk between both types of stress can be observed in the activation of mechanisms related to the PTI response. (Nejat and Mantri, 2017). It was observed that PRRs are not only important for pathogen detection, but also play a role during abiotic stresses, as observed with GbRLK, a PRR-RLK whose overexpression conferred tolerance to drought, salinity, and resistance to pathogens in *A. thaliana* and rice (Zhao *et al*., 2013; Jun *et al*. 2015). In brief, although activation of similar mechanisms occurs under biotic and abiotic stress, the combinatory effects also trigger new interactions, activation, or repression of different pathways not observed under single stress.

1.2.4. Cell wall responses to stress in plants

1.2.4.1. Cell wall composition

For many years, it was assumed that the plant cell wall was a passive structure that only provides mechanical support for the cell. However, it is now considered a dynamic, responsive, and highly controlled structure. It is connected to the cytoskeletal network and the plasma membrane and plays an essential role in cell growth and in the response to diverse stresses (Humphrey, Bonetta, and Goring, 2007).

Depending on the age of the plant, the plant cell wall is composed of two layers: the primary and the secondary cell wall. The primary cell wall is a thin layer with the flexibility to enable cell expansion as it is surrounded by growing cells. In contrast, the secondary cell wall is a thicker layer surrounding cells that have completed their expansion. The primary cell wall mainly contains microfibrils of

cellulose interconnected with a network of hemicellulose and embedded in a matrix of pectin. Pectin is abundant in the primary cell wall, but not in the secondary cell wall, which is characterised by reinforcement with lignin (Vorwerk, Somerville, and Somerville, 2004; Malinovsky, Fangel, and Willats, 2014) (**Figure 1.5**).

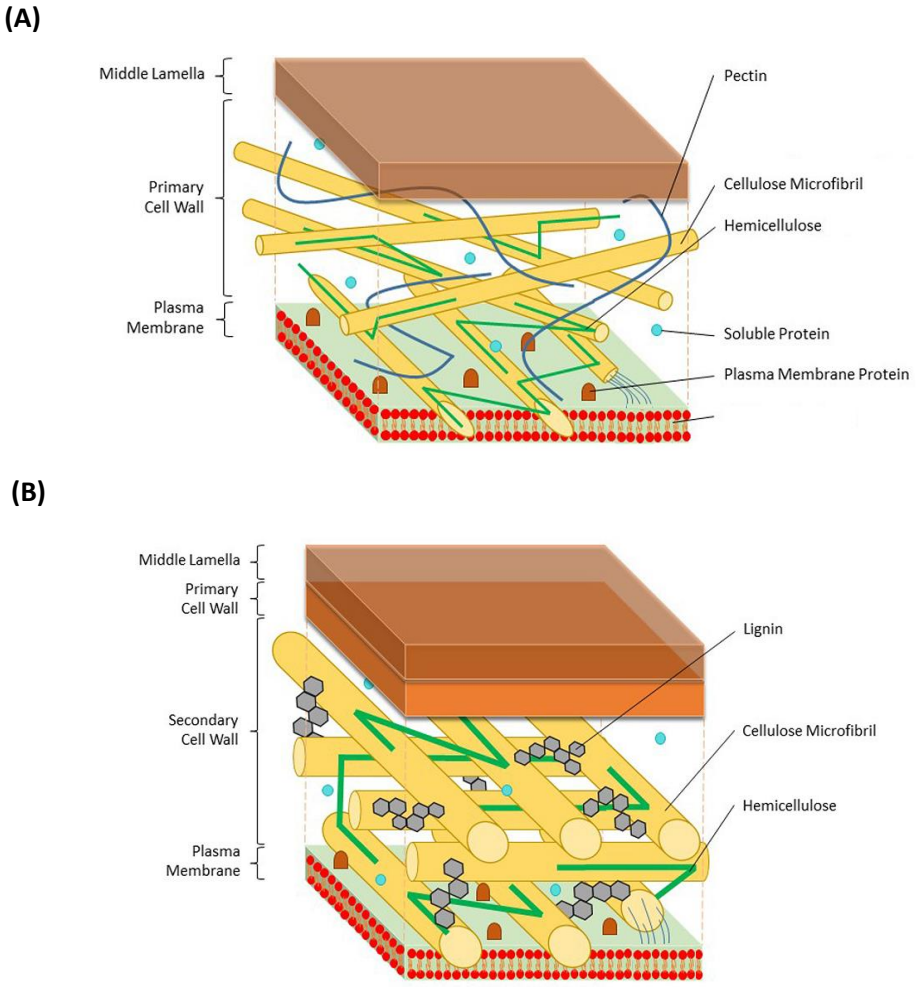


Figure 1.5 The plant cell wall. The primary cell wall is composed mainly of cellulose interconnected with hemicellulose, and embedded in a pectin matrix **(A)**. Instead of pectin, lignin is abundant in the secondary cell wall **(B)**. Adapted from Loix *et al*., 2017.

Cellulose is an insoluble and inelastic crystalline structure composed of 18 to 24 chains of β-1,4-linked D-glucose packed into microfibrils (Thomas *et al*., 2013). These fibrils have hydrophobic and hydrophilic surfaces with different affinities to attach different hemicellulose components (Cosgrove, 2014). Hemicelluloses are all the non-pectin and non-cellulosic components of the cell wall. The

hemicellulose backbone can be made up of a homopolymer chain of glucose, xylose, or mannose units, called glucan, xylan, or mannan, respectively. These units can also be mixed in the backbone to form a heteropolymeric chain, as in glucomannan (**Figure 1.6**). The amount of each type of hemicellulose can vary among phylogenetic groups. For example, xyloglucan is the major hemicellulose component of the primary cell wall of many dicots, making up ~20% of the dry mass, but this percentage drops to 2% in grasses (Park and Cosgrove, 2015). While xylan is predominant in the secondary cell wall (McCartney, 2005). The interactions between cellulose and hemicellulose components fortify the structure of the cell wall (Malinovsky, Fangel, and Willats, 2014).

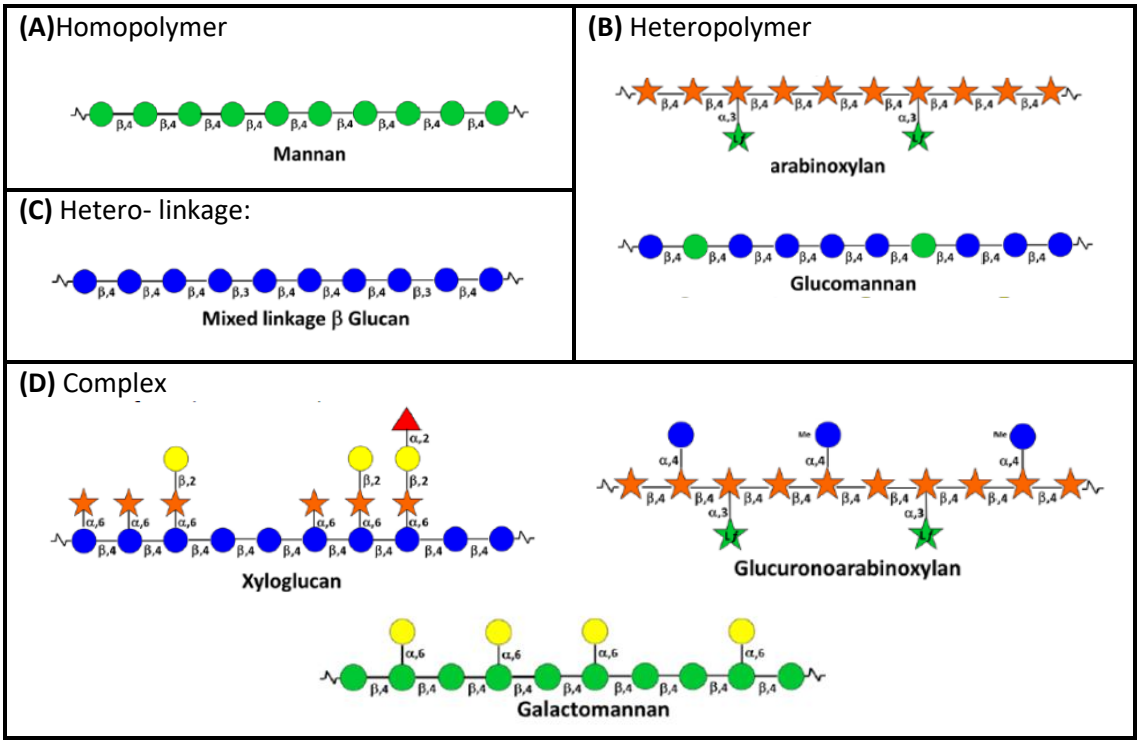


Figure 1.6. Types of cell wall hemicellulose. Green, blue, and yellow circles represent mannose, glucose, and galactose residues, respectively. Orange stars represent xylose and green stars represent arabinose residues. Modified from Joseleau and Pérez, 2016.

Pectin is the main component of the cell wall in dicotyledons and is composed of a polysaccharide chain rich in galacturonic acid that can be methylesterified and/or acetylesterified. The structure of its principal backbone is formed by homopolymers of galacturonic acid (α-(1→4)-D-Gal*p*A) or dimers of galacturonic acid and rhamnose (α-(1→4)-D-Gal*p*A- α-(1→2)-L-Rha*p*). From this backbone, depending on the side chains, pectin has four different domains: (1) the homogalacturonic acid (HG)

is composed only of α-(1→4)-D-Gal*p*A, without side chains, (2) the xylogalacturonans (XGA) composed of side chains of xylose, (3) the rhamnogalacturonans type II (RGII) composed of side chains of different glucans; and (4) the rhamnogalacturonans type I (RGI) that also contain side chain glucans, as with RGII, but with different units in the backbone (**Figure 1.7**). RG I is the most abundant pectin and represents from 20% to 35% of the pectin polysaccharides (Mohnen, 2008). Although RG II possesses different residues in the side chain that make it a complex structure, it is highly conserved among vascular plants (Matsunaga *et al*., 2004).

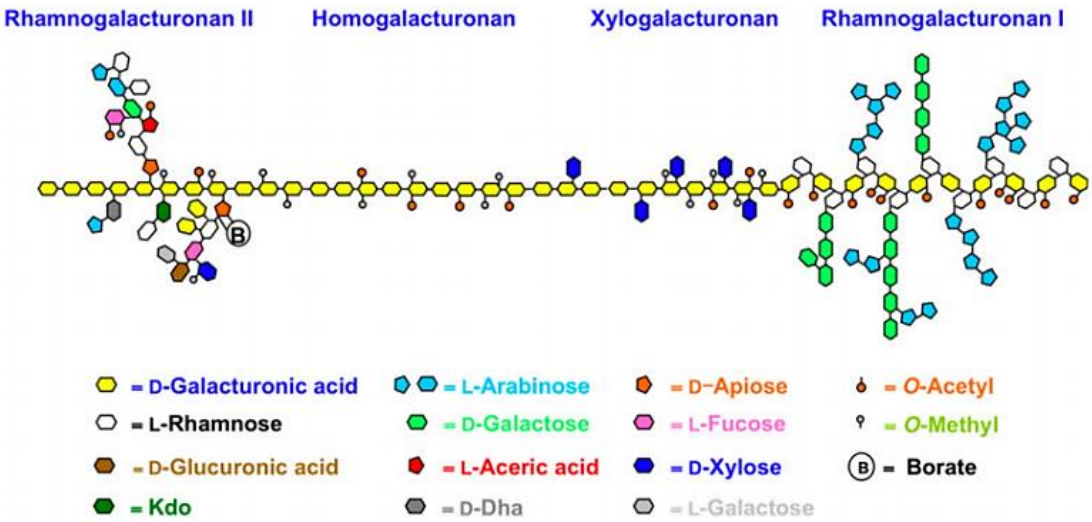


Figure 1.7. Types of cell wall pectin. Pectin is classified in 4 groups: homogalacturonan composed of D-galacturonic acid, without side chains, (2) xylogalacturonans that contain xylose as side chains, (3) rhamnogalacturonans type II (RGII) composed with side chains of different glucans; and (4) rhamnogalacturonans type I (RGI) that also contain different glucans in its side chains, as RGII, but its backbone is composed of a mixture of galacturonic acid plus L-rhamnose. Reproduced from Joseleau and Pérez, 2016.

Glycoproteins are another important cell wall component that may be structural or enzymatic. Enzymatic proteins target the glycans of the cell wall, to break bonds in the glycan network during cell elongation. Structural proteins are grouped into hydroxyproline-rich glycoproteins (HRGP), glycine-rich proteins, and proline-rich proteins. HRGPs include extensin, proline hydroxyproline-rich proteins, and arabinogalactan proteins (AGP) (Deepak *et al*., 2010).

The exact composition of the cell wall varies depending on cell function, tissue type, developmental stage, plant species, or accession within species (Tyler *et al.*, 2014; Faria-Blanc, Mortimer, and Dupree, 2018). Differences include the types and the degree of biochemical modifications, like acetylation, esterification, or methylations. These modifications affect the three-dimensional structure of the cell wall and its physicochemical properties. Plant pathogens must therefore generate different mechanisms to overcome this complex barrier (Bacete *et al.*, 2018).

1.2.4.2. Cell wall response to abiotic stress

The cell wall not only functions as a physical barrier that separates the extracellular from the intracellular space, but it also plays an important role against different stresses, including metal accumulation, lack of nutrient availability, drought, salt, flood, and cold stress (Le Gall *et al*., 2015). Several studies have documented the function of Ca^{2+} as an intracellular signal triggered by stress, but calcium also has an important role in influencing the rigidity of the cell wall. In the process of cell wall synthesis, acid pectin residues are liberated and de-esterified by pectin methyl esterase (PME), producing pectates with free carboxyl groups. These negative carboxyl groups bind Ca^{2+} ions, generating bridges between pectates and forming a gel structure called the “egg-box” (Baccini and Pérez, 2001). It has been suggested that this characteristic of storing calcium confers a buffering property to the cell wall by balancing the concentration of Ca^{2+} found in the environment since the amount of demethylesterified pectin increased in the cell wall at a higher concentration of Ca^{2+} (Voxeur and Hofte, 2016).

The cell wall can also harbour other elements, like boron. Boron in the form of borate forms ester bonds with the pectin rhamnogalacturonan II (RGII), in its apiose residue. This union generates dimers of RG II that contribute to the formation of pectin networks, similar to the crosslinking of pectates by Ca^{2+}. In *Lillium*, the increment in calcium and boron makes the cell wall more rigid and decreases the rate of pollen elongation (Funakawa and Kyoko Miwa, 2015).

Under drought, changes in cellulose and pectin, and increases in hemicellulose and lignin occur. An example of this can be observed in the reaction of plants to low water availability, in which they roll their leaves in an attempt to reduce transpiration. A photo-sensitive leaf rolling 1 (PSL1) gene encoding a pectin-degrading polygalacturonase enzyme was responsible for leaf rolling in rice mutants exhibiting this phenotype. The activity of PSL1 modified the cell wall composition and led to higher tolerance to drought in the mutant compared with the wild type (Zhang *et al*., 2021). The methylesterification status of pectin can also influence the tolerance of plants to drought. For example, the overexpression of a pectin methyl esterase inhibitor from pepper, CaPMEI, which also exhibits antifungal activity, increased drought, and oxidative tolerance (An *et al*., 2008). In response to drought, different plant species changed their cellulose synthesis, although they did not necessarily increase cellulose content (Le Gall *et al*., 2015). Therefore, the cell wall has been reported to be a dynamic structure whose modification under abiotic stress helps resist the negative impact of the stress.

1.2.4.3. Cell wall response to pathogen infection

Some pathogens, like oomycetes, invade the cell wall by using a structure called appressorium to produce a physical force in the cell wall to break it. However, pathogens can also produce cell wall degrading enzymes (CWDE) to disrupt and penetrate the plant cell wall. CWDEs includes a set of carbohydrate esterases, glycoside hydrolases, glycosyl transferases, and polysaccharide lyases, among others (Giraldo, *et al*., 2010). As part of the early plant response, papillae deposition is generated in the plant cell host at the point of the infection (Voigt *et al*., 2014). Plants use papillae to slow down pathogen invasion, allowing them to have more time to evoke additional defence responses (Voigt, 2014). The structure of the papillae contains ROS, phenolics, callose and cell wall fragments, such as cellulose, hemicellulose, pectins, lignin, and glycoproteins (Bellicampi *et al*., 2014, Voigt, 2014).

Callose is a (1,3)-β-glucan and its production under stress require the action of the callose synthase, also called either PMR4 (Powdered mildew resistance 4) or GSL5 (GLUCAN SYNTHASE-LIKE 5). Callose deposition can be induced by PAMPs such as flagellin, bacterial elongation factor EF-Tu, chitin, N-acetylglucosamine, and chitosan (Wang *et al*., 2021a). Callose deposition reinforces the cell wall and can produce complete penetration resistance against pathogens (Ellinger *et al*., 2013). However, it was also shown that the lack of callose in *Arabidopsis* mutant lines not expressing *pmr4* produced resistance in A*rabidopsis* against powdery mildew, instead of susceptibility. In this case, the resistance was given by the salicylic acid, and it was observed that this hormone was negatively regulated by callose (Nishimura *et al*., 2003). The importance of callose in pathogen resistance and their property to reinforce the cell wall have been also associated with the restriction of the CWDE dissemination into the host (Wang *et al*., 2021). Callose deposition is considered as a common response from the plant host against infection as it was observed in many plant-pathogen interactions (Voight, 2014).

Increasing of peroxidase activity associated with lignin accumulation has been associated with cell wall fortification at the site of the infection to decrease fungi entrance (Voight, 2014). Lignification has been observed to be enhanced by reducing the amount of cellulose and to produce resistance in *Arabidopsis* against powdery mildew (Cano-Delgado, *et al*., 2003). A higher amount of crosslinking lignification was observed in cotton resistant variety to *Verticillium dahliae* than susceptible variety (Xu *et al*., 2011). The production of monolignol, which are monomers of lignin, was also activated in resistant *Camelia sativa* in response to the infection with *Sclerotinia sclerotiorum* (Eynck *et al*., 2012). Production of lignin has been observed as an important mechanism against infection, it increases cell wall rigidity and could restrict the flux of toxin and degrading enzymes into the host cell, as well as nutrients from the host to the pathogen (Eynck *et al*., 2012).

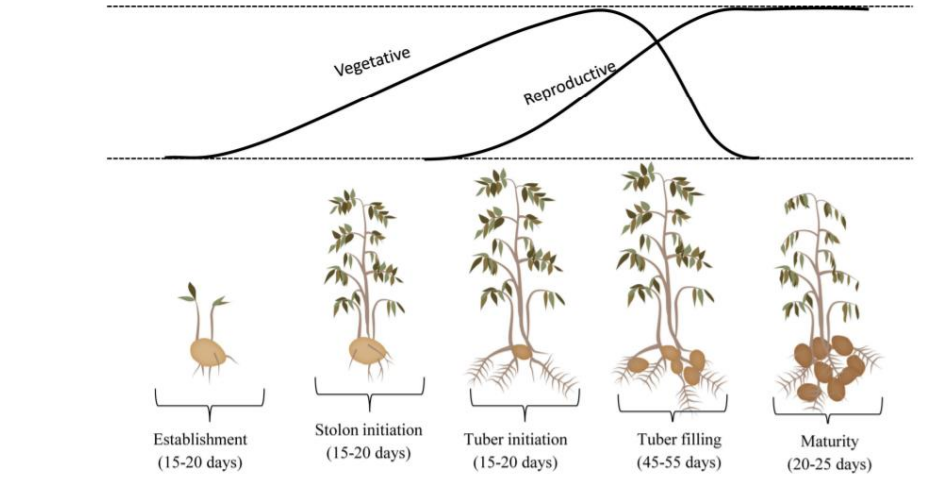
Accumulation of glycoproteins has also been observed as a plant reaction to an infection. Crosslinked extensin in the plant cell wall and its glycosylation catalyzed by peroxidases produce protection against pathogens (Castilleux *et al*., 2021). This conferred protection in Arabidopsis roots against *Phytophthora parasitica,* therefore it is important in root defence. Also in *Arabidopsis*, mutant plants with a deficiency in starch biosynthesis or mobilization and infected with the fungus *Colletotrichum higginsianum* showed that differences in pectin content or structure affected the penetration and hyphal growth of *C. higginsianum*. The study suggestes a key role for carbohydrate availability and pectin composition in penetration resistance (Engelsdorf *et al*., 2017).

In plants, the only well study DAMPs associated with cell wall damage are the pectin-derived oligogalaturonide fragments (OGAs), with a degree of polymerization of 10-16, whose putative receptors are the wall-associated kinases (WAK) (Ridley *et al*., 2001). Other DAMPs identified in plants include xyloglucan fragments (Claverie *et al*., 2018) and arabinoxylan (AX)-oligosaccharides, with a particularly strong response in *Arabidopsis* elicited by those with a specific degree of polymerisation, namely XA3XX or 3^3-α-L-arabinofuranosyl-xylotetraose (Mélida *et al*., 2020).

Different studies show that WAK receptors can confer resistance against pathogens, and are apparently involved in the cross-communication between the cytoskeleton and the cell wall (Baluška *et al.*, 2003). In *Arabidopsis*, WAKL22 was identified as a major Quantitative Trait Locus (QTL) conferring resistance against different races of *Fusarium* (Diener and Ausubel, 2005), distinct from R genes that usually confer resistance to specific races. In maize, two QTLs identified as WAK-like proteins conferred resistance against fungal diseases (Zuo *et al.*, 2014; Hurni *et al.*, 2015). However, there are key gaps in knowledge regarding cell wall composition in different plant varieties that may affect resistance and in the identification of fragments other than OGAs that may act as DAMPs to trigger the immune response.

1.3. Potato response to drought

Although potato is considered an efficient crop, producing more carbohydrates with a lower input of water compared with other crops, it is considered a drought susceptible crop because of its shallow root system. It is well known that drought has negative effects on potato crop productivity, which is measured in relation to the harvest index and dry matter content. However, drought not only reduces yield but also reduces quality, for example by increasing the incidence of tuber scab (Mane *et al*., 2008). The effect of drought on yield loss depends on the growth phase where the stress occurs (**Figure 1.8**). Likewise, the response of the plant to drought is based on the intensity, duration, and speed of stress progression (Obidiegwu *et al*., 2015).



Impact of water deficit	Delayed emergence Restrict root establishment Restrict plant development Presence of fewer stalks/stem	Restrict plant development Limited stolon initiation Limited number of stolon Restrict root growth Restricts uptake and response to nutrients	Limited foliage and plant development Limited tuber initiation Limited number of tubers	Limited plant and tuber development Restricts tuber size Promotes distorted tuber shape Faster senescence	Limited relative tuber density Limited tuber size

Figure 1.8 Drought effects in potato during different phases of the life cycle. Reproduced from Obidiegwu, *et al*., 2015.

A transcriptomic study using microarrays showed a widespread difference in gene expression during drought between the potato variety 397077.16, an improved variety that maintains its yield under

water-limited conditions, and another susceptible variety (Chanchan) (Legay *et al*., 2011). The resistant plant showed a high expression of genes related to the synthesis of protective compounds, ROS detoxification, and to the maintenance of protein structure, compared to the susceptible variety, suggesting maintenance of turgor and limitation of cellular damage as key tolerance phenotypes (Legay *et al*., 2011). In another study, the leaf transcriptome from a Chinese tolerant variety, Loshua, under drought stress was sequenced using RNASeq. The plant responded by increasing the expression of genes involved in cell membrane stabilization and fortification of the cell wall, including lipid binding and transport proteins, cell wall proteins, and wax synthases. Similarly, genes encoding transcription factors, such as MYB and AP2/ERF, WRKY; genes related to carbohydrate metabolism, such as chalcone synthase; and genes related to osmotic adjustment in cell rescue, such as those involved in proline synthesis, increased their expression under drought stress. (Zhang *et al*., 2014).

1.4. Potato response to *P. infestans*

Late blight disease, produced by *Phytophthora infestans* infection, is the main cause of global potato yield losses (Savary *et al*., 2019). This pathogen is a hemibiotroph, meaning it is biotrophic at the beginning and necrotrophic in the later stages of infection. At the beginning of the disease, it can be recognized in leaves by the appearance of pale green spots that, in later stages, turn into black necrotic areas spreading across entire leaves. In stems, late blight is observed as light brown lesions and in tubers, as rusty brown discoloration (Lal *et al*., 2018). The optimum condition for rapid development of the disease is a temperature between 12-15°C, with high humidity (>90%), cloudy weather, alternating with rain and warm temperature periods of 18-20°C (Lal *et al*., 2018).

1.4.1. Resistance genes in potato against *P. infestans*

The first group of resistance genes again *P. infestans* were identified in the native Mexican species *Solanum demissum*. Named R1 to R11, they have been recognized as dominant, single-race specific

genes providing no durable resistance. This is due to the rapid evolution of virulence proteins of *P. infestans* and the gene-for-gene nature of the interaction, where one mutation in the relevant pathogen effector can abolish the interaction, causing the host to no longer recognize the pathogen (Fry, 2008). New strategies were employed by pyramiding different R genes into new cultivars. However, these new varieties became susceptible very rapidly (Niederhauser, Alvarez-Luna, and Mackenzie, 1996; Hein *et al*., 2009).

Later, effort was invested to identify new sources of resistance genes in Mexican potato species and in native potato species growing in the Andean region of South America, including *S. bulbocastanum, S. stonoloniferum, S. americanum, S. michoacanum, and S. pinnatisectum* (Hein *et al*., 2009; Aguilera-Galvez *et al*., 2018). New combinations of the identified genes, recognizing different avirulent effectors, were generated to obtain a broader spectrum of quantitative disease resistance to *P. infestans* (Fry 2008; Corwin and Kleibenstein 2017; Willocquet *et al.* 2017). Plants with the stacked R genes were tested into the field in Belgium and the Netherlands. Transformed plants were significantly more resistant compared to the control over the two seasons, although in Belgium there was a slightly decreasing level of resistance in the second season, highlighting the substantial effect of the environment on the progression of the disease (Haesaert *et al*., 2015). The majority of the cloned R genes contain a nucleotide-binding (NB) site and leucine-rich repeat (LRR) domain that produce a hypersensitive response (HR), and as a consequence, a programmed cell death. However, the action of the R genes may be associated with regulatory elements, and the introgression of these R genes into new varieties does not also ensure the transfer of the trait. Therefore, it is necessary to identify the function of the resistance gene to decipher the resistance mechanism (Kushalappa and Gunnaiah, 2013; Yogendra *et al*., 2014a).

Resistant-related (RR) metabolite and protein production is controlled by R genes and directly suppresses pathogen invasion by their antimicrobial properties or by their accumulation in the cell

wall to reinforce it (Kushalappa, 2016). A metabolomic study was conducted to identify RR metabolites in the leaves of resistant and susceptible potato varieties after infection with *P. infestans*. A high abundance of flavonoid, fatty acid, alkaloid and phenylpropanoid chemical groups in the resistant variety was observed. Among the last group, hydroxycinnamic acid amides (HCAA) were identified, which reinforce the cell wall by accumulating in the space between the primary cell wall and the plasmalemma during pathogen attack (Yogendra *et al*., 2014b). HCAAs can also reduce digestibility of the cell wall and inhibit hyphae development (Grandmaison *et al*., 1993). In another study, the metabolomic analysis of tubers from resistant and susceptible potato varieties showed that 37 metabolites correlated with the severity of the disease (Hamzehzarghani *et al*., 2015). The amount of amino acids was higher after infection in the resistant variety and among these amino acids was L-proline, which is involved in the production of extensin to reinforce the cell wall by increasing its rigidity (Hamzehzarghani *et al*., 2015).

1.5. Transcriptomic analysis and the detection of resistance mechanisms in plants

Massive sequencing of transcriptomes, a technique called RNASeq (Wang *et al*. 2009b), has made it possible to detect in a genome new active genes, novel gene isoforms, and transcriptional changes beyond what can be found with microarrays (Unamba *et al*., 2015). For this reason, the use of RNASeq and genomic sequences has provided valuable information on the mechanisms behind plant resistance to disease or tolerance to environmental stress.

The transcriptomic analysis of wheat infected with four strains of *Zymoseptoria tritici* revealed that during the early asymptomatic period of the infection, wheat had an active response against this necrotrophic fungus (Ma *et al*., 2018a). This was observed by the upregulation of genes encoding receptors like kinase proteins, pathogen related proteins, and genes related to the synthesis of cysteine, as well as genes involved in epigenetic change (Ma *et al*., 2018a). In addition, wheat plants infected with the less virulent strain of *Z. tritici* could be protected by highly expressing a xylanase

inhibitor protein XIP-1, which inhibits xylanase secreted by this fungus. Although this response was not associated with resistance since XIP-1 was expressed when plants were severely damaged (Ma *et al*., 2018a). Transcriptomic analysis of potato leaves infected with *P. infestans* identified that in durable resistance phenotype was involved the expression of mainly 23 R genes, which need the expression of pathogenesis related proteins such as endochitinase and phenolic compounds (Hao, *et al*., 2018). In relation to abiotic stress, the transcriptomic response of potato varieties exposed to drought identified that tolerance to this stress could be influenced by the activation of mechanisms related to carbohydrate metabolism and the production of secondary metabolites, including lignin and flavonoids (Aliche *et al*,2021). In addition, activation of metabolic pathways involved in cell wall maintenance, osmotic adjustment pathways, and cell membrane were also detected (Alvarez-Morezuelas *et al*., 2022). Therefore, the use of transcriptomics is a valuable tool for identifying the molecular mechanisms that regulate stress response and tolerance in plants.

1.6. Complex trait genetic architecture in crops.

In agriculture, the majority of economically important traits, like the yield or quality in the production of a crop, tolerance to abiotic and resistance to biotic stresses, among others, are quantitative. A quantitative trait is a measurable trait with continuous variation in the population (Geldermann *et al*., 1975). A quantitative trait depends on the action of several genes that have cumulative effects on the trait, and in most cases, this cumulative effect is the sum of small effects coming from each gene (East, 1916). These genes or genomic regions that affect a quantitative trait are called Quantitative Trait Loci or QTL. Quantitative trait variation is also influenced by environmental factors, whose effects may be large compared with the effects of individual genes.

To identify the genomic position of QTLs, genetic markers are required, which are specific DNA variants such as single nucleotide polymorphisms (SNPs) whose position in the genome can be mapped. Then, QTL can be mapped using two main approaches. QTL linkage analysis uses designed

segregating populations of offspring individuals resulting from crosses between parental lines. Association mapping, also called genome-wide association study (GWAS), identifies statistical associations between markers and traits by employing existing natural populations of genetically diverse individuals and can improve the resolution of mapped QTLs. It often relies on the key concept of Linkage Disequilibrium (D), the non-random association of alleles at two different loci.

The majority of agronomically important crops are polyploid, generating challenges for quantitative trait analysis due to greater complexity in allele segregation in meiosis. These crops include tetraploids (2n=4X) such as quinoa, cotton, and potato; hexaploids (2n=6X) such as bread wheat, oat, and sweet potato; and octaploids (2n=8X) such as strawberry and sugarcane (Tang *et al.*, 2017). Polyploids are classified as allopolyploids and autopolyploids, depending on the origin of their subgenomes. Allopolyploids originate from the hybridization of genomes from two species, and during meiosis their chromosomes usually show preferential pairing (non-random) between pairs of homologous chromosomes, generating a disomic inheritance pattern as in diploids (Osabe *et al*., 2012; Doyle and Sherman-Broyles, 2017). Autopolyploids originate mainly by the fusion of unreduced gametes generating chromosome doubling, involving only one species. In meiosis, autotetrapolyploids usually show a random association between their four homologous chromosomes that can form not only bivalents but also trivalents and even quadrivalents (Choudhary *et al*., 2020), leading to complex polysomic inheritance (Lloyd and Bomblies, 2016).

1.7. Aims

The aim of this project is to identify components of the cell wall that can confer resistance against *P. infestans* and drought stresses in potato. This is because the cell wall is a dynamic structure that functions as an important barrier against pathogen infection and changes in some of its components have conferred resistance against environmental stressors in other plant species. Although there is evidence that potato can modulate its cell wall under both types of stress, there is a need to

investigate the reaction of the cell wall more deeply to find what cell wall composition can produce a resistant phenotype. Due to the wider importance of extracellular and intracellular receptors in detecting pathogen structures, cell damage, or pathogen effectors, defence genes related to these factors are also evaluated in addition to phytohormone-mediated biological processes. This project not only intends to find genes that confer resistance to each independent stressor but also to identify common genes that could confer resistance to both stressors. The methodology involves the analysis of the glycome and transcriptome to determine cell wall components and genes whose expression is regulated under both *P. infestans* infection and drought. In addition, as potato has a complex inheritance due to autotetraploidy, identifying genetic markers linked to QTLs in the analysis of genome-wide association is a challenge. Given the importance of linkage disequilibrium (*D*) in the detection of significant marks, being *D* is the non-random association of two alleles at two different loci, this project aims to provide a preliminary analysis for extending the basic definition and measure of D from diploids to autotetraploids.

CHAPTER 2: Transcriptomic response of *Solanum tuberosum* subsp. *Andigenum* under drought stress and recovery in leaf and root tissues.

2.1. Introduction

Potato is one of the world's most important crops that will be highly affected by climate change, particularly drought (Obidiegwu *et al*., 2015). Its severity is influenced by different factors, like the capacity of the humidity storage of the soil, the atmospheric evaporation demand, and the amount and distribution of precipitation (Vicente-Serrano *et al*., 2020). It is estimated that drought will cause losses of between 18% and 32% in the production of potatoes in the next 30 years (Hijmans, 2003).

The first organ that senses the low availability of water in the soil is the root. From the root, signals are produced and transported to the shoot to generate physiological changes that can give rise to an adaptative response. Root-to-shoot signals include hydraulic pressure, reactive oxygen species (ROS)/Ca^{+} waves and mobile peptides (Takahashi *et al*., 2020) including CLAVATA3/EMBRYO-SURROUNDING REGION-RELATED 25 (CLE25) (Takahashi *et al*., 2018).

Under a water deficiency, abscisic acid (ABA) controls different regulatory processes and is an important phytohormone mediating drought responses and tolerance. In the leaf, ABA induces stomatal closure and consequently less transpiration and low internal availability of CO_2. This physiological change produces a reduction of photosynthesis, and when there is less 3-phosphoglyceric acid produced in the Calvin cycle by the low amount of CO_2 substrate, NADPH and ATP accumulate, producing an energy imbalance and oxidative stress by the generation of ROS, including H_2O_2, $O^{\cdot -}_2$, $OH^{\cdot}$ and $^1O_2$ (Dahal *et al*., 2019). This toxicity produced by ROS activates chloroplast-localized antioxidants like superoxide dismutase, ascorbate peroxidase, and glutathione-S-transferase, among others (Legay *et al*., 2011). In addition, an increase in molecular chaperones

like LEAs and HSPs that prevent protein aggregation and denaturation is observed. Other physiological changes include the production of metabolites like proline, glycine, betaine, and sugar to stabilize the osmotic balance (Legay *et al*., 2011).

In potato, to determine the mechanism that confers tolerance to drought, various transcriptomic analyses have been carried out. Many of them evaluated the response of leaves to drought in one variety (Zhang *et al*., 2014), more than one variety with contrasting phenotypes (Pieczynski *et al*., 2018) or only in tolerant varieties (Chen *et al*., 2020). In these studies, the tolerant response was associated with a high expression of genes contributing to the stabilization of the cellular membrane, like lipid-binding proteins and non-specific lipid transfer proteins, and changes in the expression of genes related to modification or the reinforcement of the cell wall. These changes include the downregulation of xyloglucan endotransglucosylase (XET) (Zhang *et al*., 2014) and the upregulation of expansin-like B during mild drought with an increased fold change during severe drought (Chen *et al*., 2020). Genes encoding cell wall remodeling proteins, such as expansin, pectinesterase, pectate lyase, and XET, were also identified in the proximity of a QTL located in the potato chromosome 3 associated with drought tolerance (Schumacher *et al*., 2021a). Similarly, genes increasing their expression under drought stress included genes encoding transcription factors, such as MYB and AP2/ERF, WRKY; genes related to carbohydrate metabolism, such as chalcone synthase; genes related to osmotic adjustment, such as those involved in proline synthesis; and genes related to cell communication, such as kinases and phosphatases (Zhang *et al*., 2014). To gain a more comprehensive understanding of the tolerance response of potato to drought stress at the molecular level, the tetraploid Andean potato varieties, *S. tuberosum subsp. andigena*, are of particular interest since they are well adapted to harsh climatic conditions (Vasquez-Robinet *et al*., 2008). These varieties may provide an important primary gene pool for improving the stress responses of the more widely grown potato *S. tuberosum* subsp. *tuberosum* (Sukhotu and Hosaka, 2006). Andigena landraces can more effectively maintain photosynthesis levels under prolonged drought stress

compared with Tuberosum (Vasquez-Robinet *et al*., 2008). Vasquez-Robinet *et al*., monitored the decrease in photosynthesis of different potato genotypes under drought and found that Andean genotypes experienced less photosynthesis reduction under stress and recovered more quickly following rewatering than the Tuberosum genotypes. They also evaluated the transcriptomic change of Andean potato leaves by using microarrays. The evaluated time point was when plants undergo maximum stress after 25 days of stress (late drought) where the more tolerant genotype kept 60% of its initial photosynthesis, while the susceptible only kept 20%. The transcriptomic change of the Andean tolerant potato showed more activation of genes related to antioxidant function than the susceptible Andean genotype, a lower level of ROS, in addition to higher mitochondrial metabolic activity and higher expression of chloroplast-localized chaperones(Vasquez-Robinet *et al*., 2008).

Chen *et al* also analyzed the transcriptomic response in leaves of an Andean potato landrace under drought, but at different time points considering the response to re-watering. It was observed that with the increase in drought severity, the number of genes that change their expression increased as well. The transcriptomic analysis revealed upregulation of genes related to sugar and lipid metabolism and genes involved in signal transduction, also upregulation of genes with antioxidative function and related to the cell wall during stress. During recovery time there was upregulation of genes involved in fatty acid metabolism, sugar metabolism, flavonoid metabolism, and detoxification. (Chen *et al*., 2020).

Few studies have focused on the transcriptomic response of Andean varieties to drought in other tissues like root (Qin *et al*., 2020) or stolon (Gong *et al*., 2015). Only Torres *et al*., generated transcriptomic data from leaves and roots of two Andean potato varieties with contrasting phenotypes exposed at different time points of drought. Based on the photosynthetic activity patterns described by Vásquez-Robinet *et al*., (2008), Torres *et al*. determined how long it took, after cutting the irrigation of an aeroponic system, for the photosynthetic rate to decrease to 25% and

60% of the initial rate. These time points were defined as the early and late responses to drought, respectively. They also determined the time when plants recovered 80% of their initial photosynthetic rate after irrigation (Torres *et al*., 2013). They observed that the photosynthetic rate of the susceptible variety decreased more quickly compared to the tolerant variety. While in the tolerant variety, the decay of the photosynthetic rate to 60% occurred at 120 minutes, the decay in the susceptible occurred earlier, at 100 minutes. Moreover, after rewatering, the susceptible variety only recovered ~50% of its initial photosynthetic rate.

To identify which transcriptomic changes are associated with drought tolerance in Andigenum potato varieties, this chapter analyses the transcriptomic data generated by Torres *et al*. and compares the response of leaf and root tissues under drought stress and rewatering between tolerant and susceptible varieties. This comparison allows the identification of key genes associated with tolerance to drought and inform breeding of new *S. tuberosum* cultivars with improved yield and quality under drought stress.

2.2. Material and methods

2.2.1. Plant material and drought treatment.

Two CIP potato varieties of the subspecies *Andigenum* (*Solanum tuberosum* subsp. *Andigena*) were employed in this study. The two varieties were "Negrita" (CIP accession number: 703671) and "Wila Huaka Lajra" (CIP accession number: 703248), tolerant and susceptible to drought, respectively. Plants were grown as described by Torres *et al*., 2013 for three months until tuber initiation in an aeroponic system installed in the "Estación experimental Santa Ana (INIA - Huancayo)" located in Huancayo, Peru, where the temperature oscillation was between 6°C and 18°C.

After 3 months of normal irrigation, the two potato varieties were exposed to hydric stress to simulate a drought condition. Based on the patterns of the photosynthetic activity, the selected time point to be evaluated was when the initial photosynthetic rate decreased by 25% and 60%, defined as the early and late responses to drought, respectively, and when plants recovered 80% of their initial photosynthetic rate, after irrigation. These time points were captured in the tolerant variety for the early response at 40 minutes after drought induction (T_1), for the late response at 120 minutes after drought induction (T_2), and for the recovery phase at 20 minutes after re-watering (**Figure 2.1**). (Torres *et al*., 2013). Leaves and roots from the two varieties were collected at T_1, T_2, T_3, and before stress (T_0), with 3 biological replicates, each corresponding to an independent plant (Torres *et al*., 2013) (**Figure 2.1**).

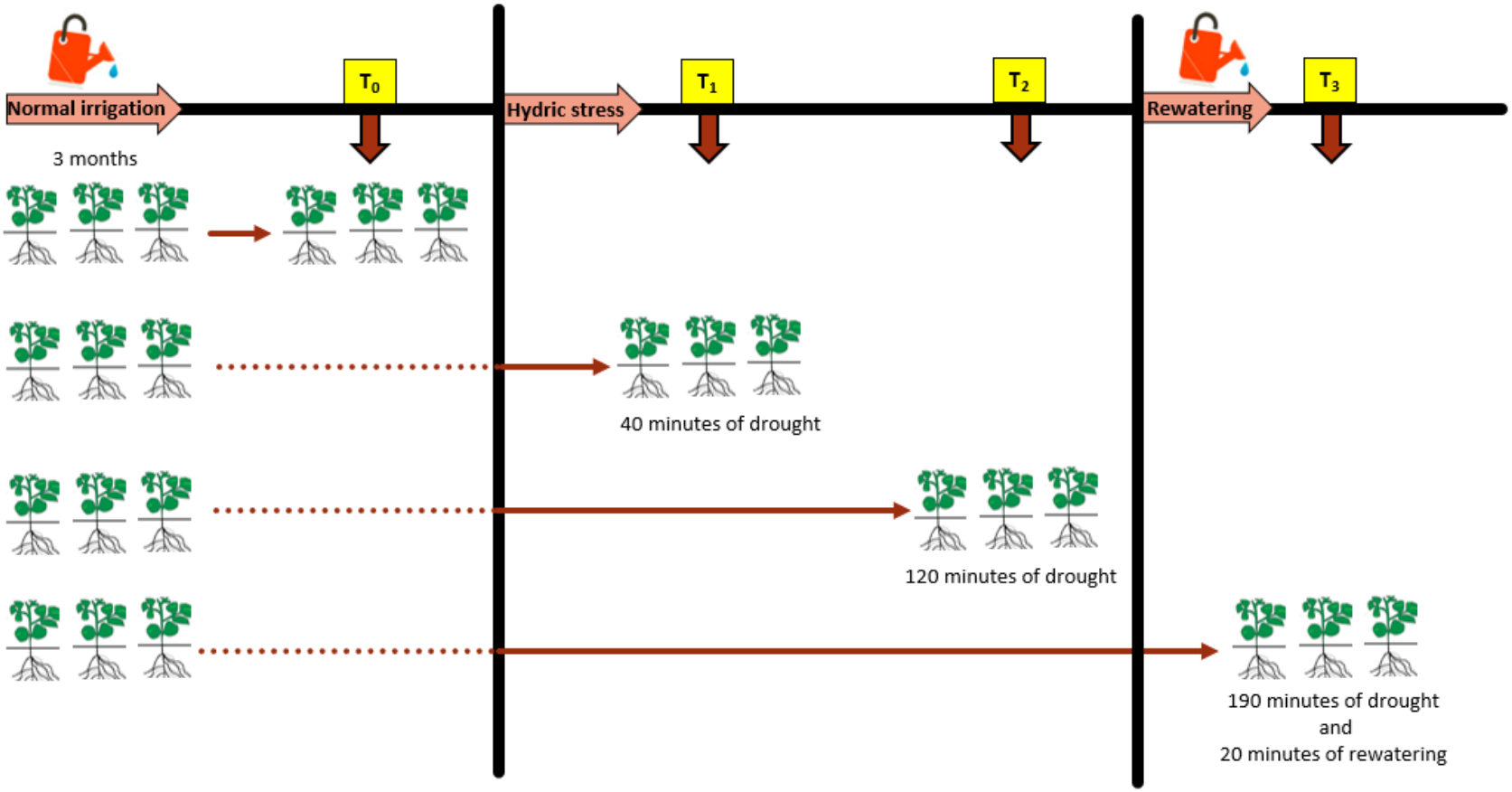


Figure 2.1 Experimental design to simulate drought. After 3 months of irrigation, potato plants were exposed to hydric stress until observing in the tolerant variety a decrease of 25% and 60%, and a recovery of 60% of their initial photosynthetic rate. These responses were observed at 40 (early response-T_1) and 120 (late response-T_2) minutes after removing the water supply, and at 20 minutes after re-watering (recovery phase-T_3). At these time points, leaf and root samples were collected from three plants of each susceptible and tolerant variety. Control plants, without hydric stress, were also collected (Control-T_0).

2.2.2. RNA extraction, sequencing, and read filtering.

All tissue from leaves was collected, polled, and stored at -80°C, as from roots. From each polled tissue, 1-2g were used to extract total RNA with Tri®Reagent (Sigma). The purity and the concentration of the RNA were determined by the OD260/OD280 and OD260/OD230 ratio using NanoDrop™ 1000fto, and RNA integrity was verified with agarose gel electrophoresis. To remove DNA contamination, RNAs were treated with DNAase using the kit DNA-free™ (Ambion). Samples were sent to Michigan State University where cDNA libraries were constructed and sequenced. The Illumina HI-Seq™ 2000 was employed to produce 48 mRNA libraries of 50nt single-end reads (Torres *et al.*, 2013) (**Table 2.1**). The quality of the reads was observed in FASTQC v.11.19 (bioinformatics.babraham.ac.uk/projects/fastqc/) and trimmed with TrimGalore v.6.5 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). During trimming, reads with a quality Phred value less than 28 were discarded, Illumina adapters were removed, and reads with a length of at least 20nt were retained.

2.2.3. Read mapping, differential gene expression analysis and functional enrichment

Good quality-trimmed reads were mapped to the potato reference genome v6.1 (Pham *et al.*, 2020) downloaded from SpudDB (http://spuddb.uga.edu/), with STAR v.2.7.2.b (Dobin *et al.*, 2013). Before mapping, index files were built for the potato genome with the option –runMode genomeGenerate and using the gff3 annotation file. For the mapping, the option –quantMode TranscriptomeSAM was used to align the reads to the genome. The number of counts per gene was obtained with HTSeq v.0.11.0 (Anders *et al.*, 2015) with the htseq-count script and the options --mode=union and --nonunique=none. The first option indicates that in the mode of counting reads by the program, the union of a set of reads that map to a gene will be considered as a part of the gene. The second option specifies to not count ambiguous reads that map in several positions of the genome.

Table 2.1 Samples for RNA-sequencing. The libraries were constructed from potato genotypes with contrasting drought tolerance phenotypes, one tolerant and one susceptible. In total 48 libraries were created from leaf and root samples.

Variety	Tissue	Time point	Replicate	Sample code	Library name
Tolerant: Negrita CIP code:703671	Leaf	Control: Before stress	1	TLC-1	AA
			2	TLC-2	BA
			3	TLC-3	CA
		T1: Early response	1	TL1-1	AI
			2	TL1-2	BI
			3	TL1-3	CI
		T2: Late response	1	TL2-1	AM
			2	TL2-2	BM
			3	TL2-3	CM
		T3: Recovery	1	TL3-1	AE
			2	TL3-2	BE
			3	TL3-3	CE
	Root	Control: Before stress	1	TRC-1	AB
			2	TRC-2	BB
			3	TRC-3	CB
		T1: Early response	1	TR1-1	AJ
			2	TR1-2	BJ
			3	TR1-3	CJ
		T2: Late response	1	TR2-1	AN
			2	TR2-2	BN
			3	TR2-3	CN
		T3: Recovery	1	TR3-1	AF
			2	TR3-2	BF
			3	TR3-3	CF
Susceptible: Wila Huaka CIP code: 703248	Leaf	Control: Before stress	1	SLC-1	AC
			2	SLC-2	BC
			3	SLC-3	CC
		T1: Early response	1	SL1-1	AK
			2	SL1-2	BK
			3	SL1-3	CK
		T2: Late response	1	SL2-1	AO
			2	SL2-2	BO
			3	SL2-3	CO
		T3: Recovery	1	SL3-1	AG
			2	SL3-2	BG
			3	SL3-3	CG
	Root	Control: Before stress	1	SRC-1	AD
			2	SRC-2	BD
			3	SRC-3	CD
		T1: Early response	1	SR1-1	AL
			2	SR1-2	BL
			3	SR1-3	CL
		T2: Late response	1	SR2-1	AP
			2	SR2-2	BP
			3	SR2-3	CP
		T3: Recovery	1	SR3-1	AH
			2	SR3-2	BH
			3	SR3-3	CH

2.2.4. Normalization and DE analysis

Gene count normalization and differential expression analysis were carried out with DESeq2 package v1.26.0 (Love *et al*., 2014) using the R statistical software v3.6.3, and Rstudio v.1.2.1335. Normalization was performed through the DESeq dispersion function, and the Wald test was applied to determine a significant differential expression of each gene between different pairs of samples. Genes with a Benjamini-Hochberg adjusted P-value below 0.05 and with an absolute shrunken log 2-fold change (LFC) >= 1 were considered as differentially expressed genes (DEGs).

2.2.5. Gene ontology analysis

The gene ontology (GO) terms associated with the annotated genes were downloaded from SpudDB (http://spuddb.uga.edu/GO). The GO enrichment analysis of the DEGs was performed with the g:profiler2 R package v0.2.0 (Raudvere *et al.*, 2019) and the function gost(). Enriched GO terms were considered if the Bonferroni-corrected *P*-values from the hypergeometric test were lower than 0.05.

2.2.6. Identification of DEGs that respond quickly to water stress.

Genes were considered as responding to the water stress if they significantly upregulated or downregulated their expression (not restricted to $|\log_2 FC| > 1$) once irrigation stopped, during the early response (T_1), and significantly changed their expression but in the opposite direction, after rewatering, during the recovery stage (T_3). The genes selected were either those whose expression increased in T_1, but remained the same or continued to increase in T_2, or those whose expression decreased in T_1 but remained the same or continued to decrease in T_2. These stress-responsive genes were identified and selected independently in each tissue of the tolerant variety. The expression of these selected genes was also observed in the susceptible tissues to find differences or similarities between the response of the two varieties.

2.3. Results.

The transcriptomic profiles of two Andigenum potato varieties with contrasting phenotypes, one tolerant and one susceptible to drought, were analysed to identify genes related to drought response and tolerance. RNA was sequenced from leaf and root samples taken at different time points of the hydric stress: before stress (control), 40 minutes after drought (T_1-early response), 120 minutes after drought (T_2-late response) and 20 minutes after rewatering (T_3-recovery phase) (**Figure 2.1**, **Table 2.1**).

2.3.1. Overview of the transcriptome profiling

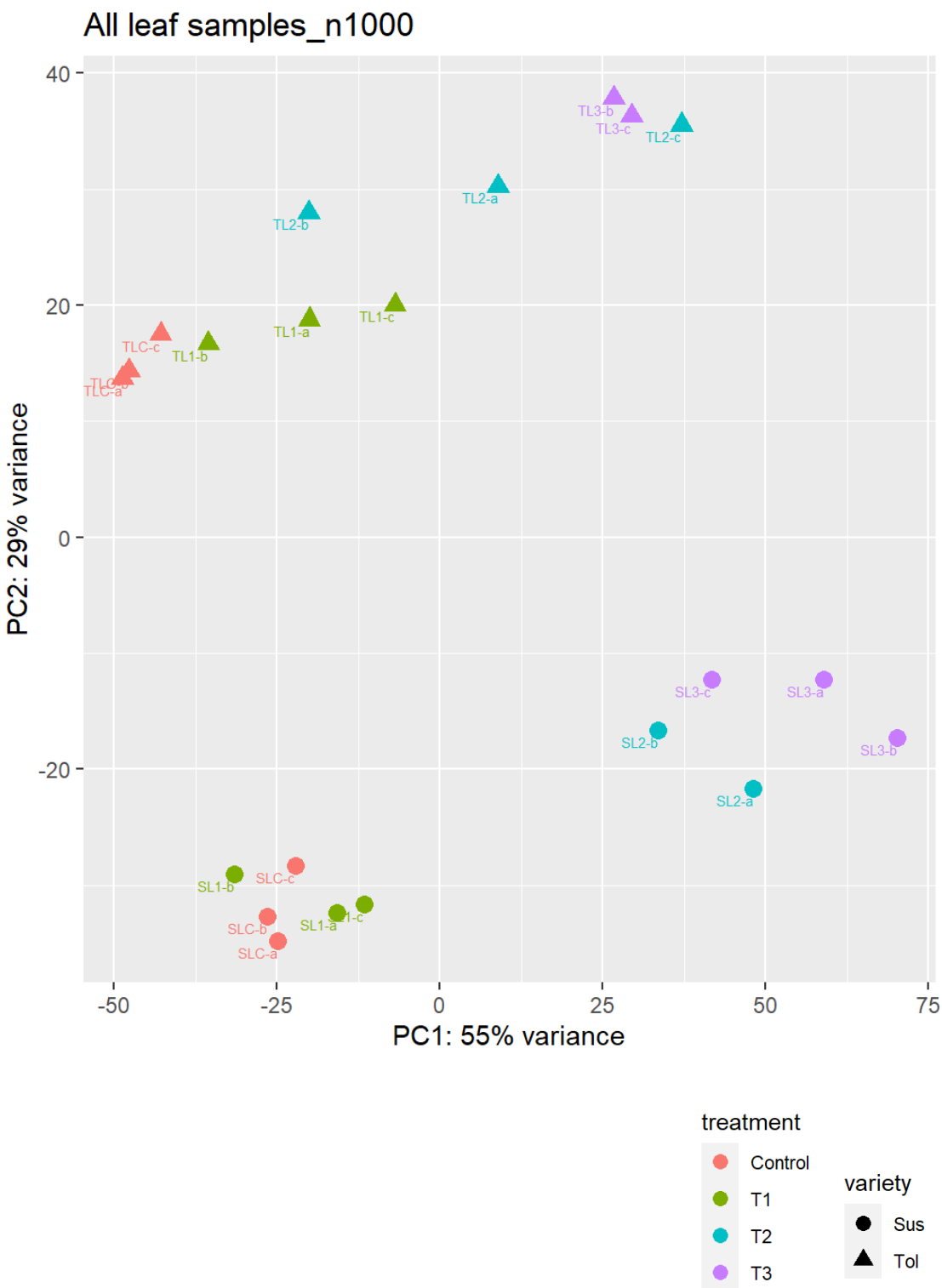
From each of the 48 RNA-seq libraries, most reads uniquely mapped to the genome reference and were used in the following analysis, where the minimum percentage of reads kept after trimming and mapping was 82.10% (**Appendix-Table 2.1**).

Principal component analysis (PCA) was carried out with the normalized counts and based on the top 1,000 genes with the greatest variance across the leaf or root samples, independently (PCA with the entire dataset can be visualized in **Appendix-Figure 2.1**). According to the PCA plot and the correlation values between pairs of replicates, some biological replicates did not appear as true replicates. Higher correlation values were expected and found between pairs of samples from the same replicates, most of them ranged from 0.98 to 1. The PCA of leaf samples showed that TL3-a and SL2-c were far apart from their respective replicates, and they did not have a high correlation values with their 2 other replicates, which were 0.97 and 0.95, respectively (yellow circles in **Appendix-Figure 2.2-A, B**, **Appendix-Figure 2.4-A, B**). In root, the PCA showed that TR2-b was far apart from their respective replicates, this sample did not have also a high correlation value with their other 2 replicates (0.97) (yellow circles in **Appendix-Figure 2.2-C**, **Appendix-Figure 2.4-C**). Therefore these samples were removed from the following analysis.

New PCA plots were constructed with the samples retained for analysis. Across component 2 (PC2) of the PCAs for leaf and root tissues, which contained 29% and 19% of the total variance, respectively, samples from the same variety were clustered separately and this clear division between the susceptible and tolerant variety samples was observed in both tissues (**Figure 2.2-A, B**). Through component 1 (PC1), which contained 55% and 56% of the total variance for leaf and root, respectively, samples were distributed following the course of the treatment. In leaf and root, control samples were closest to T_1, and increasingly distant from T_2 and T_3 samples (from left to right in **Figure 2.2-A, B**). This suggests that although T_3 was a physiological recovery phase, the expression of many genes did not return to the levels observed before the drought treatment in either tissue or either variety, though this return towards control levels was expected in the tolerant variety.

The distribution of the samples at each time point differed between both varieties, in leaf and root. In the leaf, in the susceptible variety, the control and T_1 samples were closer, generating a separate group from T_2 and T_3, though this was not observed in the tolerant variety (**Figure 2.2-A**). This indicated that in leaf during the early response (T_1), the tolerant variety changed the expression of its genes faster, since there was more variance in gene expression between control and T_1, compared to the susceptible variety. In the root, in both varieties, samples from control and T_1 formed a closer group more distant from T_2, as in susceptible leaf, and T_2 were distant from T_3 samples (**Figure 2.2-B**, and **Appendix-Figure 2.3-C, D**). In leaf, samples for T_2 were not far apart from T_3 (**Appendix-Figure 2.3-A, B**). It suggests that the difference in gene expression from the late response (T_2) to the recovery phase (T_3) is greater in the root than in the leaf. Overall, the sample distributions in both tissues (**Figure 2.2-A, B**) suggested that the main differences between varieties may be observed in the response of the leaf tissue.

(A)


All leaf samples_n1000
PC2: 29% variance
PC1: 55% variance
40
20
0
-20
-50
-25
0
25
50
75
TL3-b
TL3-c
TL2-c
TL2-a
TL2-b
TL1-c
TL1-a
TLC-c
TL1-b
TLC-b
TLC-a
SL3-c
SL3-a
SL2-b
SL3-b
SL2-a
SL1-b
SLC-c
SLC-b
SL1-a
SL1-c
SLC-a
treatment
Control
T1
T2
T3
variety
Sus
Tol

(B)

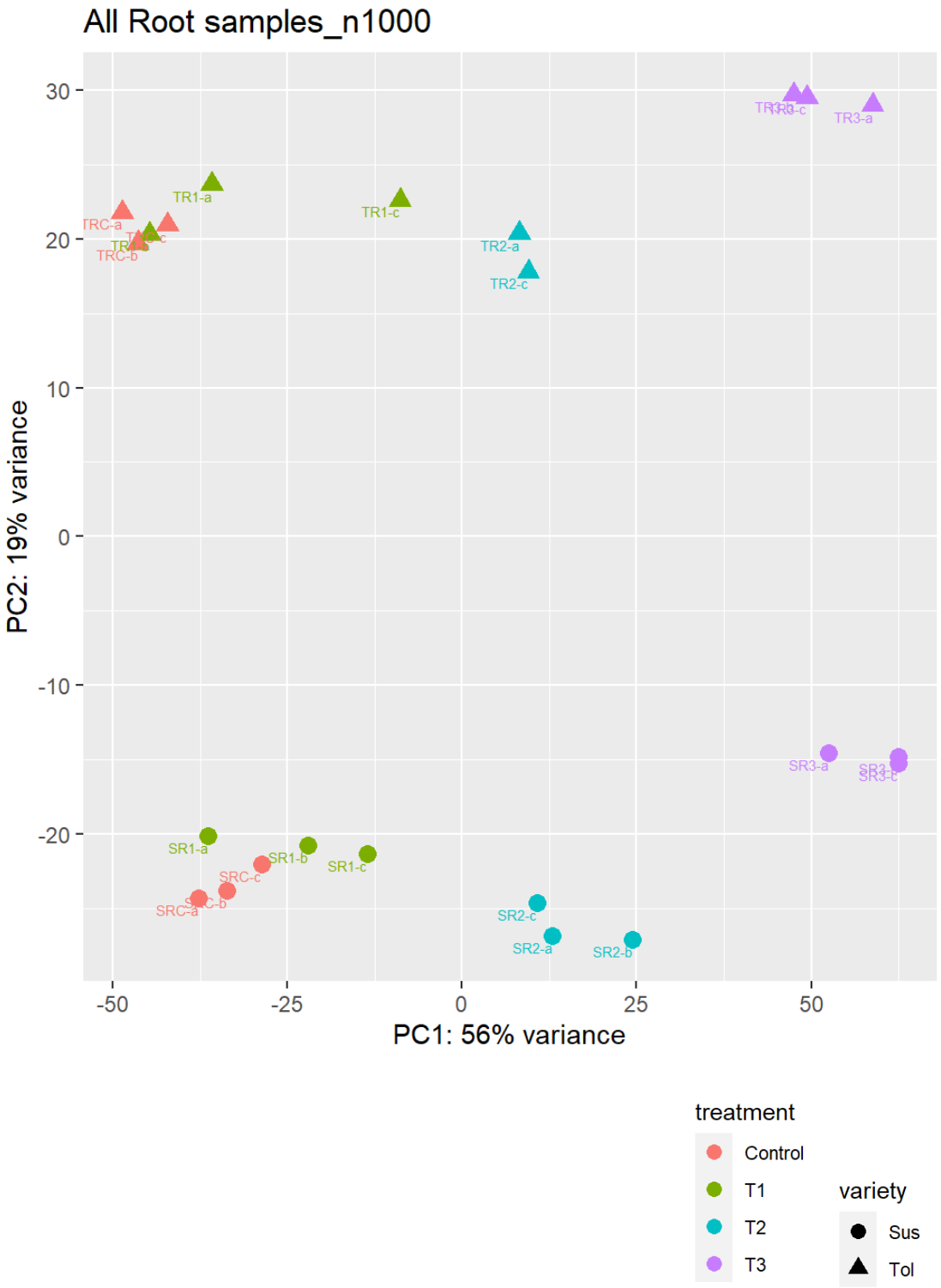


Figure 2.2 Distribution of the samples by principal component analysis (PCA) based on the transcriptomic profile. PCA plot of leaf (A) and root (B) samples in the tolerant and susceptible varieties for the top 1,000 genes showing the most variation in expression. The control time point is before the stress, while T1, T2 and T3 are the early, late and recovery responses respectively.

2.3.2. Differentially expressed genes in leaf and root.

Differentially expressed genes (DEGs) passing thresholds of |log2FC| ≥ 1 and padj. < 0.05 were identified in leaf and root by comparing the expression in each time point to control or expression between varieties at a given time point.

2.3.2.1. *Expression over time in each variety and tissue*

The tolerant variety responded faster to drought than the susceptible by upregulating more DEGs during stress and tending to recover the expression of its genes after rewatering. In both tissues and both varieties, the number of DEGs that were up or downregulated increased over time, even in the recovery phase. In the leaf, in the early response (T_1) the tolerant up-regulated and down-regulated a higher number of DEGs (389 and 102, respectively) than the susceptible (31 and 7, respectively (**Figure 2.3-A**). This difference is also reflected in the PCA plots where the control and T_1 samples were closer together in the susceptible compared to the tolerant variety (**Figure 2.2**). In the late response (T_2), there were more up-regulated genes in the tolerant than the susceptible (2471 and 1875, respectively), while the opposite occurred for downregulated genes (1059 and 1417, respectively). In the recovery phase (T_3), although in both varieties the number of DEGs was more than in T_2, the number of up and downregulated genes in T_3 was higher in the susceptible, and particularly so for the downregulated genes (**Figure 2.3-A**).

In the root, in the early response (T_1) the tolerant also up-regulates and down-regulates more DEGs (552 and 44, respectively) than the susceptible (434 and 35, respectively), as in leaf, but the difference between varieties was less. In the late response (T_2), there were more up and downregulated genes in the tolerant (2,197 and 1,800, respectively) than in the susceptible (1,628 and 1,588, respectively). In the recovery phase (T_3) of both varieties, the number of DEGs was more than in T_2, though the number of upregulated genes was higher in the tolerant, while the number of downregulated genes was much lower (**Figure 2.3-B**).

Therefore, in both tissues, the tendency of both varieties was to increase the number of DEGs under drought (T_1 and T_2); however, in the recovery phase (after rewatering) the increment began to be reduced in the tolerant (for upregulated genes in leaf or downregulated genes in root), which suggested that this variety was responding to rewatering more effectively than the susceptible. Physiologically, the tolerant variety recovered 80% of its photosynthetic rate at T_3, while the susceptible variety recovered only 50%. All these observations were consistent with those from the PCA (**Figure 2.2**), i.e., there was a progressive change in gene expression over the time course, which can be observed by the number of new genes that were differentially expressed at each time point.

The tolerant variety showed a larger early response than the susceptible variety not only in terms of the number of DEGs, but also in terms of the fold change. In the volcano plots, in the leaf, the tolerant was clearly responding with more DEGs in T_1 than the susceptible, while in the root, this difference was not as accentuated. However, in both tissues, the tolerant varieties had several DEGs with a log2FC close to or more than 5, while in the susceptible no DEGs exceed this value (**Figure 2.4**).

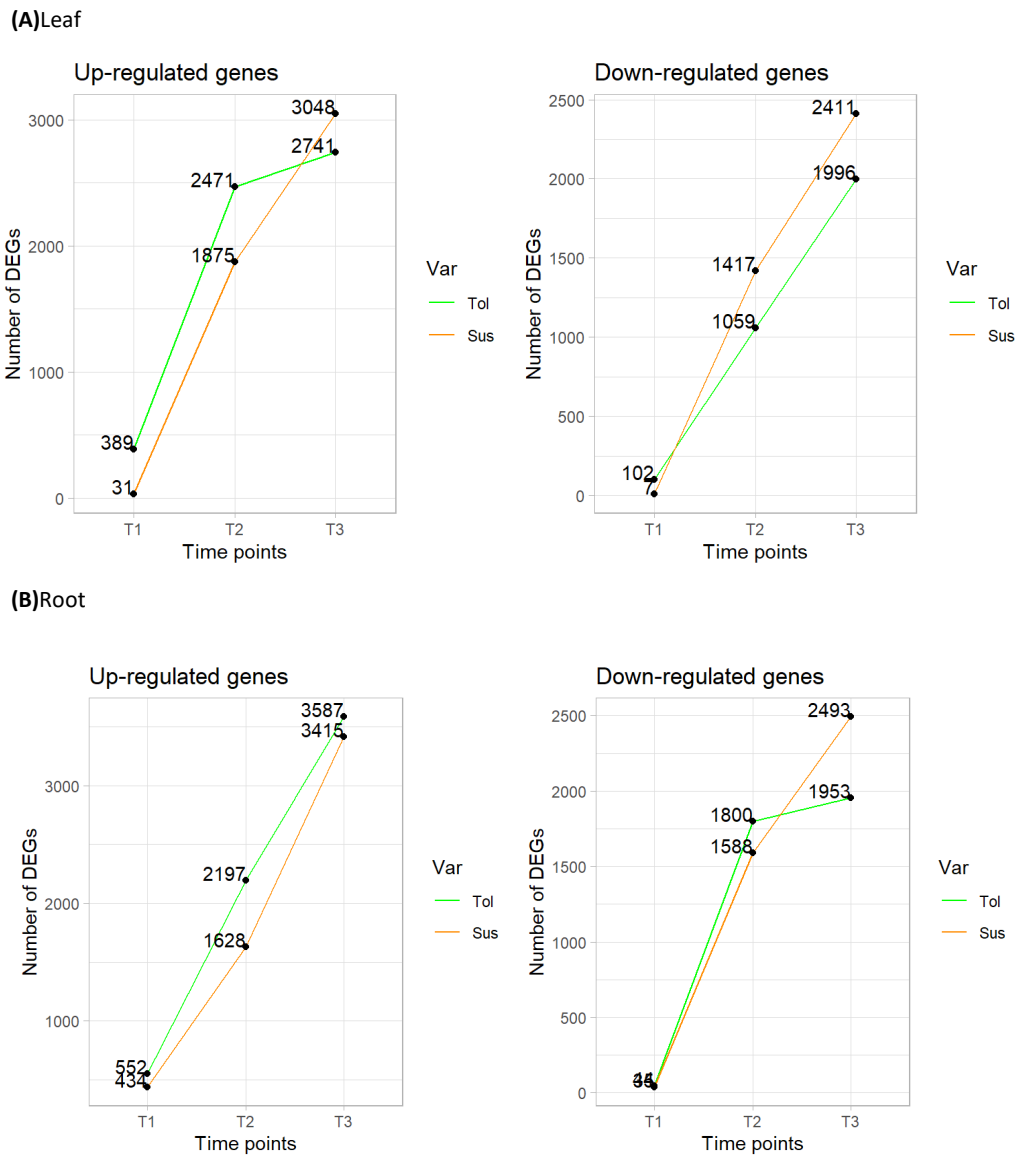


Figure 2.3 DEGs in each variety and tissue across treatments. The data are plotted separately for upregulated (padj. < 0.05 and log2FC >1) and downregulated genes (padj. < 0.05 and log2FC <-1) in leaf (A) and root (B) at T_1, T_2 and T_3 time points compared with the control.

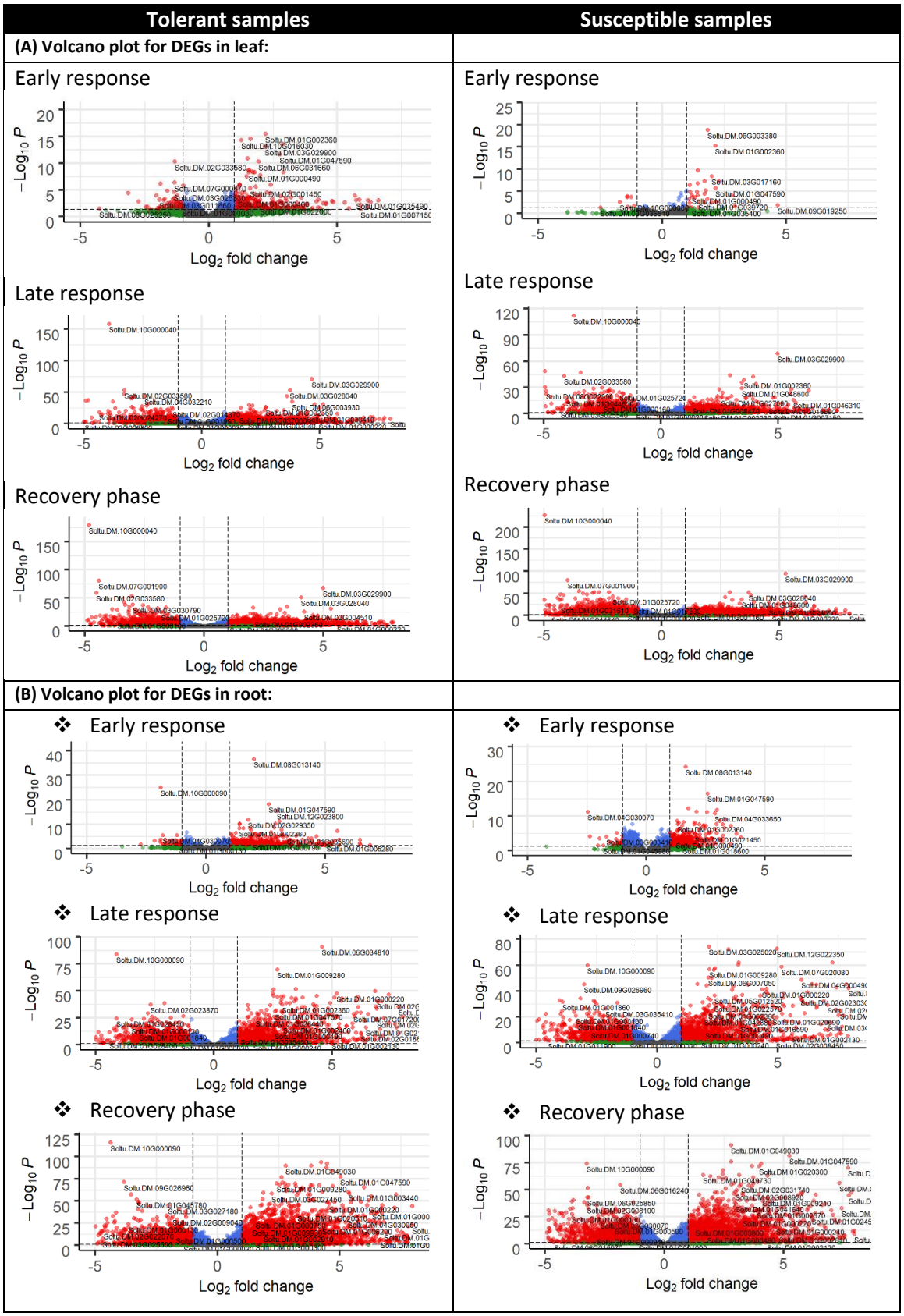


Figure 2.4 Volcano plot distribution of the gene expression in the early response, late and recovery response to drought in leaf (A) and root (B). Significance at different levels is shown in colour, where significance is defined with an absolute Log2FC of at least 1 and an adjusted p-value of 0.05, and NS denotes not significant. NS Log_2 FC p-value p – value and log_2 FC

2.3.2.2. Commonalities and differences in DEGs across stress time points.

In leaf and root tissues, the number of DEGs specific to only one time point increased over the treatment in both varieties ($T_1 < T_2 < T_3$). However, there were fewer specific DEGs at one time point than the number shared among different time points, which indicated that most of the genes that changed their expression at a particular time point continued to be changed during the rest of the treatments (**Figure 2.5**).

In the leaf, in the tolerant variety, from the 389 DE upregulated genes in the early response, 324 continued to be upregulated in the late response, and 281 over the 3-time points. In the late response, 2,147 genes were significantly upregulated, of which 1,757 were also upregulated in the recovery phase. In the recovery phase, fewer DEGs specific to this time point were upregulated in the tolerant than in the susceptible (698 vs. 1,437, respectively). For the downregulated DEGs, at the early time point, the number of DEGs unique or shared at other times were also higher in the tolerant than in the susceptible. However, the number of DEGs that began to be downregulated in T_2 was lower in the tolerant than in the susceptible (978 vs 1,410). The same was true for the group of DEGs that started to be downregulated only in T_3 (1,097 vs 1,209) (**Figure 2.5-A, B**).

In the root, in the tolerant variety, from the 552 upregulated DEGs in T_1, 359 continued to be upregulated in T_2, and 206 also in T_3. In the late response, 1,898 DEGs began to be significantly upregulated, of which 1,302 were also upregulated in the recovery phase. In this recovery phase, 1,861 began to be upregulated only at this time point. All these numbers were lower in the susceptible variety, except for the number of DEGs expressed only in T_3, where the susceptible began to upregulate more DEGs than the tolerant (2,049 vs 1,861, respectively). For the downregulated DEGs, the trend was very similar between both varieties around the different time points (**Figure 2.5-C, D**).

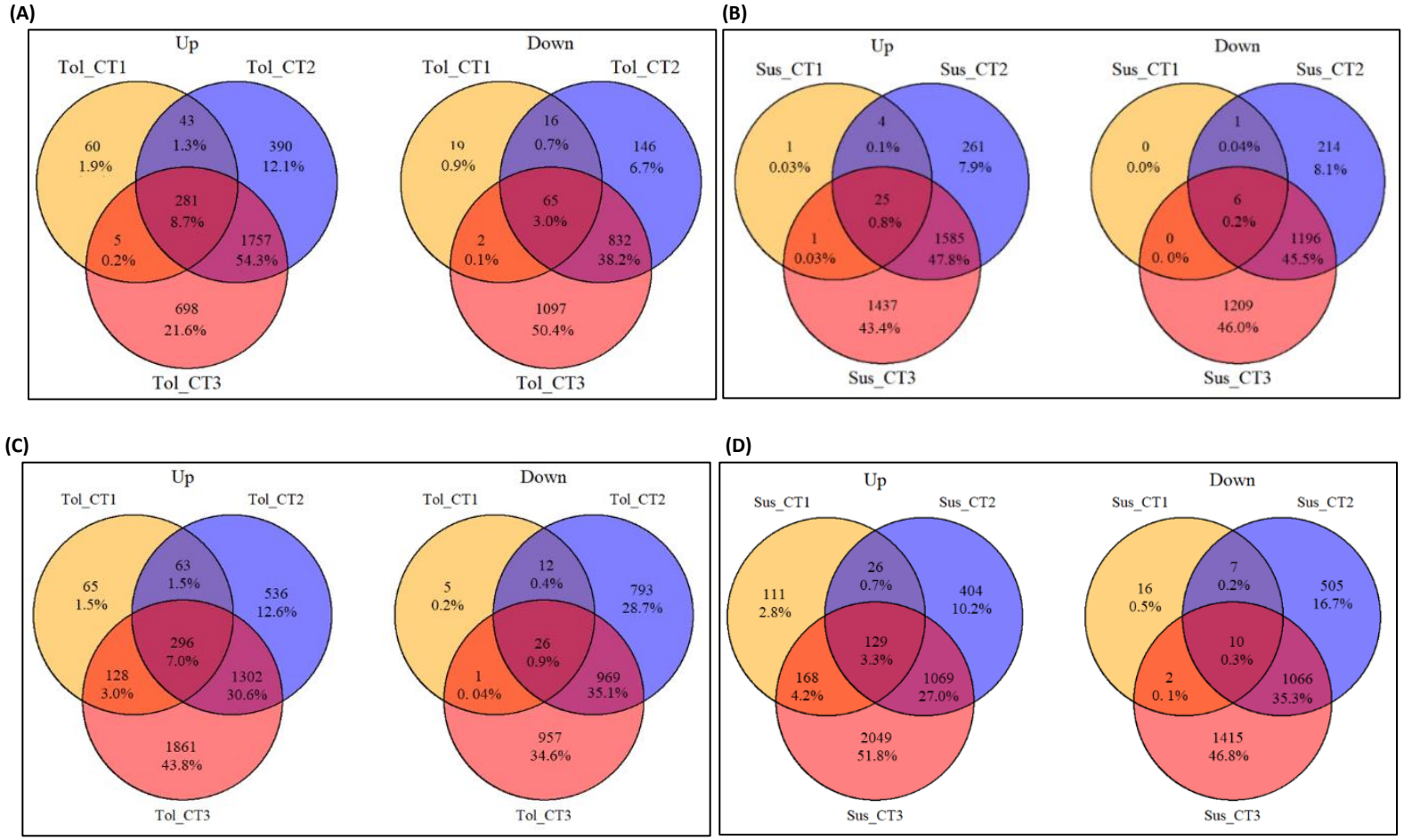


Figure 2.5 DEGs across 3 drought treatment time points in tolerant and susceptible varieties. DEGs (padj. < 0.05 and abs(log2FC) > 1) are separated by upregulated and downregulated in the tolerant (Tol) and in the susceptible (Sus) varieties in leaf (A, B) and root (C, D).

2.3.2.3. Commonalities and differences in DEGs between varieties

In leaf and root, most of the up or down regulated DEGs in the early response (T_1) were specific to the tolerant variety. This can be observed in the percentage of DEGs that were up-regulated or down-regulated in each variety (**Figure 2.6-A, B**). In leaf, there was a remarkable difference between the percentage of DEGs expressed only in the tolerant (92.1% and 93.4%, for up and down-regulated DEGs) and only in the susceptible variety (1.3% and 3.8%, for up and down-regulated DEGs). This could be expected since the number of DEGs in T_1 in the susceptible leaf was very low (only 38 DEGs in total) compared to the tolerant leaf (491 in total) (**Figure 2.3-A**, **Figure 2.6-A**, **Appendix-Table 2.2**). In the root, the major difference between the number of variety-specific DEGs was observed in the late response (T_2). In total, for up and downregulated genes, the tolerant root had 2,060 variety-specific DEGs while the susceptible root had 1,279 variety-specific DEGs (**Figure 2.6-D**, **Appendix-Table 2.3**). In the recovery phase, in both tissues, the tolerant variety showed more common than variety-specific DEGs (**Figure 2.6-E, F**; **Appendix-Table 2.3**). Indeed, the number of common genes between varieties increased over each time point and at most time points, majority of DEGs in the susceptible were shared with the tolerant variety. In leaf, there were more downregulated genes at the later time points, T_2 and T_3, in the susceptible compared with the tolerant variety.

The data suggest that, under mild stress, tolerant plants react faster to stress by altering the expression of many genes (**Figure 2.3**), which in some cases change dramatically (**Figure 2.4**). Many of these genes were unique to this variety, not being expressed in the susceptible variety, in both root and leaf (**Figure 2.6-A, B**). While during acute stress at later time points, there were more DEGs in common between both varieties (**Figure 2.6-C, D**). Even so, more genes changed expression in the tolerant than in the susceptible in both tissues (**Figure 2.3**, **Appendix-Table 2.2**).

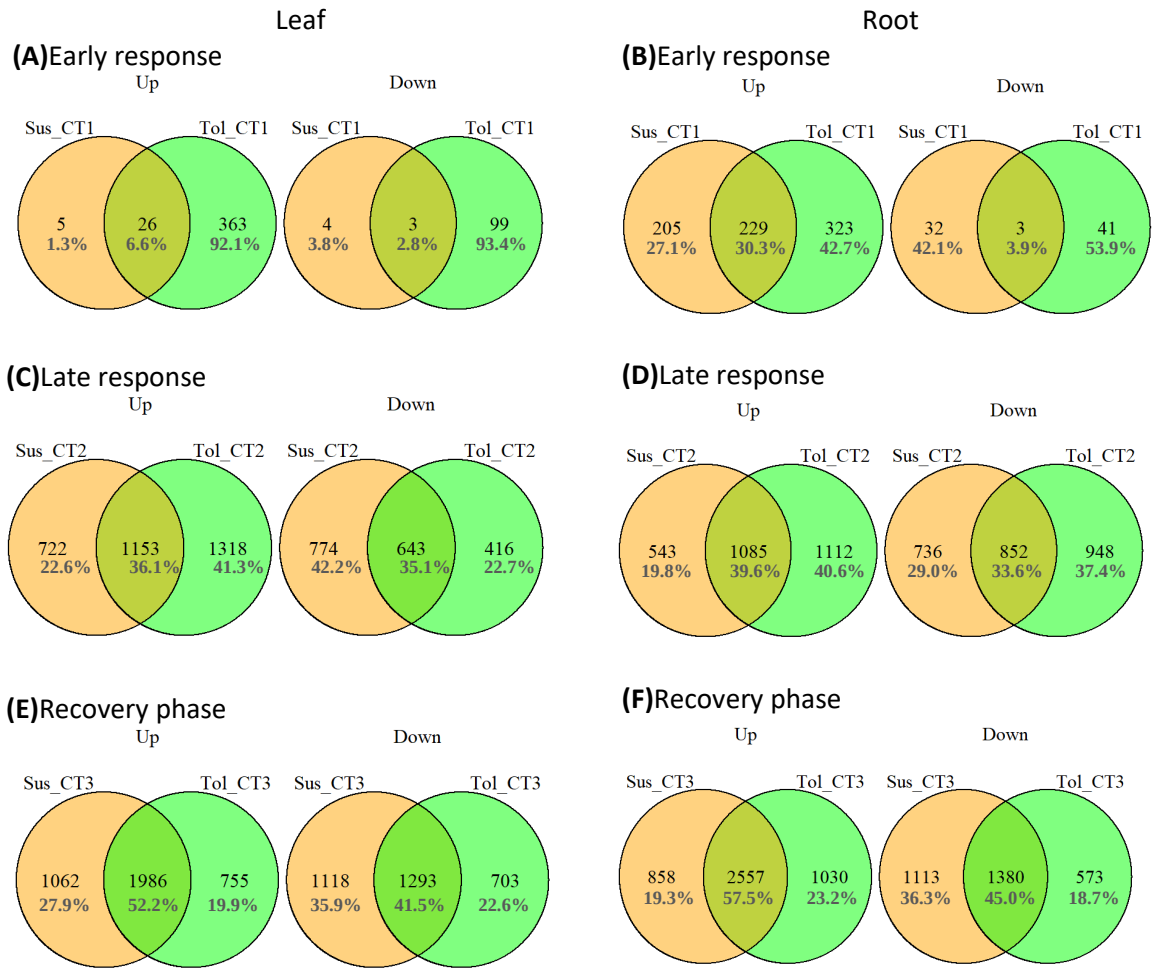


Figure 2.6 DEGs up or downregulated in only one variety or in both at each drought treatment time point. Venn diagrams show the number of DEGs unique or shared between varieties in the early response (**A, B**) late response (**C, D**) and the recovery phase (**E, F**).

2.3.2.4. DEGs specific to the tolerant variety

Since the main difference between the two varieties was observed in T_1, DEGs that respond to drought at this early time point in only the tolerant variety were selected and the top 20 with the highest fold change identified. Additionally, the gene expression between both varieties was compared to observe if these DEGs found in T_1 in the tolerant had a higher or lower expression than the susceptible variety.

Only the tolerant root, but not the susceptible one, highly changed the expression of genes involved in cell wall modification, terpene biosynthesis, and genes codifying for UDP-glycosyl-transferases

during the early, late response, and the recovery phase, although the genes involved in these processes were different in the 3 time points. For example, in T_1 upregulation of genes for lignin biosynthesis and for **expansin** was observed; in T_2 a strong downregulation of genes involved in cell wall modification, including xyloglucan and pectin components, was observed, and in T_3 this strong downregulation was observed for genes related to the hemicellulose component (cellulose synthase like G3) (**Appendix-Table 2.4, Appendix-Table 2.6, Appendix-Table 2.8).**

In only the tolerant, but not susceptible leaves, in T_1 and T_3 there was a strong upregulation of genes related to the ABA response. Upregulated genes include a protein phosphatase 2CA (PP2C) gene *Soltu.DM.06G013730*, in all 3-time points, but more strongly upregulated in T_1 and T_3. The overexpression of these genes may allow the tolerant plant to regulate the ABA response faster than the susceptible variety. Moreover, in this tissue also upregulation of DEGs involved in the transport of organic compounds was observed in T_1 (sugars, amino acids, and lipids) and in T_2 (amino acids and lipids). Among these genes, *Soltu.DM.10G018680* encoding a lipid transfer protein was highly upregulated at both time points. Also, at both times, there was strong upregulation of heat shock proteins. Genes for bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily proteins were strong downregulated in the 3-time points, with downregulation of the *Soltu.DM.08G025250* gene observed at T_1, T_2, and T_3. Among the 20 most downregulated genes in leaves only in T_3, were 4 genes encoding SAUR-like auxin-responsive protein family proteins (**Appendix-Table 2.5, Appendix-Table 2.7, Appendix-Table 2.9)**.

Genes in common between both tolerant tissues code for a dehydrin domain containing-protein (*Soltu.DM.02G006200*) in T_1 (**Appendix-Table 2.4, Appendix-Table 2.5)**, and for a hypothetical protein (*Soltu.DM.01G007060*) in T_3 (**Appendix-Table 2.8, Appendix-Table 2.9)**. While in T_2 there were no genes in common in both tissues, there were genes whose functional annotation was the

same, such as for pectin-lyase like superfamily proteins, NAD(P)-binding Rossmann-fold superfamily protein, and HXXXD-type acyl-transferase family protein (**Appendix-Table 2.6, Appendix-Table 2.7)**.

2.3.3. Gene ontology enrichment of differentially expressed genes

To identify biological functions associated with a tolerant response, GO enrichment analysis was performed with the DEGs grouped into up and downregulated genes in leaf and root tissue, at each time point (**Figure 2.9**).

2.3.3.1. Early response to drought

In the tolerant leaves, as expected by observing the number of DEGs, there were more enriched GO terms specific to this variety than in common with the susceptible leaf. Only in the tolerant leaf, but not in the susceptible leaf, the upregulated DEGs were enriched with 13 GO terms and the downregulated DEGs with 12 GO terms (**Figure 2.9-A**). While most of the 13 GO terms were also enriched in root, all the 12 GOs were specific to leave (**Figure 2.9-A)**. Most of the 13 GO enriched terms exclusive to the tolerant in leaves were processes related to the osmotic or abiotic stress ("Response to ABA"- GO:0009737, "response to water deprivation", "ABA activated signaling pathway", "regulation of stomatal movement", "response to heat", "cellular response to heat", "response to cold", "response to osmotic stress"). Also, one process related to the biotic stress ("response to chitin"), transcription factors ("negative regulation of DNA-templated transcription initiation", "negative regulation of transcription DNA-templated", "regulation of transcription, DNA-templated"), and RNA modification ("RNA modification") were included (**Figure 2.9-A, Appendix-Figure 2.5-A**).

In the "response to ABA"-GO:0009737 process, which was one of the enriched GO terms with more DEGs, there were 39 upregulated DEGs in the tolerant variety compared with only 5 in susceptible leaves (**Figure 2.7**). These included 5 genes encoding PP2C proteins, two ABA transporters (*Soltu.DM.11G011430-AtABCG25, Soltu.DM.05G023720*-AtABCG40), 3 ABI five binding proteins

(*Soltu.DM.04G000490, Soltu.DM.02G030840, Soltu.DM.05G000860*), and 2 *AtRD26* (*Soltu.DM.12G029330, Soltu.DM.07G024710*), among others, which were not upregulated in the susceptible leaf in T_1 (**Figure 2.7**, **Appendix-Table 2.10**). Two of these PP2Cs were among the 20 most upregulated DEGs in only the tolerant variety (**Appendix-Table 2.5**). Interestingly, most of the genes that responded to ABA early in the tolerant variety only began to be upregulated in the susceptible leaves during the late response to drought (T_2) (**Figure 2.7**). Together, these findings suggest an earlier, stronger ABA-mediated response in the tolerant leaves compared with the susceptible leaves.

Genes downregulated in the tolerant leaves were enriched for seven GO terms relating to DNA replication, negative regulation of transcription factor activity and cell division, likely reflecting a generalized shut down in growth occurring in leaf, which was not observed in the susceptible variety until T_2 **(Figure 2.9-A, B)**. One of the common biological processes enriched in both varieties, and only in leaf and not in root, was related to the cell wall modification ("Plant-type cell wall modification"-GO:0009827) (**Figure 2.9-A**). In this term, 6 genes encoding for plant invertase/pectin methylesterase inhibitor superfamily proteins were upregulated in the tolerant but only 3 in the susceptible. However, in T_2, 7 genes coding for this enzyme were DE in both varieties with a high log2FC (**Appendix-Table 2.11)**.

While there was very little similarity between the two varieties for gene enrichment in leaves, in root there were 21 common enriched GO terms for upregulated genes (**Appendix-Figure 2.5-B**). These included 8 terms also enriched for the upregulated DEGs of the tolerant leaves, such as the "response to ABA", "response to water deprivation", "response to osmotic stress", "cellular response to heat", "regulation of stomatal movement", "response to chitin", and "regulation of transcription, DNA-templated". Although the "response to ABA" (GO:0009737) term was enriched in both varieties, it also contained specific DEGs (log2FC > 1) for the tolerant root (**Appendix-Table 2.10**). These DEGs were almost completely different from those observed in only the tolerant leaf. It included 3 ABCG11

transporters (for cutin transport), 2 ABCG40 transporters (for ABA transport), 1 raffinose synthase family protein, and most highly upregulated, 1 galactinol synthase (*Soltu.DM.02G006360*) and 2 MYB domain proteins (*Soltu.DM.05G023310, Soltu.DM.12G001820*) (**Appendix-Table 2.10**). These 2 last DEGs were among the most 20 upregulated genes in only the tolerant variety (**Appendix-Table 2.4**).

Also, from the 21 GO terms, common terms enriched in both varieties, but only in root, included "response to hypoxia" and "response to wounding", both with a high number of DEGs, "salicylic acid (SA) catabolic process", "lignin catabolic process", "L-phenylalanine catabolic processes", and "response to oxidative stress" **(Figure 2.9-A, Appendix-Figure 2.5-B)**. In the "lignin catabolic process" (GO:0046274) and "L-phenylalanine catabolic process" (GO:0006559), both varieties upregulated six DEGs encoding PHE ammonia lyases (PALs), which are involved in the first step of production of lignin by converting phenylalanine into cinnamic acid (**Figure 2.8**). Interestingly, among the top 20 most upregulated genes in only the tolerant variety was a laccase gene (*Soltu.DM.04G028320*) and a peroxidase superfamily protein homologue to *PER52* in *A. thaliana* (*Soltu.DM.06G032730*) (**Appendix-Table 2.4**, both involved in the polymerization of lignin monomers. Laccase *Soltu.DM.04G028320* was also one of the genes with the biggest difference in expression between tolerant and susceptible varieties (**Appendix-Table 2.4**). Together, these data indicate that despite both varieties showing enrichment for a metabolic activity related to lignin, the tolerant roots experienced more changes in gene expression relating to this process than the susceptible roots (**Figure 2.8**). In the "response to oxidative stress" (GO:0006979), although being enriched in both varieties in root, more overexpression DEGs were observed only in the tolerant (16 DEGs) compared to only in the susceptible root (8 DEGs) (**Table 2.3**). These included the high overexpression of one galactinol synthase (*Soltu.DM.02G006360*) and 3 serine-type endopeptidase inhibitors (*Soltu.DM.03G003070, Soltu.DM.06G018620,* and *Soltu.DM.06G018610*), 2 of which were also among the 20 most upregulated genes in only the tolerant root (**Table 2.3**, **Appendix-Table 2.4**).

There were 10 enriched terms specific for tolerant roots including the “response to salt stress” (GO:0009651) and to the “defence response to fungus” (GO:0050832) **(Figure 2.9, Appendix-Figure 2.5-B)**. In the defence against fungus, 4 genes annotated as CHI-B in *Arabidopsis (Soltu.DM.02G022920, Soltu.DM.02G022960, Soltu.DM.07G005390, Soltu.DM.07G005400*) were only upregulated in the tolerant root, during T_1 and T_2, but not in the recovery phase (**Table 2.4**).

Since the response to ABA was one of the main differences observed between the two varieties in leaf, and the process related to this hormone was also observed in root, the expression of NCED genes was analysed in both tissues (**Table 2.2**). In roots, *Soltu.DM.07G022620 (AtNCED3*) and *Soltu.DM.08G006990 (AtNCED5*) were overexpressed in both varieties across all three-time points. At*NCED3* was also upregulated in leaves of both varieties at the later stress time points. Specifically, in leaf the *Soltu.DM.08G015120* gene, homologous to At*NCED3* and At*NCED1,* was not upregulated in roots, but was overexpressed only in the tolerant leaf during all 3-time points (**Table 2.2**, **Figure 2.7**).

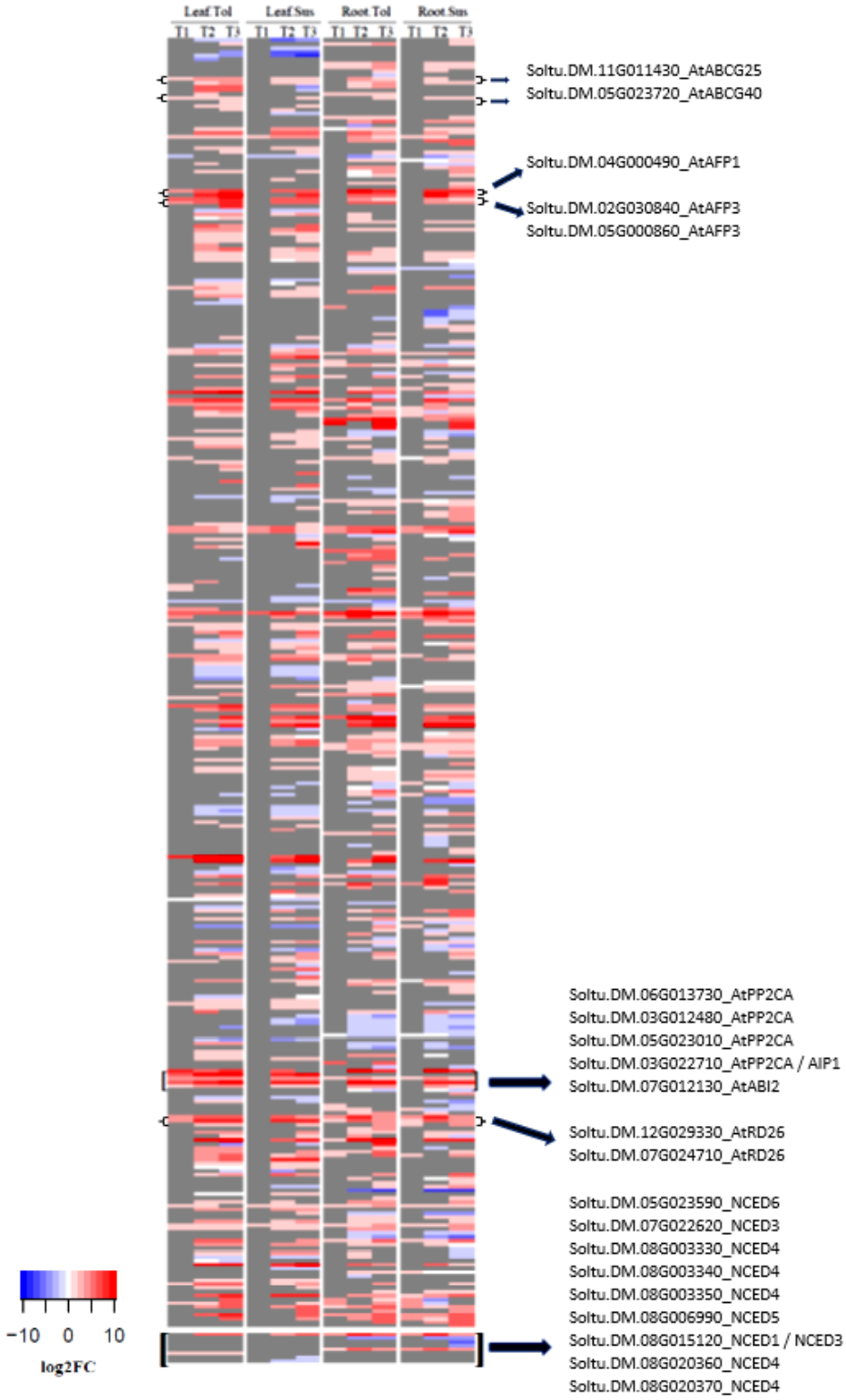


Figure 2.7 Heatmap of the log2 fold change values of the DEGs related to the process response to ABA (GO:0009737) and of the NCED genes. The values are the log2FC of each DEG and in grey are the genes with no significant change in the respective time point, tissue, and variety. In the heatmap it is indicated genes encoding for ATP Binding Cassette Subfamily G (AtABCG25 ABCG40), ABA-insensitive (ABI) five binding protein (AtAFP1, AtAFP3), protein phosphatase 2C/ AKT1 interacting protein phosphatase 1 (AtPP2CA/AIP1), responsive to desiccation 26 (AtRD26) and for 9-cis-epoxycarotenoid dioxygenase (NCED6,3,4,5,1/3). The DEGs expressed only in the tolerant leaves and root can be found in **Appendix-Table 2.10**.

Table 2.2 Expression of NCED genes annotated in the potato genome across the different time points, tissues, and varieties. The table shows the log2FC from control to each time point of each NCED gene. The (-)s are for genes that did not have a significant change from control to the respective time point.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Leaf.Tolerant			Leaf.Susceptible			Root.Tolerant			Root.Susceptible			*A. thaliana* GeneName
		T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	
Soltu.DM.05G023590	nine-cis-epoxycarotenoid dioxygenase	-	3.90	-	-	3.87	-	-	6.80	4.01	-	7.06	-	NCED6
Soltu.DM.07G022620	nine-cis-epoxycarotenoid dioxygenase	-	3.92	4.64	-	3.03	3.96	3.16	6.20	3.50	3.35	5.12	2.64	NCED3
Soltu.DM.08G003330	nine-cis-epoxycarotenoid dioxygenase	-	-	-	-	-	-	-	-3.26	-2.90	-	-2.16	-3.10	NCED4
Soltu.DM.08G003340	nine-cis-epoxycarotenoid dioxygenase	-	-	-	-	-	-	-	-	-	-	-	-3.79	NCED4
Soltu.DM.08G003350	nine-cis-epoxycarotenoid dioxygenase	-	-	-	-	-	-	-	-	-	-	-	-2.36	NCED4
Soltu.DM.08G006990	nine-cis-epoxycarotenoid dioxygenase	-	-	-	-	-	-	1.86	3.74	2.53	2.23	3.79	3.46	NCED5
Soltu.DM.08G015120	carotenoid cleavage dioxygenase	1.24	1.51	1.02	-	-	-	-	-	-	-	-	-	NCED1 / NCED3
Soltu.DM.08G020360	nine-cis-epoxycarotenoid dioxygenase	-	-	-	-	-	-1.59	-	-	-	-	-	-	NCED4
Soltu.DM.08G020370	nine-cis-epoxycarotenoid dioxygenase	-	-	-	-	-1.44	-1.35	-	-	-	-	-	-	NCED4

Table 2.3 16 DEGs inside the "response to oxidative stress" (GO:0006979) only upregulated in the tolerant root. The (-)s are for genes that did not have a significant change from control to the respective time point.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Root.Tolerant			Root.Susceptible			*A. thaliana* Gene Name
		T1	T2	T3	T1	T2	T3	
Soltu.DM.03G003070	serine-type endopeptidase inhibitors	6.45	-	-	-	-	-	AT1G72060
Soltu.DM.06G018620	serine-type endopeptidase inhibitors	5.33	-	-	-	-	-	AT1G72060
Soltu.DM.06G032730	Peroxidase superfamily protein	4.93	-	-	-	-	-	PER52
Soltu.DM.02G006360	galactinol synthase	4.74	7.13	7.43	-	4.01	5.16	GOLS1
Soltu.DM.06G018610	serine-type endopeptidase inhibitors	4.63	-	-	-	-	-	AT1G72060
Soltu.DM.02G023240	Rhodanese/Cell cycle control phosphatase superfamily protein	2.53	1.73	-	-	1.80	-	STR15
Soltu.DM.01G040810	Peroxidase superfamily protein	2.24	-	3.73	-	-	3.53	PER28
Soltu.DM.01G000790	PLANT CADMIUM RESISTANCE	1.96	-	2.28	-	-	-	PCR2
Soltu.DM.03G030760	blue-copper-binding protein	1.85	1.93	-	-	-	1.80	BCB
Soltu.DM.01G011520	Peroxidase superfamily protein	1.62	-	-	-	-1.78	-1.46	PER59
Soltu.DM.02G033230	Raffinose synthase family protein	1.57	6.94	8.22	-	4.98	5.57	RFS5
Soltu.DM.06G031880	HSP20-like chaperones superfamily protein	1.54	2.56	4.29	-	1.77	3.73	HSP17.6B
Soltu.DM.06G010770	Peroxidase superfamily protein	1.51	4.04	5.78	-	3.37	4.81	PER52
Soltu.DM.04G027640	Peroxidase superfamily protein	1.26	-	-	-	-	-	PER12
Soltu.DM.04G037090	chloroplastic drought-induced stress protein of 32 kD	1.22	-	-	-	-	1.29	CDSP32
Soltu.DM.12G004810	catalase	1.11	1.10	-	-	-	-	CAT2

Table 2.4 DEGs annotated as chitinase, Ca interacting proteins, or WRKY inside the "response to fungus" category (GO:0050832). The table shows the chitinases and calcium interacting in GO:0050832 that was DE since T_1 or T_2 in any tissue or variety. Also, all the WRKY found in GO:0050832. The (-)s are for genes that did not have a significant change from control to the respective time point.

Potato genome v6.1	Potato genome v6.1 Gene Annotation	Leaf.Tolerant			Leaf.Susceptible			Root.Tolerant			Root.Susceptible			*A. thaliana* Gene Name
		T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	
Soltu.DM.02G022920	Chitinase family protein	-	2.19	2.84	-	-	2.12	1.90	2.94	3.76	-	3.53	4.33	CHI-B
Soltu.DM.02G022960	basic chitinase	-	-	-	-	-	-	1.61	1.73	-	-	-	-	CHI-B
Soltu.DM.07G005390	basic chitinase	-	-	-	-	-	-	3.46	3.50	-	-	-	-	CHI-B
Soltu.DM.07G005400	basic chitinase	-	-	-	-	-	-	3.52	3.63	-	-	-	-	CHI-B
Soltu.DM.10G026220	Calmodulin	-	1.35	1.34	-	1.23	1.59	-	1.43	1.95	-	-	1.15	CAM7
Soltu.DM.04G012040	Calcium-binding EF-hand family protein	-	1.39	2.13	-	-	1.76	-	1.91	2.93	1.13	-	2.30	CML36
Soltu.DM.10G026210	Calmodulin	-	1.14	1.27	-	1.29	1.65	-	1.21	1.72	-	-	1.23	CAM7
Soltu.DM.10G027990	Calcium-binding EF-hand family protein	-	1.35	1.75	-	-	1.70	-	0.94	1.80	-	-	1.45	CML36
Soltu.DM.09G009490	WRKY DNA-binding protein	2.26	4.14	5.45	-	-	4.95	1.71	1.26	3.16	1.88	-	2.78	WRKY33
Soltu.DM.08G015910	WRKY DNA-binding protein	-	4.19	4.16	-	-	-	-	2.26	-	-	-	1.59	WRKY40
Soltu.DM.03G030960	WRKY DNA-binding protein	-	2.24	2.49	-	-	2.17	2.26	2.30	6.16	3.24	2.36	6.52	WRKY40
Soltu.DM.06G018840	WRKY DNA-binding protein	-	2.43	3.00	-	-	2.30	1.38	1.28	2.89	1.43	-	2.56	WRKY33
Soltu.DM.09G011140	WRKY DNA-binding protein	-	2.43	2.68	-	-	-	-	2.39	1.41	-	-	-	WRKY70
Soltu.DM.08G012710	WRKY DNA-binding protein	-	4.13	4.80	-	3.59	4.34	2.03	3.77	4.41	-	-	2.31	WRKY50
Soltu.DM.06G024270	WRKY DNA-binding protein	-	1.62	1.86	-	1.29	2.69	1.65	-	4.03	1.75	-	4.18	WRKY40
Soltu.DM.03G013350	WRKY DNA-binding protein	-	-	1.57	-	-	-	-	1.86	1.63	-	-	1.74	WRKY70
Soltu.DM.04G023540	WRKY DNA-binding protein	-	3.96	4.53	-	2.31	1.98	-	3.84	3.88	-	2.55	2.37	WRKY50
Soltu.DM.04G028130	WRKY DNA-binding protein	-	2.85	3.30	-	-	1.71	-	3.12	4.13	-	1.62	3.78	WRKY51
Soltu.DM.08G015900	WRKY DNA-binding protein	-	2.23	3.32	-	-	2.78	-	4.71	3.91	-	2.85	3.82	WRKY40
Soltu.DM.10G018560	WRKY DNA-binding protein	-	-	-	-	-	-	-	-	-	-	-2.53	-	WRKY70
Soltu.DM.10G021890	WRKY DNA-binding protein	-	-	-	-	-	-	-	3.12	-	-	-	-	WRKY70
Soltu.DM.12G007400	WRKY DNA-binding protein	-	-	-	-	-	-	-	4.31	7.68	-	-	4.41	WRKY51
Soltu.DM.12G007390	WRKY DNA-binding protein	-	-	-	-	-	-	-	-	6.42	-	-	4.10	WRKY51

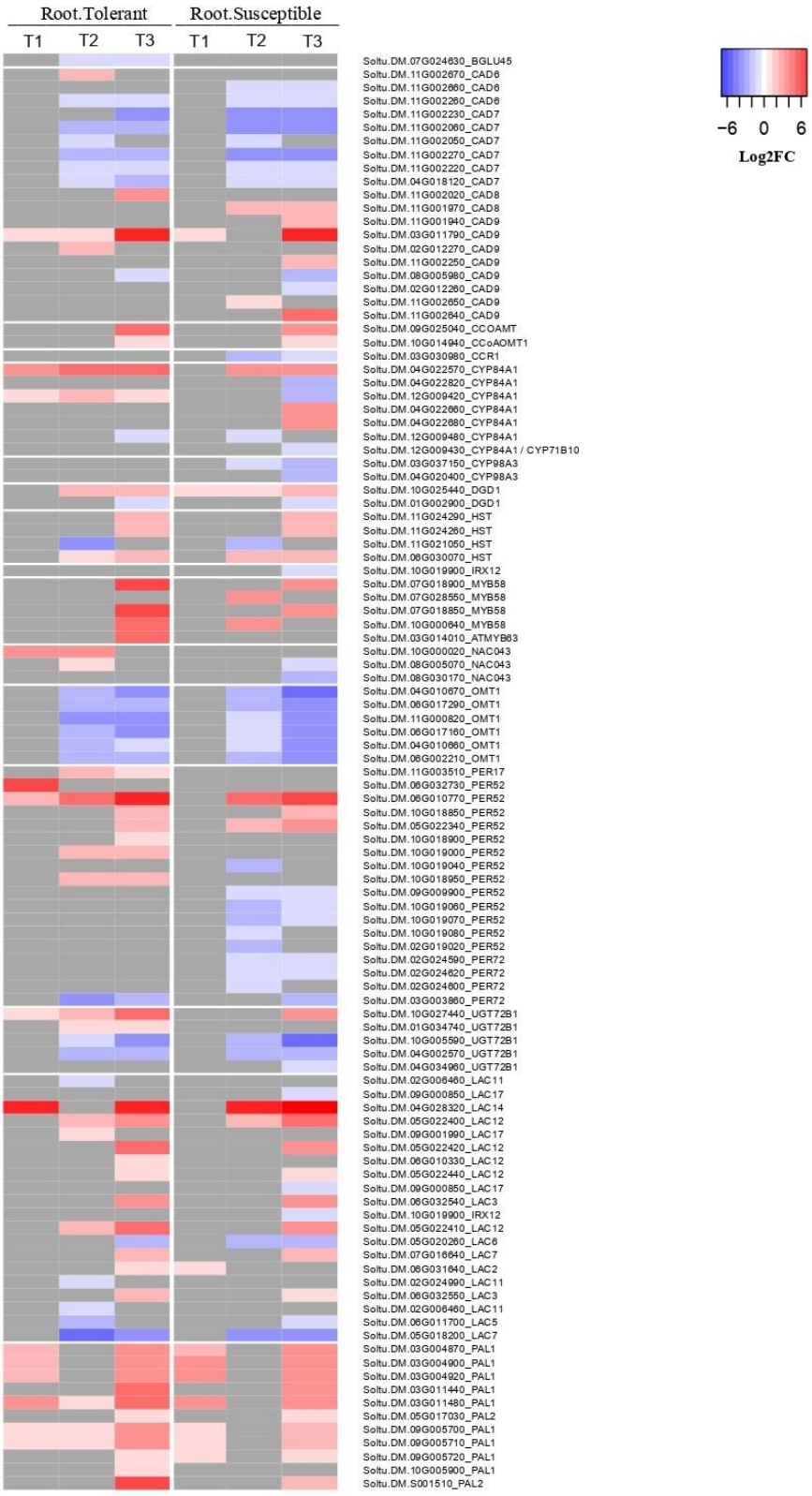


Figure 2.8 Expression of the DEGs inside the "lignin biosynthetic process"(GO:0009809), the "lignin catabolic process" (GO:0046274) and the genes annotated as laccase in the potato genome v6.1. in the root of any of the two potato varieties. The heatmap shows the log2FC of the genes in the respective time point and variety.

2.3.3.2. Late response to drought

In the late response to drought, there were more shared enriched biological processes across both variety and tissue (**Figure 2.9-B**). There were 7 GO terms enriched for upregulated genes in both varieties and tissues, all of them related to the response to abiotic stress, such as ABA activated signalling pathway (GO:0009738), the responses to ABA (GO:0009737), osmotic stress (GO:0006970), hypoxia (GO:0071456), salt stress (GO:0009651), heat (GO:0009408) and water deprivation (GO:0009414) (**Figure 2.9-B**).

In the tolerant leaf, several GO terms enriched in the early response (T_1) continued to be enriched in the late response (T_2), but they began to be enriched in the susceptible variety only in T_2. In leaf, in the tolerant variety, from the 27 enriched GO terms for the upregulated DEGs in T_2, 10 were already enriched in T_1 (**Appendix-Figure 2.5-A**). In contrast, none of the 17 GO terms enriched in the susceptible leaves in T_2 were previously enriched in T_1, and 6 of these 17 terms were already enriched in the T_1 in the tolerant ("RNA modification"- GO:0009451, "response to heat"- GO:0009408, "response to abscisic acid"- GO:0009737, "response to water deprivation"- GO:0009414, "abscisic acid-activated signalling pathway"- GO:0009738, and "response to osmotic stress"- GO:0006970) (**Appendix-Figure 2.5-A**). A similar pattern could also be observed for downregulated genes related to growth, with genes involved in DNA replication and cell population proliferation reducing their expression in the tolerant leaf since T_1, but not until T_2 in the susceptible leaves (**Appendix-Figure 2.5-A**). Similarly, in root, for upregulated genes, there were GO terms enriched in the tolerant variety at the early stage of stress that were not enriched in the response of the susceptible variety until T_2. However, there were only 2 terms that followed this trend, which were the response to salt stress (GO:0009651) and the ABA activated signalling pathway (GO:0009738) (**Appendix-Figure 2.5-B**).

More GO terms enriched in only the tolerant variety were also observed at this time point compared with T_1. For the upregulated genes, 19 GO terms were exclusively enriched in the tolerant leaves and only 9 in the susceptible leaves (**Figure 2.9-B**, **Appendix-Figure 2.5-A**). Of the 19 GO terms enriched only in the tolerant leaves, 4 were also enriched at the early time point (regulation of stomatal movement, regulation of transcription, response to cold and response to chitin). The remaining 15 GO terms included 2 processes in response to hormones (jasmonic acid signalling pathway- GO:2000022 and salicylic acid biosynthetic process- GO:0080142) and 5 terms related to the biotic defence response, such as response to chitin (GO:0010200), wounding (GO:0009611), fungi (GO:0050832), bacteria (GO:0042742) and oomycetes (GO: 0002239) (**Figure 2.9-B**, **Appendix-Figure 2.5-A**), compared with only one enriched process related to biotic stress (GO:0010200: response to chitin) in tolerant leaves at T_1. Another interesting result in T_2 was observed in only the susceptible leaf, which was enriched with processes involved in protein refolding (**Appendix-Figure 2.5-A)**. It suggests that greater protein damage could have occurred at this time in only the susceptible leaves, for which more control of their folding could be needed. In root, for the upregulated genes, while 8 GO terms were enriched in only the tolerant variety, 4 were enriched only in the susceptible (**Figure 2.9-B**, **Appendix-Figure 2.5-B)**. These 8 GO terms exclusive to the tolerant roots also included processes that respond to biotic stress, such as the responses to fungus (GO:0050832) and the response to wounding (GO:0009611), already enriched in T_1.

The defence response to fungus process (GO:0050832), enriched in the tolerant leaves and roots, but not the susceptible tissues, contained *WRKY* genes. In leaf, 3 were significantly upregulated in both varieties and 7 uniquely in the tolerant, while in root, 4 were significantly upregulated in both varieties and 8 uniquely in the tolerant, including *Soltu.DM.12G007400 (AtWRKY51),* which is also one of the top 20 most upregulated genes in the late response of root (**Table 2.4**, **Appendix-Table 2.6**). Another WRKY gene *Soltu.DM.08G028850* (*AtWRKY53*) was also in the top 20 DEGs uniquely upregulated in the tolerant leaves (**Appendix-Table 2.7**). Inside the response to fungus process, there

were also 3 genes whose products interact with calcium, including two calmodulin genes upregulated in all but the susceptible roots (*Soltu.DM.10G026220*, *Soltu.DM.10G026210*) during T_2 (**Table 2.4**).

Both varieties showed evidence for additional cell wall remodelling processes during the late response. In the tolerant and susceptible roots, downregulated genes were enriched for the xyloglucan metabolic process (GO:0010411) and cell wall biogenesis (GO:0042546), both including a large number of xyloglucan endotransglucosylase/hydrolase genes (**Figure 2.9-B**, **Appendix-Figure 2.5, Appendix-Table 2.12**). Only the tolerant root was enriched for genes involved in cell wall modification (GO:0042545), with the downregulation of 10 DEGs encoding pectin methylesterase inhibitors in T_2, of which only 4 were downregulated in the susceptible (**Figure 2.9-B**, **Appendix-Figure 2.5, Appendix-Table 2.12**). It was previously observed that among the top 20 genes downregulated only in the tolerant variety were three cell wall-related genes, including one xyloglucan endotransglucosylase/hydrolase (*Soltu.DM.12G025120*), one plant invertase/pectin methylesterase inhibitor superfamily (*Soltu.DM.02G001870*), and one pectin lyase-like superfamily gene (*Soltu.DM.04G014020*) (**Appendix-Table 2.6**).

2.3.3.3. Recovery phase to drought

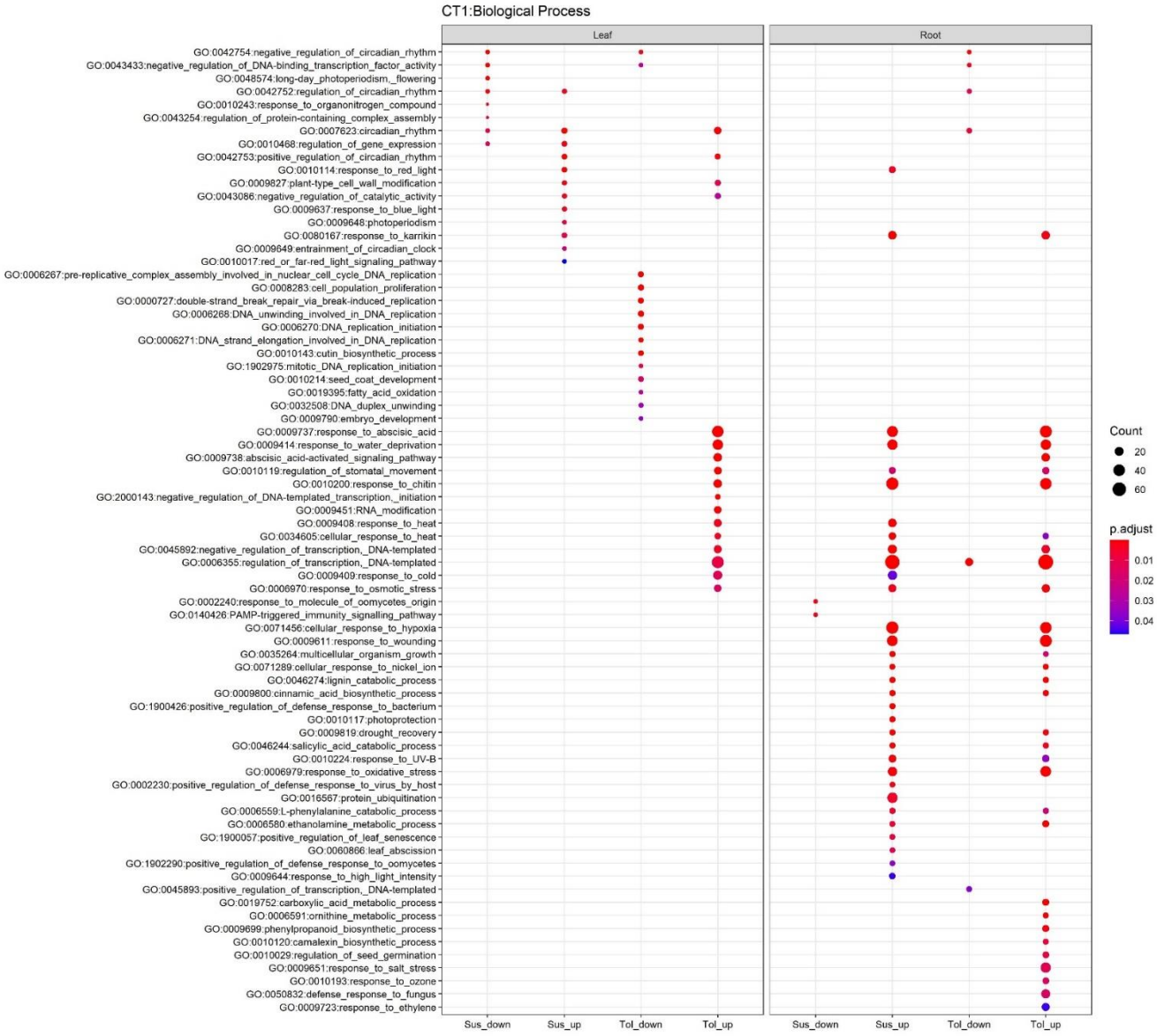
Some of the enriched GO terms observed in the late response continued to be enriched in the recovery phase. A widespread upregulation of genes related to the responses to ABA (GO:0009737), hypoxia (GO:0071456), salt stress (GO:0009651), and water deprivation (GO:0009414), was still observed across both tissues and both varieties (**Figure 2.9-C**).

Only 1 GO term began to be enriched in T_3 for the upregulated genes ("response to Karrikin"), in all but the susceptible leaves. 9 terms began to be enriched for the downregulated genes, from which 6 were for translation processes only in the tolerant leaves ("translation", "ribosomal large subunit assembly", "ribosomal large subunit biogenesis", "ribosomal small subunit assembly", "cytoplasmic translational", and "rRNA processing") (**Appendix-Figure 2.6-A.1, A.2**). Although none of these terms

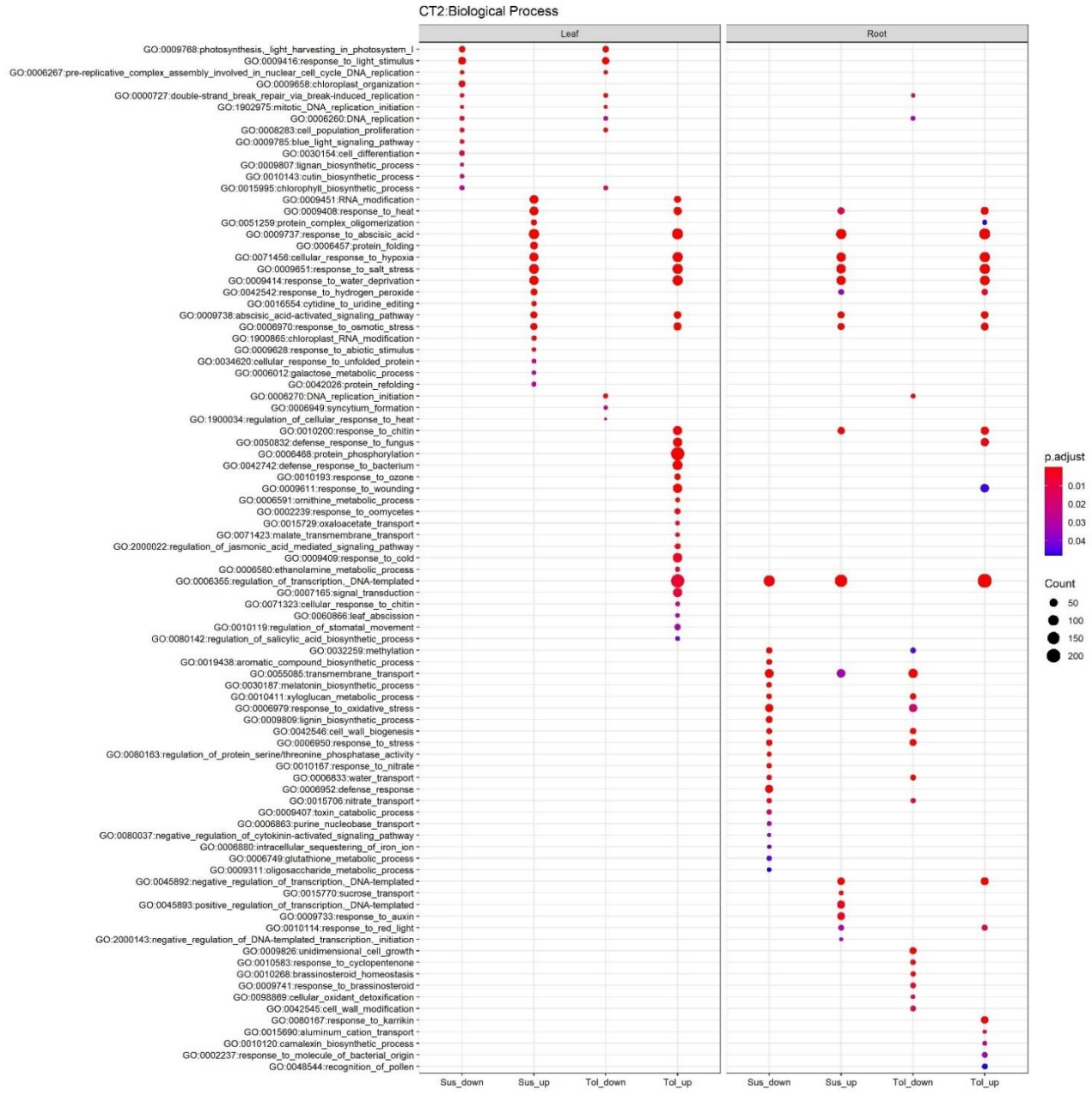
was enriched previously during stress in the tolerant leaves, this indicated that a decrease in protein translation, or synthesis, during the recovery phase occurred in the tolerant leaves, but not in the susceptible leaves.

Some GO terms became enriched for downregulated genes in T_3, where they had previously been enriched for upregulated genes in T_2, or vice versa. For example, the "xyloglucan metabolic process" (GO:0010411), which was enriched in the late response for downregulated genes in the roots of both varieties, but enriched in the recovery phase for upregulated genes. Other interesting GO terms in the tolerant in root were those related to hormone metabolism. While the "salicylic acid catabolic process" (GO:0046244) was enriched among upregulated genes only in the early response to drought in both varieties, the opposite term "regulation of salicylic acid biosynthetic process" was enriched only in the recovery phase among upregulated genes in the tolerant leaf and root (**Appendix-Figure 2.6-B.1, B.2**). The "regulation of jasmonic acid mediated signalling pathway" (GO:2000022) was enriched among upregulated genes only in the tolerant leaf in the late response, but was enriched in all but the susceptible leaf at T_3. Similarly, GO terms relating to biotic defence including defence response to fungus (GO:0050832) and defence response to bacterium (GO:0042742) were previously enriched in the late response only in the tolerant variety but became enriched for both tissues and varieties in T_3. These observations continue to indicate the delayed response to drought stress in the susceptible compared with the tolerant variety.

(A)


CT1:Biological Process
Leaf
Root
GO:0042754:negative_regulation_of_circadian_rhythm
GO:0043433:negative_regulation_of_DNA-binding_transcription_factor_activity
GO:0048574:long-day_photoperiodism,_flowering
GO:0042752:regulation_of_circadian_rhythm
GO:0010243:response_to_organonitrogen_compound
GO:0043254:regulation_of_protein-containing_complex_assembly
GO:0007623:circadian_rhythm
GO:0010468:regulation_of_gene_expression
GO:0042753:positive_regulation_of_circadian_rhythm
GO:0010114:response_to_red_light
GO:0009827:plant-type_cell_wall_modification
GO:0043086:negative_regulation_of_catalytic_activity
GO:0009637:response_to_blue_light
GO:0009648:photoperiodism
GO:0080167:response_to_karrikin
GO:0009649:entrainment_of_circadian_clock
GO:0010017:red_or_far-red_light_signaling_pathway
GO:0006267:pre-replicative_complex_assembly_involved_in_nuclear_cell_cycle_DNA_replication
GO:0008283:cell_population_proliferation
GO:0000727:double-strand_break_repair_via_break-induced_replication
GO:0006268:DNA_unwinding_involved_in_DNA_replication
GO:0006270:DNA_replication_initiation
GO:0006271:DNA_strand_elongation_involved_in_DNA_replication
GO:0010143:cutin_biosynthetic_process
GO:1902975:mitotic_DNA_replication_initiation
GO:0010214:seed_coat_development
GO:0019395:fatty_acid_oxidation
GO:0032508:DNA_duplex_unwinding
GO:0009790:embryo_development
GO:0009737:response_to_abscisic_acid
GO:0009414:response_to_water_deprivation
GO:0009738:abscisic_acid-activated_signaling_pathway
GO:0010119:regulation_of_stomatal_movement
GO:0010200:response_to_chitin
GO:2000143:negative_regulation_of_DNA-templated_transcription,_initiation
GO:0009451:RNA_modification
GO:0009408:response_to_heat
GO:0034605:cellular_response_to_heat
GO:0045892:negative_regulation_of_transcription,_DNA-templated
GO:0006355:regulation_of_transcription,_DNA-templated
GO:0009409:response_to_cold
GO:0006970:response_to_osmotic_stress
GO:0002240:response_to_molecule_of_oomycetes_origin
GO:0140426:PAMP-triggered_immunity_signalling_pathway
GO:0071456:cellular_response_to_hypoxia
GO:0009611:response_to_wounding
GO:0035264:multicellular_organism_growth
GO:0071289:cellular_response_to_nickel_ion
GO:0046274:lignin_catabolic_process
GO:0009800:cinnamic_acid_biosynthetic_process
GO:1900426:positive_regulation_of_defense_response_to_bacterium
GO:0010117:photoprotection
GO:0009819:drought_recovery
GO:0046244:salicylic_acid_catabolic_process
GO:0010224:response_to_UV-B
GO:0006979:response_to_oxidative_stress
GO:0002230:positive_regulation_of_defense_response_to_virus_by_host
GO:0016567:protein_ubiquitination
GO:0006559:L-phenylalanine_catabolic_process
GO:0006580:ethanolamine_metabolic_process
GO:1900057:positive_regulation_of_leaf_senescence
GO:0060866:leaf_abscission
GO:1902290:positive_regulation_of_defense_response_to_oomycetes
GO:0009644:response_to_high_light_intensity
GO:0045893:positive_regulation_of_transcription,_DNA-templated
GO:0019752:carboxylic_acid_metabolic_process
GO:0006591:ornithine_metabolic_process
GO:0009699:phenylpropanoid_biosynthetic_process
GO:0010120:camalexin_biosynthetic_process
GO:0010029:regulation_of_seed_germination
GO:0009651:response_to_salt_stress
GO:0010193:response_to_ozone
GO:0050832:defense_response_to_fungus
GO:0009723:response_to_ethylene
Sus_down
Sus_up
Tol_down
Tol_up
Sus_down
Sus_up
Tol_down
Tol_up
Count
20
40
60
p.adjust
0.01
0.02
0.03
0.04

(B)


CT2:Biological Process
Leaf
Root
GO:0009768:photosynthesis,_light_harvesting_in_photosystem_I
GO:0009416:response_to_light_stimulus
GO:0006267:pre-replicative_complex_assembly_involved_in_nuclear_cell_cycle_DNA_replication
GO:0009658:chloroplast_organization
GO:0000727:double-strand_break_repair_via_break-induced_replication
GO:1902975:mitotic_DNA_replication_initiation
GO:0006260:DNA_replication
GO:0008283:cell_population_proliferation
GO:0009785:blue_light_signaling_pathway
GO:0030154:cell_differentiation
GO:0009807:lignan_biosynthetic_process
GO:0010143:cutin_biosynthetic_process
GO:0015995:chlorophyll_biosynthetic_process
GO:0009451:RNA_modification
GO:0009408:response_to_heat
GO:0051259:protein_complex_oligomerization
GO:0009737:response_to_abscisic_acid
GO:0006457:protein_folding
GO:0071456:cellular_response_to_hypoxia
GO:0009651:response_to_salt_stress
GO:0009414:response_to_water_deprivation
GO:0042542:response_to_hydrogen_peroxide
GO:0016554:cytidine_to_uridine_editing
GO:0009738:abscisic_acid-activated_signaling_pathway
GO:0006970:response_to_osmotic_stress
GO:1900865:chloroplast_RNA_modification
GO:0009628:response_to_abiotic_stimulus
GO:0034620:cellular_response_to_unfolded_protein
GO:0006012:galactose_metabolic_process
GO:0042026:protein_refolding
GO:0006270:DNA_replication_initiation
GO:0006949:syncytium_formation
GO:1900034:regulation_of_cellular_response_to_heat
GO:0010200:response_to_chitin
GO:0050832:defense_response_to_fungus
GO:0006468:protein_phosphorylation
GO:0042742:defense_response_to_bacterium
GO:0010193:response_to_ozone
GO:0009611:response_to_wounding
GO:0006591:ornithine_metabolic_process
GO:0002239:response_to_oomycetes
GO:0015729:oxaloacetate_transport
GO:0071423:malate_transmembrane_transport
GO:2000022:regulation_of_jasmonic_acid_mediated_signaling_pathway
GO:0009409:response_to_cold
GO:0006580:ethanolamine_metabolic_process
GO:0006355:regulation_of_transcription,_DNA-templated
GO:0007165:signal_transduction
GO:0071323:cellular_response_to_chitin
GO:0060866:leaf_abscission
GO:0010119:regulation_of_stomatal_movement
GO:0080142:regulation_of_salicylic_acid_biosynthetic_process
GO:0032259:methylation
GO:0019438:aromatic_compound_biosynthetic_process
GO:0055085:transmembrane_transport
GO:0030187:melatonin_biosynthetic_process
GO:0010411:xyloglucan_metabolic_process
GO:0006979:response_to_oxidative_stress
GO:0009809:lignin_biosynthetic_process
GO:0042546:cell_wall_biogenesis
GO:0006950:response_to_stress
GO:0080163:regulation_of_protein_serine/threonine_phosphatase_activity
GO:0010167:response_to_nitrate
GO:0006833:water_transport
GO:0006952:defense_response
GO:0015706:nitrate_transport
GO:0009407:toxin_catabolic_process
GO:0006863:purine_nucleobase_transport
GO:0080037:negative_regulation_of_cytokinin-activated_signaling_pathway
GO:0006880:intracellular_sequestering_of_iron_ion
GO:0006749:glutathione_metabolic_process
GO:0009311:oligosaccharide_metabolic_process
GO:0045892:negative_regulation_of_transcription,_DNA-templated
GO:0015770:sucrose_transport
GO:0045893:positive_regulation_of_transcription,_DNA-templated
GO:0009733:response_to_auxin
GO:0010114:response_to_red_light
GO:2000143:negative_regulation_of_DNA-templated_transcription,_initiation
GO:0009826:unidimensional_cell_growth
GO:0010583:response_to_cyclopentenone
GO:0010268:brassinosteroid_homeostasis
GO:0009741:response_to_brassinosteroid
GO:0098869:cellular_oxidant_detoxification
GO:0042545:cell_wall_modification
GO:0080167:response_to_karrikin
GO:0015690:aluminum_cation_transport
GO:0010120:camalexin_biosynthetic_process
GO:0002237:response_to_molecule_of_bacterial_origin
GO:0048544:recognition_of_pollen
Sus_down
Sus_up
Tol_down
Tol_up
p.adjust
0.01
0.02
0.03
0.04
Count
50
100
150
200

(C)

Figure 2.9 Gene Ontology pathway enrichment in the Biological Process category for DEGs. The enriched processes are shown for the early (**A**) and late (**B**) response to hydric stress, and for the recovery phase (**C**). The left and right panels show results in leaf and root tissues for up- and down- regulated DEGs in susceptible (sus) and tolerant (tol) varieties. Significant enrichment is considered at Benjamini-Hochberg adjusted $p < 0.05$.

2.3.4. Gene responding quickly to water in the tolerant leaf or root.

To identify drought-responsive genes in the tolerant variety, genes were selected whose expression significantly increased in the early response (not restricted to $|\log_2 FC| > 1$), was maintained or continued to be increased in the late response, and returned partially or fully towards the levels in the non-stressed control in response to rewatering. In total, more genes with a quick response to drought were found in the tolerant roots (176 genes) than in leaves (45 genes). From the 45 genes in leaf, 39 showed upregulation and recovery and 6 showed downregulation and recovery; from the 145 genes in root, 131 showed upregulation and recovery and 45 showed downregulated and recovery. The expression of all these genes was also observed in the susceptible variety.

In leaf, the 45 genes that responded quickly to drought in the tolerant were involved in several processes, such as the response against pathogens, cell wall modification, starch breakdown, transport, calcium-binding; other genes spanned various functions, including cellulose-degradation, molecular chaperones, or transcription factors. From these 45 genes, 25 had more extreme changes compared to the susceptible variety, having a difference of more than 0.80 log2FC compared with the susceptible at T_1, including 21 upregulated genes and 4 downregulated genes (**Table 2.5**).

Soltu.DM.06G031870, a HSP20-like chaperones superfamily protein and *Soltu.DM.08G010200*, a cytochrome P450, family 71 subfamily B polypeptide, were particularly highly upregulated at T_1, with log2FC values of > 5 and > 4 respectively. Both were in the top 20 upregulated genes in the tolerant and both were significantly more upregulated in T_1 compared with susceptible variety, which barely changed its expression. The susceptible variety only upregulated these genes from T_2 onwards, indicating a delayed response to the drought stress. Comparing the expression of both varieties in each time point, *Soltu.DM.08G010200* did not have a significant difference before the stress (control samples), but was expressed at a significantly higher level in the tolerant variety compared with the susceptible at both T_1 and T_2, with a log2FC of 4.83 and 2.99 log2FC, respectively. This log2

foldchange in T_2 was less because the susceptible started to significantly upregulate its expression at this time point.

In leaf, among the 25 genes with a quick response to water in the tolerant, 12 genes were not differentially expressed from control to T_1, or control to T_2, in the susceptible variety. These genes were involved in various processes including the response to pathogens (*Soltu.DM.12G00530*), cellulose degradation (*Soltu.DM.01G028100*), calcium-binding (*Soltu.DM.01G032110*), transcription regulation (*Soltu.DM.09G019660*), among others. 2 of the upregulated genes from control to T_1 (*Soltu.DM.01G028100* - "beta glucosidase" and *Soltu.DM.01G032110* - "EF hand calcium-binding protein family") had a higher expression in the tolerant than in the susceptible leaves during T_1 and 1 downregulated gene from control to T_1 (*Soltu.DM.08G002160* - "FAD-dependent oxidoreductase family protein") had a lower expression in the tolerant than the susceptible in T_1.

In root, the quick responding genes in the tolerant included transcription factors, genes that respond to pathogens, genes related to hormones including ABA, genes involved in calcium-binding, transport, signalling cascades, molecular chaperones, starch degrading enzymes, among others. From these 45 genes, 42 had more extreme changes compared to the susceptible variety, having a difference of more than 0.80 log2FC compared with the susceptible at T_1, including 38 upregulated genes and 4 downregulated genes (**Table 2.6**). Comparing the expression of these 42 genes between the two varieties at each time point, 7 upregulated and 2 downregulated genes from control to T_1 had a higher and lower expression, respectively, in the tolerant than the susceptible in T_1 (**Table 2.6**). Among the 7 genes were 3 basic chitinases (*Soltu.DM.07G005400, Soltu.DM.07G005390, Soltu.DM.02G022960*), which were not DE in the susceptible at any time, and 1 beta-1,3-glucanase (*Soltu.DM.02G033060*), which was not upregulated in the susceptible variety until the recovery phase, T_3.

Among the most highly upregulated genes from control to T_1 in the tolerant were *Soltu.DM.05G002810*, encoding an alpha/beta-Hydrolases superfamily protein and *Soltu.DM.09G019250*, encoding an EID1-like protein, whose expression also significantly changed in the susceptible across the 3-time points, but with a lower log2FC.

Of the 42 genes shown in **Table 2.6**, 18 genes were not differentially expressed in the susceptible root from control to T_1 or T_2, and 15 were not differentially expressed at any time point. These 15 genes that were responding to drought stress only in the tolerant roots included, in the upregulated genes, 2 NAC-domain containing proteins (*Soltu.DM.07G014750* and *Soltu.DM.10G000020*), 3 basic chitinases previously mentioned (*Soltu.DM.07G005400*, *Soltu.DM.07G00539*, and *Soltu.DM.02G022960*), 1 nitrate transporter (*Soltu.DM.06G030890*); among the downregulated genes was 1 flavanone 3-hydroxylase (*Soltu.DM.02G023850*).

Table 2.5 Genes with a quick response to water in the tolerant variety in leaf. The expression of these genes selected in the tolerant (Leaf.Tol) is also shown in the susceptible variety (Leaf.Sus). The padj > 0.05 are in grey and the highest log2FC are in red, while the lowest are in yellow. An asterisk denotes genes whose expression was significantly higher (if upregulated from control to T_1) or lower (if downregulated from control to T_1) in the tolerant than in the susceptible during T_1.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Leaf.Tolerant						Leaf.Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj.	LFC	padj.	LFC	padj.	LFC	padj.	LFC	padj.	LFC	padj.
Upregulated genes from control to T1													
Cell wall modification													
*Soltu.DM.03G015500**	Plant invertase/pectin methylesterase inhibitor superfamily protein	3.17	3.32E-05	4.51	1.20E-12	3.71	1.76E-07	1.78	4.60E-01	5.16	1.01E-12	5.71	6.09E-19
*Soltu.DM.03G015510**	Plant invertase/pectin methylesterase inhibitor superfamily protein	2.9	5.30E-09	5.13	8.02E-32	4.56	2.29E-20	1.98	3.29E-03	5.55	1.59E-28	6.08	2.12E-42
Chaperones													
Soltu.DM.06G031870	HSP20-like chaperones superfamily protein	5.45	2.74E-02	5.9	2.10E-03	4.68	3.17E-02	-0.06	1.00E+00	6.15	4.80E-03	5.51	2.59E-03
Transcription factor													
Soltu.DM.06G015000	heat shock transcription factor A6B	2.42	3.37E-03	3.42	1.23E-07	2.65	3.19E-04	1.57	5.06E-01	3.38	3.40E-06	2.93	3.04E-06
Pathogen response													
Soltu.DM.12G005300	bifunctional nuclease in basal defense response	2.28	2.54E-02	2.5	1.62E-03	1.39	1.44E-01	0.31	1.00E+00	1	3.58E-01	1.54	5.35E-02
Starch breakdown													
*Soltu.DM.08G023420**	chloroplast beta-amylase	2.4	1.09E-06	2.86	3.65E-11	2.11	2.24E-05	0.39	1.00E+00	1.78	6.95E-04	1.86	2.24E-05
Cellulose degrading enzyme													
*Soltu.DM.01G028100**	beta glucosidase	1.43	1.02E-02	1.54	5.25E-04	0.85	1.10E-01	0	1.00E+00	0.13	8.70E-01	-0.18	7.46E-01
Transport													
Soltu.DM.04G031660	MATE efflux family protein	2.2	7.60E-03	2.3	4.84E-04	1.34	8.62E-02	0.73	1.00E+00	1.38	8.91E-02	1.6	1.31E-02
Calcium binding													
*Soltu.DM.01G032110**	EF hand calcium-binding protein family	1.3	3.09E-02	1.92	1.20E-05	1.25	1.26E-02	0.03	1.00E+00	0.74	2.44E-01	1	3.66E-02

Others													
*Soltu.DM.08G010200**	cytochrome P450, family 71, subfamily B, polypeptide	4.18	1.53E-03	4.52	3.17E-05	3.5	4.35E-03	1.09	1.00E+00	3.28	2.55E-02	3.19	1.03E-02
Soltu.DM.12G009420	Cytochrome P450 superfamily protein	2.47	3.41E-02	2.5	5.73E-03	1.49	1.65E-01	0.25	1.00E+00	0.69	6.03E-01	0.59	5.67E-01
*Soltu.DM.04G034610**	cytochrome P450, family 82, subfamily C, polypeptide	1.71	9.32E-04	1.86	1.32E-05	1.23	1.39E-02	0.5	1.00E+00	1.62	1.22E-03	1.77	2.12E-05
Soltu.DM.09G002950	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	2.22	1.88E-02	2.62	3.04E-04	1.51	7.76E-02	0.55	1.00E+00	0.8	3.87E-01	1.04	1.30E-01
Soltu.DM.09G002960	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	1.97	1.47E-02	2.41	1.08E-04	1.55	3.28E-02	0.04	1.00E+00	0.4	6.93E-01	1.09	9.27E-02
Soltu.DM.09G002970	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	1.82	8.93E-04	2.71	3.57E-10	2.13	1.20E-05	-0.33	1.00E+00	0.24	7.19E-01	0.83	5.15E-02
*Soltu.DM.04G007210**	alpha/beta-Hydrolases superfamily protein	2	3.69E-02	3.63	1.06E-07	1.34	1.22E-01	0.56	1.00E+00	2.58	1.87E-03	3.45	3.28E-07
Soltu.DM.01G026800	Subtilase family protein	1.85	2.38E-03	2.81	3.08E-09	2.01	2.29E-04	0.35	1.00E+00	1.19	4.87E-02	0.96	5.81E-02
Soltu.DM.01G050660	GAST1 protein homolog	1.82	4.70E-02	2.03	3.57E-03	1.46	6.90E-02	-0.15	1.00E+00	-0.18	8.85E-01	0.75	3.17E-01
Soltu.DM.02G020310	deoxyhypusine synthase	2.39	4.28E-02	2.96	6.83E-04	2.31	1.67E-02	0.19	NA	0.63	5.91E-01	0.94	2.65E-01
Soltu.DM.03G029920	Tetratricopeptide repeat (TPR)-like superfamily protein	1.21	2.40E-02	1.48	2.53E-04	0.96	3.56E-02	0.16	1.00E+00	1.29	2.13E-03	1.3	2.62E-04
Soltu.DM.01G040460	hypothetical protein	1.54	4.29E-02	2.3	2.75E-05	1.56	1.38E-02	0.29	1.00E+00	2.08	5.94E-04	1.32	1.42E-02
Downregulated genes from control to T1													
Transcription factors													
Soltu.DM.09G019660	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-1.5	4.60E-02	-1.8	1.56E-03	-0.39	6.13E-01	-0.36	1.00E+00	-0.09	9.31E-01	-0.15	8.40E-01
Others													
Soltu.DM.10G000510	terpene synthase	-1.75	8.65E-03	-2.54	7.95E-07	-0.8	2.31E-01	-0.29	1.00E+00	-1.9	2.71E-03	-3.55	2.97E-11
*Soltu.DM.08G002160**	FAD-dependent oxidoreductase family protein	-1.48	1.94E-03	-1.93	1.19E-06	-1.2	5.58E-03	0.17	1.00E+00	-0.58	2.66E-01	-0.66	9.92E-02
*Soltu.DM.02G024270**	B-box type zinc finger family protein	-1.47	1.72E-02	-4.35	2.05E-19	-3.82	2.88E-13	-0.6	1.00E+00	-3.96	1.01E-13	-4.89	1.94E-24

Table 2.6 Genes with a quick response to water in the tolerant variety in root. The expression of these genes selected in the tolerant (Root.Tol) is also showed in the susceptible variety (Root.Sus). The padj > 0.05 are in grey and the highest log2FC are in red while the lowest in yellow. An asterisk denotes genes whose expression was significantly higher (if upregulated from control to T_1) or lower (if downregulated from control to T_1) in the tolerant than in the susceptible during T_1.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Root.Tolerant						Root.Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj.	LFC	padj	LFC	padj	LFC	padj.	LFC	padj	LFC	padj
Upregulated genes from control to T1													
Transcription factors													
Soltu.DM.04G000840	NAC-like, activated by AP3/PI	3.78	4.64E-06	5.19	4.94E-12	3.96	1.57E-08	2.08	5.53E-03	3.82	2.15E-10	2.46	5.93E-05
Soltu.DM.07G014750	NAC domain containing protein	3.55	1.87E-02	4.43	2.67E-04	2.64	3.02E-02	-0.12	NA	1.04	3.67E-01	-0.46	7.06E-01
Soltu.DM.10G000020	NAC domain containing protein	2.31	4.74E-02	2.65	3.95E-03	1.38	1.45E-01	1.26	NA	0.9	3.62E-01	-1.33	1.86E-01
Soltu.DM.01G044140	xylem NAC domain	1.44	4.01E-02	2.62	1.53E-06	1.4	6.23E-03	0.63	3.87E-01	1.61	1.75E-03	0.8	1.33E-01
Soltu.DM.02G018840	MYB-like	3.32	1.93E-03	6.22	2.49E-11	3.94	2.67E-06	1.91	9.09E-02	4.44	1.74E-07	2.72	1.57E-03
Soltu.DM.07G019030	MYB-like	2.11	8.09E-03	5.61	2.31E-18	4.31	7.81E-14	1.31	9.54E-02	3.28	1.90E-08	3.31	5.37E-09
Soltu.DM.01G040220	basic region/leucine zipper motif	1.45	2.46E-02	1.92	2.85E-04	1.19	1.68E-02	-0.15	8.60E-01	1.93	1.06E-05	1.57	2.74E-04
Pathogen response													
*Soltu.DM.07G005400**	basic chitinase	3.52	3.12E-04	3.63	8.11E-05	1.25	1.75E-01	-0.17	9.16E-01	1.17	2.52E-01	-0.54	5.97E-01
*Soltu.DM.07G005390**	basic chitinase	3.46	1.03E-03	3.5	4.17E-04	1.31	1.77E-01	-0.25	8.82E-01	0.82	4.93E-01	-0.73	4.81E-01
*Soltu.DM.02G022960**	basic chitinase	1.61	1.37E-04	1.73	1.61E-05	0.53	1.89E-01	0.24	6.94E-01	-0.33	5.30E-01	-0.21	6.50E-01
*Soltu.DM.02G033060**	beta-1,3-glucanase	3.14	3.43E-06	3.73	1.08E-08	1.21	6.55E-02	-0.49	6.17E-01	-0.59	4.95E-01	-2.96	2.04E-05
Soltu.DM.03G017710	PAR1 protein	2.16	1.24E-02	3.03	3.44E-05	0.73	3.69E-01	0.14	NA	1.45	4.29E-02	-0.05	9.60E-01
Soltu.DM.09G027690	MLP-like protein	2.91	2.20E-05	3.26	8.56E-07	2.21	1.89E-04	0.83	3.36E-01	1.39	3.82E-02	1.23	5.04E-02
Transport													
Soltu.DM.06G030890	nitrate excretion transporter1	1.96	4.89E-	2.12	8.61E-	1.13	1.65E-	0.38	NA	0.47	6.52E-	0.62	4.54E-

			02		03		01				01		01
Soltu.DM.04G001100	nitrate transporter 1:2	1.52	2.36E-02	2.88	9.88E-08	1.74	4.19E-04	0.61	3.95E-01	1.52	3.34E-03	1.47	2.57E-03
Signalling cascade													
Soltu.DM.07G017180	mitogen-activated protein kinase kinase kinase	1.51	1.18E-02	4.79	2.77E-28	3.02	3.48E-13	0.56	3.78E-01	3.59	1.95E-21	2.25	7.99E-09
Others													
Soltu.DM.05G002810	alpha/beta-Hydrolases superfamily protein	5.59	1.68E-04	6.07	9.85E-06	5.13	2.81E-05	3.55	9.58E-03	2.78	2.82E-02	4.37	7.78E-05
Soltu.DM.09G019250	EID1-like	5.51	5.73E-08	8.07	3.01E-16	6.5	1.33E-13	3.99	1.44E-04	7.2	2.38E-16	5.62	1.41E-10
Soltu.DM.01G044850	RING/U-box superfamily protein	1.2	4.48E-02	1.35	6.25E-03	0.84	6.62E-02	-0.07	9.32E-01	-0.03	9.74E-01	-0.45	3.47E-01
*Soltu.DM.06G012790**	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	2.64	1.38E-02	5.39	6.80E-12	3.84	5.45E-07	0.9	NA	3.59	6.90E-06	2.98	1.67E-04
Soltu.DM.11G010780	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	2.27	3.93E-05	2.74	7.52E-08	2.2	2.08E-06	0.52	4.18E-01	0.57	3.07E-01	0.38	4.63E-01
Soltu.DM.04G021610	NAD(P)-binding Rossmann-fold superfamily protein	2.59	3.59E-03	3.08	1.28E-04	2.55	2.93E-04	1.05	2.96E-01	1.36	9.91E-02	0.15	8.77E-01
Soltu.DM.08G030040	homeobox	2.33	1.19E-04	6.03	1.71E-30	4.75	7.95E-24	1.27	5.11E-02	5.26	5.19E-29	3.73	3.91E-15
Soltu.DM.05G006430	homeobox	1.6	2.95E-04	4.11	5.91E-27	2.43	2.72E-12	-0.03	9.74E-01	2.38	1.08E-11	0.7	7.07E-02
Soltu.DM.03G030760	blue-copper-binding protein	1.85	2.60E-02	1.93	6.64E-03	0.73	3.27E-01	0.83	NA	1.34	5.78E-02	1.8	3.61E-03
Soltu.DM.02G018100	serine carboxypeptidase-like	1.8	1.86E-03	2.87	1.86E-08	1.44	2.35E-03	0.42	5.56E-01	-0.57	3.40E-01	-0.6	2.44E-01
Soltu.DM.01G026820	Subtilase family protein	1.69	4.93E-03	2.56	3.16E-07	0.82	1.32E-01	0.81	NA	0.56	3.85E-01	-0.55	3.63E-01
Soltu.DM.01G043360	plantacyanin	1.16	1.27E-03	1.3	1.06E-04	0.46	1.60E-01	-0.13	8.03E-01	-0.4	2.92E-01	-0.32	3.53E-01
Soltu.DM.06G015140	Protein of unknown function (DUF1677)	2.73	1.31E-03	4.25	1.74E-09	3.01	7.33E-06	1.81	NA	3.94	7.57E-09	2.59	2.44E-04
*Soltu.DM.10G027280**	Protein of unknown function, DUF617	2.72	1.70E-07	6.01	2.09E-41	4.9	5.93E-31	1.71	2.45E-03	5.2	1.30E-35	3.76	1.04E-18
Soltu.DM.09G020460	Protein of unknown function (DUF506)	1.52	1.19E-02	2.69	1.02E-07	1.36	3.61E-03	0.71	2.74E-01	2.18	1.89E-06	1.62	3.48E-04
Soltu.DM.03G024470	conserved hypothetical protein	4.04	2.30E-04	8.2	2.01E-17	5.86	1.33E-11	2.3	4.59E-02	7.03	2.03E-16	4.25	1.03E-06
Soltu.DM.06G024460	conserved hypothetical protein	2.85	1.87E-09	3.9	8.68E-17	0.54	3.15E-01	1.67	9.59E-04	3.05	3.32E-13	0.14	8.08E-01

Soltu.DM.03G025840	hypothetical protein	2.6	2.65E-03	4.32	3.54E-09	3.07	4.76E-06	1.33	1.22E-01	2.13	1.73E-03	2.31	2.71E-04
Soltu.DM.05G002980	hypothetical protein	2.53	1.37E-02	3.54	5.99E-05	2.72	4.70E-04	0.76	5.30E-01	2.43	3.45E-03	2.78	3.04E-04
Soltu.DM.10G026720	conserved hypothetical protein	2.26	2.40E-02	3	2.02E-04	1.3	1.24E-01	1.33	NA	2.12	4.54E-03	1.5	4.51E-02
Soltu.DM.02G033970	conserved hypothetical protein	1.69	1.37E-02	4.79	6.81E-19	4.21	1.99E-18	0.76	2.88E-01	3.45	6.77E-13	2.38	8.91E-07
*Soltu.DM.12G003800**	conserved hypothetical protein	1.32	4.03E-02	1.46	7.03E-03	0.11	8.66E-01	0.47	5.17E-01	0.78	1.69E-01	0.9	7.10E-02
Downregulated genes from control to T1													
Flavonoid synthesis													
Soltu.DM.02G023850	flavanone 3-hydroxylase	-2.15	5.06E-04	-2.85	1.23E-05	-0.72	1.58E-01	-0.92	NA	-0.23	7.40E-01	0.68	1.49E-01
Others													
*Soltu.DM.01G049280**	conserved hypothetical protein	-2.75	1.60E-02	-3.37	7.92E-04	-0.76	4.67E-01	-0.84	5.18E-01	-1.46	1.58E-01	-1.98	2.44E-02
*Soltu.DM.10G000080**	circadian clock associated	-1.92	1.24E-03	-4.9	1.40E-12	-3.96	3.85E-14	-0.57	4.18E-01	-2.76	1.90E-08	-2.71	1.59E-08
Soltu.DM.02G027940	Protein of unknown function (DUF1230)	-0.95	3.28E-02	-1.52	4.12E-05	-0.87	8.73E-03	0.16	7.81E-01	-0.41	3.31E-01	-0.08	8.60E-01

2.4. Discussion

Climate change will lead to more severe or prolonged drought periods, which will negatively impact potato production (Hijmans, 2003). To address this challenge, the transcriptomic response in leaf and root of two Andean potato varieties with contrasting phenotypes was analysed, one tolerant and one susceptible to drought, and key genes and pathways associated with tolerance have been identified.

2.4.1. Tolerant variety responds faster to drought than the susceptible in both tissues

Both tissues showed a faster change in gene transcription in the tolerant variety in response to drought than in the susceptible variety. This was observed in the higher number of DEGs in leaves and in the higher LFC of DEGs in roots of the tolerant compared with the susceptible variety since the early response to the hydric stress. This suggests that the rapid recognition of the lack of water by the tolerant root may cause it to emit more signals that would be perceived more quickly by the leaf. In turn, tolerant leaves would be able to trigger different mechanisms that would allow the plant to cope with drought more quickly, than the susceptible ones, from an early time point of the stress. The delayed response of the susceptible leaves was confirmed by observing the different biological processes related to the response to hydric stress that began to be enriched in the late response, while they were already enriched in the tolerant variety since the early time point.

One of the early responses shown by the tolerant leaves but not shown until the late response in the susceptible leaves, was the widespread downregulation of genes involved in DNA replication and cell division, which suggested a generalised shut down/arrest of cell growth. This behaviour is an important mechanism enabling plants to conserve energy under stress, and this reduction, under water deficit, has been observed to occur independently from changes in photosynthesis (Granier and Tardieu, 1999, Skirycz and Inzé, 2010).

2.4.2. Rapid drought-induction of ABA related genes in tolerant leaves and roots

One of the most obvious differences between the tolerant and susceptible varieties was the more rapid upregulation of ABA-responsive genes in the early time point, in both the tolerant leaves and roots compared with the susceptible variety. Tolerant leaves upregulated a large number of DEGs (33 genes) that were not upregulated in the susceptible variety at the early time point, while tolerant roots upregulated a different set of 19 genes from those in the leaf that were also not upregulated in the susceptible variety. In tolerant leaves, the upregulated genes included 5 PP2C genes, 2 ABA transporters including the ATP-binding cassette family G25 (ABCG25), and 3 ABI five binding (AFP) proteins. Two of these PP2Cs were among the 20 most upregulated DEGs in the tolerant variety. Similarly in roots, the upregulated genes included 5 ABA related transporters, a raffinose synthase family protein, and most highly upregulated was a galactinol synthase gene (*Soltu.DM.02G006360*); 2 MYB domain proteins were among the top 20 most upregulated genes. Most of these genes that responded to ABA early in the tolerant variety only began to be upregulated in the susceptible leaves or roots during the late response to drought (T_2), indicating an earlier, stronger ABA-mediated response in the tolerant compared with the susceptible variety.

ABA is a phytohormone that regulates different physiological processes under drought, inducing transcriptional reprogramming that leads to a variety of outcomes including stomatal closure and synthesis of osmoprotectants (Sah *et al*., 2016). ABCG25 is located in the plasma membrane of vascular tissues and functions as an ABA exporter to allow the mobilization of this phytohormone toward the guard cells (Ma *et al*., 2018b). AFP proteins are negative regulators of ABA response (Lynch *et al*., 2017), including PP2C proteins. While the overexpression of ABCG25 could indicate that there is more effect of the ABA hormone in the tolerant leaves, the overexpression of AFP and PP2C genes would suggest otherwise. In the absence of ABA, PP2C interacts with and inactivates SnRK2, but in the presence of ABA, PP2C is inactivated by PYR/PYL/RCAR receptors, allowing the release of

SnRK2. Free SnRK2 phosphorylates and activates itself and other downstream factors, including SLAC1 and SLAH3 transporters involved in ion import into guard cells, and transcription factors, such as AREB/ABF, to mediate stomatal closure and decrease water transpiration (Ali *et al*., 2020). Therefore, more PP2C proteins would maintain more inactive SnRK2 and limit or reduce the downstream effects of the ABA signal.

It has been reported that higher levels of ABA can be detrimental to plants in various ways, for example by accelerating senescence and increasing disease susceptibility (Gietler *et al*., 2020). Also, plants under drought stress still need an adequate amount of CO_2 to maintain photosynthesis (Jung, Nguyen, and Cheong, 2020), which would be also important to maintain crop yield. Therefore, although the ABA mediated response may be important in response to drought, so too is its effective regulation to make sure that levels are properly modulated so as not to confer a threat. This regulation can occur by regulating the production of ABA or by regulating the response to ABA through the action of PP2C proteins (Jung, Nguyen, and Cheong, 2020). Interestingly, the expression of PP2C can in turn be induced by ABA, specifically by the action of the transcription factors AREB/ABF that are activated in the ABA signalling pathway. This may be considered as an important form of negative feedback regulation within the ABA response pathway (Jung, Nguyen, and Cheong, 2020). Previous works in potato have also found an increase in PP2C gene expression under drought stress in the leaves of tolerant potato plants (Chen *et al*., 2020) and in the stolon tissue (Gong *et al*., 2015). Interestingly, in the potato plants evaluated here, the rate of photosynthesis decreased more rapidly in the susceptible than in the tolerant variety. The tighter regulation of ABA levels through upregulation of PP2C proteins likely allowed the tolerant variety to maintain the higher rate of photosynthesis under prolonged stress.

On the other hand, the early enrichment of genes that respond to ABA in the tolerant variety indicated that there might be more production of this phytohormone in this variety, though ABA

levels were not measured in this study. This could be related to the expression of *NCED* genes. In leaf, an early upregulation of a gene annotated as *NCED1/NCED3* in *Arabidopsis* was observed in only the tolerant variety, and maintained across all three time points, while in the susceptible variety this gene was not upregulated at all. Since *NCED3* catalyses the rate-limiting step in the ABA biosynthetic pathway, this difference might explain the earlier accumulation of ABA in the tolerant compared with the susceptible leaves. The role of *NCED3* in drought tolerance was previously reported in *Arabidopsis,* in which the antisense suppression of this gene produced a drought-sensitive phenotype (Iuchi *et al*. 2001). Interestingly, the roots of both varieties, which are enriched in the process of ABA response since T_1, overexpressed two NCED genes, *NCED3* and *NCED5*, throughout the drought stress. Previous work also reported that *NCED5* contributes together with *NCED3* to the synthesis of ABA in response to water deficit (Frey *et al*., 2012). Therefore, in our varieties, the enrichment of the processes that responded to ABA was correlated with *NCED* gene expression, which would enable more ABA to be produced at the earlier time point in the roots of both varieties, and potentially more ABA to be produced in tolerant compared with susceptible leaves (*Soltu.DM.08G015120*).

2.4.3. Transcriptomic adjustment during the recovery phase

In the recovery phase, when the plants recovered 80% of their photosynthesis, the increased number of upregulated genes suggests that a transcriptomic adjustment to reach a similar gene expression to control plants was still in process. Interestingly, during this recovery phase, some biological processes enriched in this time point were also enriched during drought, in T_1 and T_2, meaning that regulation of some biological processes observed during drought was also important during rewatering. This can be observed in the biological process of "ABA response". Under drought, ABA is a key regulator of water status to help plant survival, for which its level increases quickly under stress and is rapidly degraded and deactivated once the stress ends to allow the plant to follow its normal growth (Zhang *et al*., 2006). Thus, it is not surprising that the ABA signalling pathway had been regulated both

during droughts and during recovery. While upregulation of PP2C in response to drought would function as a negative feed-back of ABA signalling to keep part of photosynthesis activated, after rewatering, PP2C would be important to stop the ABA signalling pathway to allow a full recovery of photosynthesis and plant growth. Indeed, PP2C was strongly upregulated in T_1 and T_3, particularly in the leaf tissue of the tolerant variety. In the recovery phase, new synthesis of ABA could still be carried out by NCED3, and this hormone was probably still transported by their transporters since genes encoding this protein were still upregulated in root during the recovery phase, but with less amount than the late response. Therefore, the transcriptomic data is showing that modulation of ABA response would be an important mechanism to withstand and recover from drought. Because at the transcriptomic level, the recovery time is still in process, and although it can be observed that some genes tended to recover their expression, such as genes encoding for NAC domain-containing proteins, to observe a clear pattern of recovery, the sampling should have been after a longer period of water replenishment.

2.4.4. Conserved lignification of root tissue is enhanced in the tolerant variety

Conserved lignification of root tissue might be enhanced in the tolerant variety. During the early response to drought, there is a conserved upregulation of several genes involved in lignification in both tolerant and susceptible roots, including six phenylalanine ammonia lyases (PALs), which catalyse the first step in lignin biosynthesis. This is consistent with the widely reported role of lignin in enhancing tolerance to drought and other abiotic stresses in many plant species (Moura *et al*., 2010; Chun *et al*. 2019; Karlova *et al*., 2021). Lignin provides rigidity to the cell wall and forms a hydrophobic barrier around the xylem to reduce water loss through leakage and thus facilitates more effective water transport through the plant (Karlova *et al*., 2021). However, only the tolerant variety strongly upregulated other genes involved in lignin polymerization. These included a laccase, which is a multicopper oxidase homologous to *A. thaliana LAC14* and was in the top 20 most strongly

upregulated genes, and four peroxidase superfamily proteins homologous to *PER52* in *A. thaliana*, one of which was all in the top 20 and all of which are involved in the polymerisation of monolignols to produce the final lignin polymer (Chun *et al*., 2019). These results suggest that the tolerant variety responds to drought stress by more strongly inducing the expression of lignin biosynthetic genes in order to reinforce the plant cell wall and minimise water losses.

2.4.5. Rapid induction of genes to limit ROS-related damage in the tolerant variety.

It is well known that ROS accumulation is one of the first responses to stress in plants. Although at high concentrations it can produce severe damage to different cellular structures like proteins, lipids, and nucleic acid, at lower concentrations it functions as a stress signal that allows plants to respond to adverse conditions (Halliwell, 2006; Petrov *et al*., 2015). Although the roots of both varieties responded early to oxidative stress, the roots of the tolerant variety upregulated double the number of genes relating to this process in the early response to drought. These genes included one galactinol synthase and 3 serine-type endopeptidase inhibitors. These 3 genes showed more than a log2FC > 4 in the early response of the tolerant variety, but a negligible change in the susceptible variety. Serine-type endopeptidase inhibitors (SPI) are enzymes that regulate the action of proteases to avoid excessive protein degradation that could result in cellular damage (Clemente *et al*., 2019). The expression of protease inhibitors was observed to be highly induced under abiotic stress in *Arabidopsis*, and its overexpression conferred resistance against drought, salt, cold, and oxidative stress (Zhang, Liu, and Takano, 2008). Also, *Arabidopsis* transgenic lines overexpressing an SPI gene had less oxidative damage than the wild type under drought, showing less lipid peroxidation and more antioxidant activities (Malefo, *et al*., 2020). Upregulation of these genes in the tolerant variety could therefore play a key role in avoidance of cellular damage. This is consistent with the upregulation of genes involved in protein refolding which was only observed in the susceptible leaf,

suggesting that this variety had suffered greater protein damage by the late drought stress time point.

Galactinol synthase is involved in the synthesis of raffinose; this enzyme converts UDP-galactose into galactinol, which in turn is converted into raffinose by the raffinose synthase enzyme (Taji *et al*., 2002). In addition to upregulating a galactinol synthase, only the tolerant variety upregulated a raffinose synthase gene (*Soltu.DM.02G033230*) in the early drought response. Both genes were also upregulated in the susceptible variety, but not until the later stage of drought stress and to a lesser degree. It was observed that the accumulation of galactinol and raffinose in plants protects against ROS-related damage under stress (ElSayed, Rafudeen, and Golldack, 2014). The high expression of these antioxidant proteins in only the tolerant variety could be alleviating the oxidative damage produced under drought. Variety-specific accumulation of raffinose and galactinol is supported by other studies that show conserved accumulation in some varieties such as Alegria, Milva, Desiree and Saturna (Sprenger *et al*., 2016), but no accumulation in other Andean varieties, Sullu and SS2613 (Evers *et al*., 2010).

2.4.6. Biotic stress related response of the tolerant variety conserved across tissues

In the late response to drought, more genes involved in the response against pathogens, including fungi, bacteria and oomycetes, changed their expression in both tissues. Crosstalk between the response to abiotic and biotic stresses in plants involves processes that respond to hormones, such as ABA, salicylic acid, or jasmonic acid, as well as ROS generation as a signal of stress (Fujita *et al*., 2006). Overexpression of transcription factors, such as MYB, NAC, HSF, and WRKY are also involved in this crosstalk (Fujita *et al*., 2006, Bai *et al*., 2018). Such crosstalk was clearly observed between these two types of stress, since the early response to drought in roots and increasingly so in the late response, particularly in leaves. Among these genes involved in the crosstalk, the tolerant variety upregulated more WRKY genes in both tissues compared with the susceptible variety. In the potato

genome, 129 genes were annotated as a putative *WRKY*, whose expression responded to different types of stress, such as heat, salt, and drought, and to salicylic acid treatment (Zhang *et al*., 2017a). Specifically, the expression *Soltu.DM.08G028850*, annotated as *AtWRKY53* in *Arabidopsis*, was one of the most highly upregulated genes in the tolerant leaves but was not upregulated in the susceptible leaf until the recovery phase. The same was observed for *Soltu.DM.12G007400 (AtWRKY51)* in the late response of root. Members of the *WRKY* protein family are involved in the regulation of the ABA pathway, and their overexpression can promote drought tolerance in tomato, tobacco, and rice (Bai et al. 2018). It was reported that expression of *AtWRKY53* was modulated under biotic stress, induced by SA but repressed by JA, and was involved in plant senescence (Zentgraf and Doll, 2019). In contrast to the result observed here, the upregulation of this specific *AtWRKY53* under drought was correlated with reduced drought tolerance. This association was made because the overexpression of this gene decreased the hydrogen peroxide level and the stomatal closure in *Arabidopsis* lines that did not survive after drought and rewatering treatment (Sun and Yu, 2015). Here, less reduction in the rate of photosynthesis observed in the tolerant variety could be correlated with reduced stomatal closure, which could be a benefit for this our variety.

Other upregulated genes in the tolerant variety relating to biotic stress responses included genes encoding basic chitinases and genes relating to calcium signalling. Under both biotic and abiotic stress, the fluctuation of calcium functions as a signal, activating stress-responsive calcium sensors like calmodulins (CaMs) or calcineurin B-like proteins (CBLs), calcium-dependent protein kinases (CPK) and calcium/calmodulin-dependent protein kinases (CCaMKs) (Ku *et al*., 2018). The tolerant variety showed stronger upregulation of genes involved in calcium signalling in both tissues, including two calmodulin genes upregulated in all but the susceptible roots (*Soltu.DM.10G026220, Soltu.DM.10G026210*), and a BCL-2-associated athanogene 6 (*BAG6*) upregulated in all but the susceptible leaves. BAG proteins including *BAG6* mediate the response to multiple kinds of stress in *Arabidopsis*, including the response to salt stress (Arif *et al*., 2021).

Three basic chitinases were only upregulated in the tolerant root during the drought stress (T1 and T2) and largely recovered after rewatering. In contrast, the susceptible variety did not upregulate chitinases at any time point. Chitinases are enzymes that participate in the first line of the plant defence response during PAMP-triggered immunity (PTI) by degrading chitin, a major component of the fungal cell wall. However, chitinases are not only induced under pathogen attack, but also under salt, cold, and drought stress (Takenaka *et al*., 2009) and play a role in plant growth and development. In potato, a class I chitinase was identified by a yeast functional screening approach, as a part of the group of genes that confer drought tolerance (Kappachery *et al*., 2013). In clover leaves, chitinases and β-1,3 glucanases increase their expression under drought during the early stage of stress, and were significantly correlated with an increase of proline, with a suggested role in the detoxification from ammonia that accumulated under drought (Lee *et al.*, 2008). Here also, the tolerant variety upregulated a beta-1,3-glucanase (*Soltu.DM.02G033060*), which then recovered its expression after rewatering, while the susceptible variety did not upregulate this gene until the recovery phase. β-1,3 glucanases hydrolyse glycosidic bonds in the glucans of the fungal cell wall, to protect against fungal pathogens (Oide *et al*., 2013). The upregulation of such genes involved in the conserved pathways between biotic and abiotic stress responses may underly the improved response to drought stress seen in the tolerant variety.

2.4.7. Cell wall remodelling in response to drought stress

The identified DEGs showed that changes in the cell wall are occurring during the evaluated 3-time points in both varieties. In addition to lignin, according to the enriched biological processes, there were other components of the cell wall that were probably modified during the stress treatment. In the tolerant leaves, more plant invertase/pectin methylesterase inhibitor superfamily proteins (INV/PMEI-SP) were overexpressed since T_1. This family includes pectin methylesterase inhibitors (PMEI) and invertase inhibitors (INVI) proteins that regulate the PME and INV enzymes, respectively

(Coculo and Lionetti, 2022). Since the tolerant variety showed stronger upregulation of PMEIs in leaf, this may translate into more inhibition of the action of PMEs. Under drought, one of the most important mechanisms generated by the plant is the regulation of stomatal aperture/closure. In *Arabidopsis*, the activity of PME upon methylesterified pectin was observed to be important for a proper stomatal aperture under heat stress and drought (Wu *et al*., 2017). *Arabidopsis* mutants not expressing PME34, whose activation depended on ABA, showed an enhance stomatal aperture and lethal phenotype to heat stress (Wu *et al*., 2017). In pepper, the overexpression of CaPMEI1 increased tolerance to drought in *Arabidopsis* plants (An *et al*., 2008). Therefore, the regulation of PME by PMEI is an important factor influencing stomatal opening during drought stress.

In contrast to the early response in leaf, in the late response to drought the roots of the tolerant variety showed more widespread and stronger downregulation of INV/PMEI-SP genes than the susceptible roots, including one gene in the top 20 most downregulated genes (*Soltu.DM.02G001870*). PME demethylesterifies oligomers of the pectin backbone, then these blockwise demethylesterified pectins may bind to each other by crosslinking with Ca^{2+} to form a rigid structure called the “egg-box” in which calcium ions interact with molecules of water to keep the cell wall hydrated (Wormit and Usadel, 2018). Therefore, downregulation of PMEIs may facilitate formation of the egg box structure and maintenance of cell wall hydration. However, different experiments have shown contradictory results in *Arabidopsis* regarding the relationship between root growth and PMEI activity. While in some cases root growth was promoted by overexpression of PMEI (An *et al*., 2008), in others it was promoted by inhibition of PMEI (Wormit and Usadel, 2018). In rice, the high expression of PMEI provoked a negative effect on plant growth, producing dwarfed plants (Nguyen *et al*., 2017). In transgenic potato expressing a Petunia PME, whose activity was more pronounced in leaf and tubers, more plant growth at the early stage but no difference in growth after 35 days was observed (Pilling, Willmitzer, and Fisahn, 2000). In the case of invertase inhibitors, their expression was favourable against drought in maize (Chen *et al*., 2019), contrary to

observations in cucumber where the overexpression of vacuolar invertase reduced drought tolerance (Chen *et al*., 2022). Therefore, further investigation is needed to understand the species and tissue-specific effects of PMEIs, PMEs and invertase inhibitors in the abiotic stress response.

Among the most strongly upregulated genes in root during the early response to drought was expansin like-B1 (EXLB1). Expansins are a class of non-enzymatic cell wall proteins that play a role in the regulation of cell growth and expansion (Marowa *et al.*, 2016). The high overexpression of an expansin like-B1 gene was also observed previously in the stolon of potato variety Ningshu under drought stress (Gong *et al*., 2015). In *Brassica rapa*, BrEXLB1 was preferentially expressed in root, and under drought stress its expression was highly elevated, contributing to enhanced root growth that was positively associated with drought tolerance (Muthusamy *et al*., 2020). In maize, Exp1, Exp5 and ExpB8 were upregulated in the root under low water potential, allowing the plant to continue elongating its roots under stress (Wu *et al*., 2001). Here, while both varieties upregulated expansin-like B1 during the early response to drought stress, the response was much stronger in the tolerant than in the susceptible variety, with a log2 FC of > 6 and > 2 respectively. This gene continued to be more strongly overexpressed in the tolerant variety throughout the late and recovery responses to drought stress.

A common response in the root between both varieties was the downregulation of xyloglucan endotransglucosylase/hydrolase genes (XTH) in the late response to drought. However, even though the response was common, more XTHs were downregulated in the tolerant variety and to a greater extent than in the susceptible variety. XTHs have the capacity to cleave and re-ligate the xyloglucans fragments and their increased expression has been correlated with an increase in drought tolerance (Le Gall *et al*., 2015). In wheat, the expression and the activity of XTHs in the root under drought were different depending on the evaluated region. The expression of XTH in the apical zone was downregulated more in susceptible cultivars, while no significant change was observed in

the subapical zones (above the apical zone) (Iurlaro *et al*., 2016). Downregulation of XTHs was also observed in Arabidopsis leaves under drought stress, however, upregulation of XTHs was observed in roots of rice and leaves of hot pepper under hydric stress (Iurlaro *et al*., 2016). Then it will be important to differentiate the expression of these enzymes in the different root zones to better understand the outcome of the up or downregulation.

2.4.8. Genes that respond rapidly to drought and rewatering.

Among the genes responding rapidly to drought and rewatering, in leaf and root, were genes related to the response to pathogens. In the root, it included the 3 basic chitinases previously mentioned, and a beta-1,3-glucanase, while in the leaf was a gene for a bifunctional nuclease in basal defence response. In addition, genes that code for NAC domain-containing proteins were among the group of genes with a quick response to water in the tolerant root, whose expression increased under drought but decreased after rewatering. NAC is a large transcription factor family that, due to its high diversity, is involved in several biological processes in plants. These processes include plant development, cell division, senescence, cell wall formation, plant immunity and the response to abiotic stress. Several NAC proteins respond to hydric stress and regulate genes in the ABA-dependant pathway (Shen *et al*., 2017, Jiang *et al*., 2019). In rice, the expression of OsNAC2 was associated with the increase in ABA by activating the expression of the NCED3 gene (Jiang *et al*., 2019). A NAC gene in potato, StNAC053, became highly expressed under ABA and drought treatment. When overexpressed in *Arabidopsis* transgenic lines, this StNAC053 enabled the plants to better tolerate drought than wild type plants (Wang *et al*., 2021b).

Nitrate transporters also responded quickly to the availability of water in tolerant roots. Nitrate excretion transporter1 (NAXT1), mainly expressed in the cortex of mature roots (Segonzac *et al*., 2007), is responsible for nitrate (NO_3^-) efflux from the root into the external medium and it is stimulated by a cytoplasmic acidic PH (Aslam *et al*., 1995, Segonzac *et al*., 2007). Under drought, it

was observed that some nitrate transporters are involved in ABA transport and stomatal closure (Kanno *et al*., 2012). In both tolerant leaf and root, genes for 2-oxoglutarate (2OG) and Fe (II)-dependent oxygenase superfamily protein were also upregulated quickly under drought and downregulated with rewatering. This superfamily protein is involved in several processes in plants that include DNA repair, histone demethylation, biosynthesis or catabolism of enzymes, such as gibberellin, ethylene, auxin, and salicylic acid, and in the metabolism of secondary metabolites like flavonoids, and coumarin, and subsequently affect the response to biotic or abiotic stresses (Farrow and Facchini, 2014).

The analysis discarded some samples because they did not seem like true biological replicates, which is why part of the collected samples only had two biological replicates. Even though it may compromise the statistical power of the analysis and the DGE identification accuracy, the results of this research provide a list of promising candidate genes whose expression and function need to be tested experimentally. Indeed, following RNASeq analysis, a validation step is commonly carried out to confirm the role of candidate genes in a particular process, which in this case is drought resistance.

2.4.9. Conclusions and future work

There are commonalities and differences in the transcriptomic response between potato varieties that differ in their tolerance to drought stress, many of which involve genes related to the plant cell wall. Most strikingly, both leaves and roots of the tolerant variety show more widespread and stronger upregulation of genes relating to the ABA response in the early response to stress, compared with the susceptible variety, indicating the speed of response may be crucial. Similarly, there is a general early shut down in growth in the tolerant variety which is not seen until the late response to drought in the susceptible variety. The tolerant roots upregulate many more genes involved in the response to oxidative stress than susceptible roots, enabling the maintenance of

protein integrity and early accumulation of metabolites including galactinol and raffinose that may enhance desiccation tolerance. In addition, the tolerant roots show stronger upregulation of genes involved in lignin biosynthesis, which likely strengthens the cell walls and maintains water transport/minimizes water loss under drought stress. In the late response to stress, the tolerant variety upregulates many genes involved in the response to various biotic stresses, including WRKY family proteins, chitinases and glucanases that may modulate hormone signalling and facilitate detoxification of cells under drought stress.

All these results regarding transcriptomic change of genes raises several hypotheses that, although they need to be experimentally validated with microscopy and/or reverse genetics studies, become an important resource for future research related to abiotic factors in potatoes.

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CHAPTER 3: Varietal variation in potato cell wall composition and its relationship with response to *Phytophthora infestans*

3.1. Introduction

The plant cell wall is an important structure that borders the cell to give mechanical support and protect it against external factors. It is composed mainly of a network of polysaccharides and more than a passive barrier, the cell wall is a dynamic structure whose composition is regulated under plant cell growth and development and plays an important role in pathogen defence (Humphrey, Bonetta, and Goring, 2007). The plant cell wall consists of cellulose and hemicellulose chains, embedded in a pectin matrix, containing structural proteins and cell wall modifying enzymes, and lignin. Cellulose is a linear and unbranched homopolysaccharide chain composed of β(1,4)-linked glucose (glucan) that is able to polymerize, with up to thousands of chains forming a cord-like structure. Hemicelluloses are all the non-pectin and non-cellulosic components of the cell wall. The hemicellulose backbone can be composed of a homopolymer or heteropolymer of glucose, xylose, or mannose units, with or without side chains. Types of hemicellulose include xyloglucan, xylan, mannans, and glucomannans. Crosslink between cellulose and xyloglucan occurs at specific locations or hotspots. Pectins are composed of α(1,4)-linked galacturonic acids, that can be unbranched or with side chains. Unbranched homogalacturonic acids are called homogalacturonan (HG). If the backbone is formed with galacturonic acid alternating with rhamnose residues, they are called rhamnogalacturonan I (RG-I), but if they have a homogalacturonan backbone but with complex side chains, they are termed RG-II. Pectins are synthesized in a methyl-esterified form, but *in muro* can undergo demethyl-esterification by pectin methyl esterases (PMEs) (Joseleau and Pérez, 2016; Lampugnani, *et al*., 2018; Zhang *et al*., 2020). Either the tissue type or the plant developmental stage influences the exact components of the cell wall (Faria-Blanc N., Mortimer J., and Dupree P., 2018).

It was previously reported that *Arabidopsis* lines with different cell wall compositions differ in their resistance to pathogens (Engelsdorf *et al*., 2017, Molina *et al*., 2021). *Arabidopsis* mutant lines with a defect in starch turnover showed that reduced level of rhamnose and RG-I was associated with high *Colletotrichum higginsianum* penetration while the high amount of pectin with low penetration of this hemibiotrophic fungus (Engelsdorf *et al*., 2017). A study of 34 cell wall mutant lines of *Arabidopsis* found that 85.3% of them displayed altered resistance to the bacterium *Ralstonia pseudosolanacearum*, the necrotrophic fungus *Plectosphaerella cucumerina*, or the biotrophic oomcycete *Hyaloperonospora arabidopsidis* compared to the wild types (Molina *et al*., 2021). Using a nonparametric Classification and Regression Tree, the correlation between cell wall components and the level of resistance against these three pathogens was observed. There was a positive correlation between resistance to *P. cucumerina* and the level of fucosylated xyloglucan. This correlation was also seen between resistance to *H. arabidopsidis* and the level of fucosylated xyloglucan and the amount of galactomannan. In addition, there was a negative correlation between resistance to *R. pseudosolanacearum* and the amount of an undefined epitope in RGI (Molina *et al*., 2021). Consequently, these findings suggest that pre-existing cell wall composition can contribute to plant pathogen resistance.

This complex structure of the cell wall is the first barrier that a pathogen must cross to infect the plant. To achieve it, pathogens secrete cell wall degrading enzymes (CWDE), which at the early stages of the infection, largely act on the pectin components. After pectin degradation, the rest of the cell wall components are exposed to the following arsenal of CWDE (Lionetti, Cevone, and Bellicampi, 2012). Plants can recognize any disruption or damage in the integrity of the cell wall architecture, which is referred to as a damage-associated molecular pattern (DAMP), and, in response, can trigger an immune response. Moreover, some components of the cell wall are deposited at the site of infection to reduce the infection. This is the case of the callose, which is a b-(1,3)-D-glucan polysaccharide. Callose deposition is a rapid response generated by plants after a pathogen attack

and occurs after hours of the infection to strengthen the cell wall and function as a physical barrier against pathogen entrance (Wang *et al*., 2021). Callose can also be induced by pathogen-associated molecular patterns (PAMP) from bacteria, such as flagellin (Flg22) and bacterial elongation factor EF-Tu (Elf18), or from fungal cells, such as chitin, a β-(1,4)-linked polymer of N-acetylglucosamine, and chitosan (Wang *et al*., 2021). Callose deposition induced by PAMP is mediated by PRR recognition and induced by indole glucosinolates, ROS, and RNAi regulatory protein Argonaute1 (AGO1), although AGO1 can also repress this deposition (Wang *et al*., 2021a). Also, different outcomes in callose deposition can be produced by different percentages of sucrose, vitamins in the growth medium, and different light intensities (Luna *et al*. ,2011).

The effect of callose accumulation on resistance to pathogens is controversial. In *Arabidopsis,* callose deposition has been correlated with a decrease in *C. higginsianun* accumulation (Shimada *et al*., 2006, Voigt and Somerville, 2009) and with a complete penetration resistance against powdery mildew (Ellinger *et al*., 2013). However, Jacob *et al*. found that a lack of callose enhanced resistance to powdery mildew species and to *Peronospora parasitica*. (Jacobs *et al*., 2003). On the other hand, callose deposition not only is involved in the restriction of pathogen entrance, but it can also restrict nutrient uptake by the pathogen from the host cell (Voigt and Somerville, 2009). Therefore, callose accumulation is an important mechanism that is part of cell wall defence against pathogens.

One of the most important non-cereal crops in the world is potato and this is mainly due to its nutritional value (Zhang *et al*., 2017b), however different pathogens affect the productivity of this crop. The main cause of potato yield loss is caused by *P. infestans* (Savary *et al*., 2019). Transcriptomic, proteomic and metabolomic approaches have revealed that components from the potato apoplast are regulated under *P. infestans* infection. For example, Ali *et al*. (2014) reported that after *P. infestans* infection, a higher abundance of apoplastic proteins was observed in the resistant varieties compared with the susceptible variety, mainly at 24 hours post-infections (Ali *et*

al., 2014). Also, upregulation of genes influencing the cell wall thickness was observed at 48h post-infection in potato resistant genotypes, which includes the upregulation of genes related to extensin biosynthesis, expansin genes, and enzymes that modify pectin and xyloglucan components (Yogendra *et al*, 2016). These studies show that there is a differential response of the cell wall between resistant and susceptible potato varieties in response to infection with *P. infestans*. However, there have been no studies on whether the constitutive composition of the cell wall influences the level of resistance to *P. infestans*. Furthermore, most studies of potato cell wall were carried out specifically in tubers because of their commercial importance (Hoff *et al*., 1969; McMillan, *et al*., 1993; Ralet *et al.*, 2016; Fiorillo *et al*., 2021).

The availability of several monoclonal antibodies that detect different cell wall structures, like glycans and proteins (Smallwood *et al*., 1994; Smallwood *et al*., 1996; Willats, Marcus, and Knox, 1998; McCartney, Marcus, and Knox, 2005), have made it possible to develop a high-throughput methodology to detect the composition of the cell wall in plants. This methodology, known as "Comprehensive microarray polymer profiling" or CoMPP, is a semi-quantitative methodology designed by Moller *et al*. that combines the microarray technique with immunoblotting to profile specific glycome epitopes (Moller *et al*., 2007) and has been deployed in plants (Tyler *et al*., 2014; Lionetti *et al*., 2017; Guo *et al*., 2019).

This chapter aims firstly to identify using CoMPP whether potato varieties differ in the composition of their leaf cell wall, and secondly to find out if there are significant differences in composition between varieties with differing levels of resistance/susceptibility to *P. infestans*. This study provides an important first step in understanding the potential role of specific cell wall components in resistance against *P. infestans,* to inform the development of disease-resistant varieties.

3.2. Material and methods

3.2.1. Selected potato varieties

Twenty-five potato varieties were selected from Agriculture and Horticulture Development Board (AHDB) Potato Variety Database (varieties.adhb.org.uk/varieties) depending on the level of resistance/susceptibility to *P. infestans.* This database provides a score of the response of different potato varieties against different pathogens in leaf and tuber tissues. According to the database and the score made in foliage, the 13 most susceptible and 12 most resistant varieties to *P. infestans* were selected (**Table 3.1**).

Table 3.1 Potato varieties selected from the Potato Variety Database based on their resistance/susceptibility to P. infestans. The numbers are the score of resistance/susceptibility in tuber and leaf in each variety according to the Potato Variety Database (varieties.adhb.org.uk/varieties). The score ranges from 1 (the most susceptible) to 9 (the most resistant). In blue and red are the most susceptible and the most resistant varieties, respectively. Varieties employed in the cell wall analysis are indicated with an asterisk.

Variety name	Resistance to late blight on tubers (*Phytophthora infestans*)	Resistance to late blight on foliage (*Phytophthora infestans*)
ALMERA*	3	2
ANNABELLE*	1	2
DUKE OF YORK*	2	2
HOME GUARD*	2	2
INTERNATIONAL KIDNEY*	2	2
NICOLA	3	2
ORCHESTRA	3	2
ROYAL KIDNEY	2	2
SHARPE'S EXPRESS	2	2
SOFIA	2	2
INNOVATOR	1	3
RANGER RUSSET	2	3
RUSSET BURBANK	1	3
JULIETTE*	6	6
PENTLAND SQUIRE*	5	6
REMBRANDT	7	5
SARPO MIRA*	9	7
SARPO SHONA*	4	7
SETANTA	9	5
TOLUCA*	6	8
VALOR	7	5
CHICAGO	5	6
EXCALIBUR	7	5
CARA	7	5
MARKIES	7	5

The degree of resistance/susceptibility to *P. infestans* of these 25 selected varieties were later experimentally tested using a detached infection assay. For the cell wall analysis, the 10 most

susceptible and resistant varieties as scored for the foliage, were employed (marked with an asterisk in **Table 3.1**).

3.2.2. Potato field trials

Potato plants grew in Newcastle University's Nafferton farm in 2017 and 2019 and each variety was under two cultivation systems, conventional and organic. In each system, each potato varieties were planted in two duplicates blocks and, in each block, there were plots with 3 internal replicates per variety (**Figure 3.1**). In total, 300 potato varieties were growing in the field that were part of a bigger experiment carried out with the potato panel from the James Hutton Institute, which included the 25 varieties selected from the Potato Variety Database (**Table 3.1**). In the conventional system, plants were fertilized with Nitram (containing Ammonium Nitrate) at 180 N Kg/ha, Phosphorus at 134 Kg/ha, and Potassium at 200 Kg/ha. In the organic system, nutrients were supplied using 180 N Kg/ha of farmyard manure. These nutrients were applied one week and three weeks before planting in the conventional and the organic systems, respectively. To control fungus, Mencozeb, 1.7 Kg/ha, and Shirlan, 300 ml/ha were applied 4 times in the conventional system during plant growing, while copper oxychloride 1.7L/ha was used seven times in the organic system during the cultivation period.

Based on the number of blocks, internal replicates, and cultivation systems, with just the 25 selected potato varieties, there were a total of 300 plants growing in the field (300 plants = 25 [varieties] x 2 [systems: organic vs conventional] x 2 [block 1 and 2] x 3 [internal replicates]).

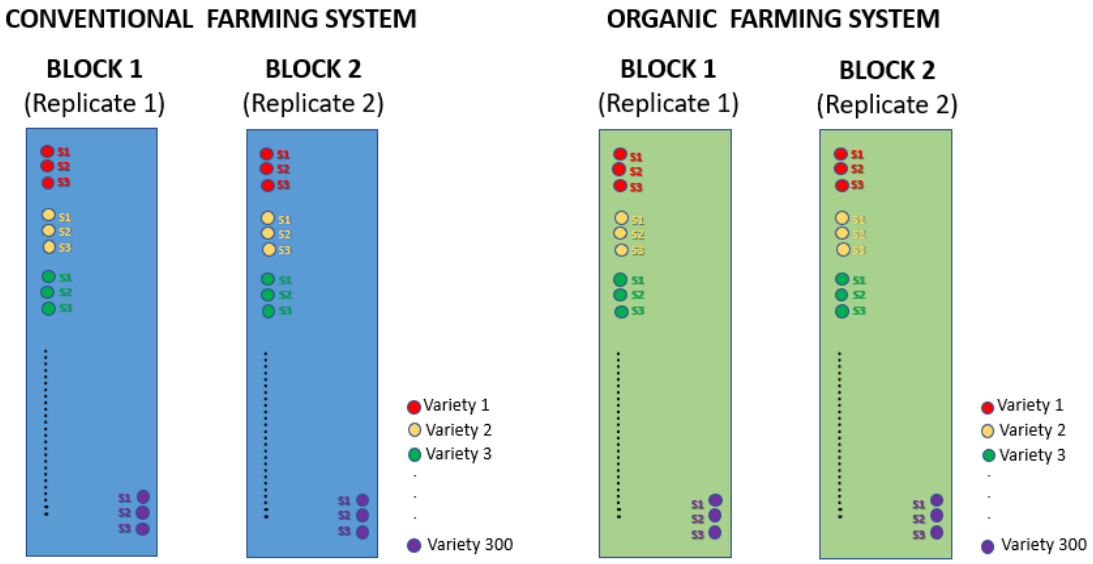


Figure 3.1 Schematic of the potato plants growing in Nafferton farm. Each spot (red, yellow, green, purple) represents one plant in the field. The 300 varieties included the 25 selected varieties (**Table 3.1**) from the Potato Variety Database.

3.2.3. *P. infestans* strain

The Blue 13 strain of *P. infestans* was used to infect potato plants in a detached infection assay. This strain was provided on mycelium infected potato leaves by The National Institute of Agricultural Botany (NIAB), Cambridge.

3.2.4. Detached infection assays

Detached infection assays were carried out using samples collected from the organic plots only.

3.2.4.1. Sample collection.

Healthy leaves from the 25 selected potato varieties (**Table 3.1**) were collected after the plants had begun to flower, on August 14th, 2019. Samples were harvested into plastic trays containing a 10-fold layer paper of damp tissues paper. One composite leaf, the youngest and the healthiest, was collected per plant. From all 25 varieties growing in the organic system, it was expected to collect 150 composite leaves (150 leaves = 1[composed leaf] x 25 [varieties] x 1 [org. system] x 2 [block 1

and 2] x 3 [intern. replicates]). However, one week before collecting the samples, plants showed symptoms of infection with *P. infestans* (late blight disease) and *Alternaria sp.* (early blight disease), and some of them were destroyed (**Figure 3.2**). Therefore, less than 150 samples were collected. No samples from Duke of York could be collected since plants in the two blocks died from infection found in the field.

Figure 3.2 Potato plant growing in the field in Newcastle in August 2019. On the left, a group of potato plant destroyed by early and late blight can be observed. On the right, these plants are observed more closely, and necrotic lesions are clearly visible.

Trays containing harvested leaf material were covered with transparent plastic bags to maintain the humidity. All the biological material was transported from the field to the University of Birmingham the same day and were stored at room temperature in the laboratory. The infection was carried out the following morning.

3.2.4.2. Infection assay

The infected potato leaves carrying the strain Blue 13 (NIAB samples), sent by NIAB, were washed with 5mL of distilled water to isolate the sporulating mycelium in a Falcon. Then, the solution was mixed and incubated for 1h 30min at 4°C to induce zoospore release from sporangia. After this period, zoospores were harvested by collecting 5 drops of 1µL of zoospore solution and placing on slides to observe and count the number of zoospores under the microscope under 20X magnification.

On average, the concentration was $3.4x10^6$ spores per mL, which was diluted to obtain a fresh suspension of $5x10^5$spores per mL for the infection.

Per composite leaf, the underside of five leaflets were infected in two points, each one with 1mL drop of zoospores at the concentration of $5x10^5$spores/mL (**Figure 3.3**). Three leaflets were employed to observe the progress of the infection and two leaflets were used the callose deposition assay.

Figure 3.3 Potato composite leaf at the beginning of the infection *with P. infestans*. The black circles mark the site of the infection in each leaflet.

Infected plants were incubated at 18˚C in the dark and scoring was performing at 1-day, 2-days, 4-days and 5-days post infection.

3.2.4.3. Semi-quantitative scale for the infection response

The score of the plant reaction ranged from 0 to 4 depending on the following reaction. The score was 0 when there was no visible sign of infection; 1, when the sign of infection was observed as black dots on the underside; 2, when the signs of infection (black dots) were observable on both the underside and upper side of the leaflet; 3, when the signs of infection were more than 5mm beyond the boundary of the droplet; and 4 when the leaf collapsed.

The score (0 - 4) of the infected leaves was transformed into a percentage, where the value of 4 was equal to 100%, and from the 3 leaflets, the average of the percentage was calculated per composite leaf. Then, the average and the standard error were calculated per variety (average of block 1 and 2).

3.2.4.4. Staining of callose deposition

Leaf samples from the infected potato varieties were double stained with aniline blue and calcofluor to visualize the reaction of the plant against *P. infestans* in relation to callose formation. Aniline blue is a fluorescent marker for callose in plant tissue suitable to visualize the early events of pathogen attack, since callose is an early visible marker. In contrast, calcofluor binds cellulose from the pathogen cell wall and it allow recognition of zoospores around the infected areas where callose is produced. Before staining, samples were decolorized. Each area where the inoculum was dropped was cut from the leaflet and incubated with 98% ethanol in the dark for 5 days, and 100% ethanol in the dark for 2 more days until the leaves became transparent or light brown. Then, the staining protocol was as described by Ton and Mauch-Mani, 2004. After discarding ethanol, the samples were incubated first with 0.07M phosphate buffer (pH=9) for 30 min at room temperature. Then, a second incubation with a solution of 3mL freshly prepared 0.05% aniline blue plus 1 mL 0.001% calcofluor was carried out for 15 min in the dark at room temperature. After discarding this last solution, samples were kept overnight in the dark with 0.05% aniline blue. The samples were put in slides with 0.05% aniline blue and visualized under a microscope with UV light equipped with a digital camera and collected with the GXCAPTURE software. Samples were observed at the 20X magnification and callose was distinguished as green-fluorescent areas, while pathogen zoospores were blue (due to the aniline and calcofluor staining, respectively). Image analysis and callose measurement was performed using Adobe Photoshop CC 20.0.6. The percentage of callose formed was calculated with the following formula:

$$\%\ of\ callose = \frac{Area\ of\ callose}{Total\ image\ area \times spore\ count} \times 100$$

By employing this formula, the percentage of callose deposition per spore was calculated in each image. Then all the percentages obtained from plants of same variety were averaged to obtain the percentage of callose formation per variety.

3.2.5. Cell wall analysis of potato leaves by CoMPP

The 10 most susceptible and resistant plants, according to the Potato Variety Database were employed for CoMPP (marked with an asterisk in **Table 3.1**). From each variety the 4th leaf, starting from the upper part, was collected and stored at -80°C in Newcastle in 2017. At the time of sampling, potato plants did not show any sign of infection, for which healthy plant were collected. They were transferred, with dry ice, to the University of Birmingham in 2018 and stored at -80°C ready for the first step in cell wall extraction (alcohol insoluble residual (AIR) extraction – Section 3.2.5.1). The second step was carried out in Newcastle (cell wall extraction – Section 3.2.5.2). No evaluation of response to *P. infestans* was performed during the 2017 trial. Furthermore, biological replicates could not be included in this analysis because the -80°C freezers, where two complete groups of replicates were stored, were damaged and samples were lost. In total, 40 plants were analyzed by COMPP (40 plants = 10[varieties] x 2 [system: organic vs. conventional] x 2 [blocks 1 and 2]). Each sample was divided into two equal parts after AIR extraction to provide technical replicates, resulting in a total of 80 samples.

3.2.5.1. Alcohol insoluble residuals (AIR) extraction

Before the cell wall extraction of CoMPP, it is necessary to undertake AIR extraction to remove salts, low molecular weight metabolites, glycan-degrading enzymes, lipids, and pigments that could affect the COMPP analysis (Fangel *et al*., 2021) and produce a preparation enriched for glycans and glycoproteins. For AIR extraction, two leaflets from each sample stored at -80°C were transferred into 50mL Falcon tubes and dried for 48 hours in the freeze dryer machine CHRIST Beta 1-8 LSCplus. 100mg of each dried sample was weighed and ground until a fine powder was obtained. Then, the

powder was mixed with 1.5mL of 70% ethanol and centrifuged at 14000g for 10 minutes. The supernatant was discarded and this process was repeated 5 times. The obtained pellet was washed first with 1,5mL of methanol:chloroform (1:1) and then with 1,5mL of acetone. After each wash, the pellet was centrifuged at 14000g for 10 minutes. Finally, the pellet was air dried and stored at -80°C until the day before cell wall extraction. Then, approximately 2 x 10mg from each sample were weighed out to provide technical replication.

3.2.5.2. Cell wall extraction

After AIR extraction, the procedure to extract the cell wall components employs two principal solvents: CDTA, to extract pectin, and NaOH, to extract non-cellulosic components such as hemicellulose, and glycoprotein (Moller et al., 2007). In each AIR sample tube were added 2 – 4 beads (Solid-glass beads 2mm, Sigma) plus 30µl of CDTA (diamino-cycle-hexano-tetra-acetic acid, 50mM, pH 7.5) per each mg of samples. In the tissuelyser, the tubes were shaken at 27s-1 (27 shakes in 1 second) for 2 minutes and at 10s-1 for 2 hours. Then, they were spun down at full speed for 10 minutes. The supernatant was transferred to a new tube (called C samples) and stored in the fridge for later use. The pellet was mixed with 30µL of NaOH (4M + 0.1%NaBH4) per mg of samples and shacked and spun down as with CDTA. The supernatant was transferred to a new tube (called N samples) and stored in the fridge.

3.2.5.3. Membrane printing

C and N samples were transferred into two different 384 well plates. In each plate, each sample was added in 4 consecutive dilutions (0.5X, 0.1X, 0.02X, and 0.004X) in a total volume of 40µL and made with PBS buffer directly in the wells. Buffer without samples was included in both plates as a control. The two plates were placed for 8 hours in the microarray robot (ARRAYJET) to print all the samples onto a single nitrocellulose membrane. Additionally, the robot made a printing replicate of all the samples on the membrane. A blue ink solution was printed bordering the area where all the samples were located to visualize the position of all the samples on the membrane (**Appendix - Figure 3.1**).

This was repeated 18 times to produce 18 membranes, 17 to be incubated with a specific antibody and one without any antibody to provide a control (**Table 3.2**). These 17 antibodies recognize the major components of the cell wall and are commonly used for cell wall analysis in William's lab, Newcastle University, where this experimental part was conducted.

Table 3.2 Antibodies employed for the detection of the cell wall components. In total, 17 antibodies were employed to detect the different component of the potato leaf cell wall. A control was also included in the experiment, which is a membrane containing all the samples but incubated with only buffer.

Well	Antibody	Polysaccharide	Epitope
(1)	JIM5	Pectin	HG partially/de-esterified
(2)	JIM7	Pectin	HG partially esterified
(3)	LM18	Pectin	HG partially/de-esterified
(4)	LM19	Pectin	HG partially/de-esterified
(5)	LM8	Pectin	Xylogalacturonan
(6)	LM5	Pectin	(1→4)-β-D-galactan
(7)	LM6	Pectin	(1→5)-α-L-arabinan
(8)	LM21	Hemicellulose	(1→4)-β-D-(galacto)(gluco)mannan
(9)	LM23	Hemicellulose	Terminal (1→4)-β-D-xylan
(10)	LM15	Hemicellulose	Xyloglucan (XXXG motif)
(11)	LM24	Hemicellulose	Galactosylated xyloglucan
(12)	LM25	Hemicellulose	Xyloglucan / unsubstituted β-D-glucan
(13)	LM10	Hemicellulose	(1→4)-β-D-xylan
(14)	LM11	Hemicellulose	(1→4)-β-D-xylan/arabinoxylan
(15)	JIM20	Glycoprotein	Extensin
(16)	LM2	Glycoprotein	AGP, β-linked GlcA
(17)	LM1	Glycoprotein)	Extensin
(18)	No antibody	Control (Buffer)	-

3.2.5.4. Incubation with antibody.

Each membrane was incubated with 2mL of milk solution for 2 hours. Then, 200uL of one antibody (**Table 3.2**,

Figure 3.4) was added to the membrane following incubation at room temperature for 1 hour and 30 minutes with constant shaking after which the solution was taken out. To remove unbound antibodies, the membranes were washed 3 times with 2mL of PBS buffer. After discarding PBS, a fresh solution of 100mL of [buffer + milk] (5% milk) with 1µL of secondary antibodies was added to each membrane and mixed for 1 hour 30 minutes at room temperature. To reveal the blots, 2mL of NBT/BCIP (Nitro Blue Tetrazolium/ 5-Bromo-4-Chloro-3-Indolyl-Phosphate) were added to each well

and incubated for 5 to 20 minutes. To stop the reaction, each membrane was placed in water for approx. 2 minutes and then placed on a piece of paper to let dry overnight.

Figure 3.4 Incubation with antibodies. Each printed membrane with all the samples were incubated in milk (step 2 in the protocol, left). Blot revelation was carried out with NBT/BCIP (step 11 in the protocol, right).

3.2.5.5. Data transformation.

After antibody incubation, all the membranes were pasted onto a fresh sheet of paper ready for scanning with a Canon 9000F Mark II scanner. After scanning, the program "Array-Pro Analyzer" was employed to detect spots in each membrane and assign an intensity value. All the data, from the 18 pieces of membranes were saved into .txt files. Each file contained the raw intensity and the background intensity, which are the intensity of each spot and the intensity around the spot, respectively. Then, the net intensity was calculated as the raw intensity minus background intensity. The net intensity was averaged with the two robot replicates (**Appendix-Figure 3.1**). This data was imported into the program MADP v1.108. Data were normalized by considering the value of the buffer equal to 0. The two printing replicates were averaged, and a new normalization was performed with the average of the 4 dilutions (D1, D2, D3, D4) as follows: {[(D1 + D2 + D3 + D4)/4]/max. value in the table} * 100.

3.2.5.6. Statistical data analysis and visualization

To evaluate the consistency between the two technical replicates, the Pearson and the Spearman correlation was calculated, for each antibody across all the samples. The Pearson correlation (parametric) evaluates the linear relationship between two continuous variables, while the Spearman correlation (non-parametric) evaluates the degree of association between two ranked variables. Then the data was separated by organic and conventional samples, and a new correlation was calculated separately for each antibody. A PCA analysis was carried out to observe not only the dispersion of technical replicates, but also if some potato varieties can generate groups depending on if they are resistant or susceptible and depending on the growing system (conventional or organic system). For the PCA analysis, the blot intensities for each antibody were taken as the variables and the data was centred and normalized (z-score).

For each antibody, the Shapiro test was used to determine if the samples followed a normal distribution and the Bartlett's test was used to detect the homogeneity of the variance (homoscedasticity), with a p-value cut-off of 0.05. To determine if a significant difference in the cell wall composition existed between potato varieties with different degrees of tolerance to *P. infestans* (resistant, susceptible, and intermediate varieties), an analysis of variance was carried out if both normality assumptions were met, otherwise the non-parametric Kruskal-Wallis test was used. *Post-hoc* pairwise tests were also carried out to determine the significant difference between groups (resistant, susceptible, intermediate) using the parametric t test or non-parametric Wilcoxon rank sum test, as appropriate. A False Discovery Rate adjusted p-value less than 0.05 was considered significant.

3.3. Results

3.3.1. Detached infection assay on potato leaves with *Phytophthora infestans*.

The greatest diversity in the reaction of potato varieties to the infection was observed two days post-infection, where clearly defined areas of necrosis appeared in some leaves of some varieties. Also, after two days of infection, the necrosis was rapidly spread across entire leaves in susceptible varieties, as in Sharpe's Express. In contrast, in the resistant varieties, Sarpo Mira and Sarpo Shona, after the second day only very few symptoms of infection appeared (**Figure 3.5**). After 5 days, more than the half of the total varieties showed collapsed leaves, scored as 100% infected (**Figure 3.5**).

The level of resistance/susceptibility obtained in this experiment after 2 days post-infection was compared with the level given in the Potato Database. The most notable differences in the plant response were observed with Chicago, Rembrandt, and Toluca. Although in general, the reaction of the varieties was largely consistent with the data stored in the database (**Figure 3.6**). According to the plant reaction (axis Y in (**Figure 3.6**), potato varieties can be grouped as resistant (from 0% to 20% damage in the leaf), intermediate (from more than 20% to 80%) and susceptible (from more than 80% to 100%). Given these thresholds given by a visual inspection of the graph (**Figure 3.6**), in total from the 25 varieties, there are 9 susceptible varieties, 14 with intermediate response and 2 resistant varieties (**Figure 3.6, Table 3.3**).

Table 3.3 Varieties grouped as resistant, intermediate, and susceptible to *P. infestans* according to the detached infection assay. According to the result obtained from the detached infection assay after 2 days post-infection, varieties were classified as susceptible (blue), intermediate (yellow) and resistant (red) to *P. infestans*. The varieties that have an asterisk are those that were used for cell wall analysis. In light grey and in dark grey are the susceptible and the resistant varieties, respectively, according to the Potato Database. All this data corresponds to reaction observed in leaf. Duke of York was not evaluated in the detached infection assay, but it has a degree of damage of 100% (**Figure 3.5, Figure 3.6**) from the beginning of the infection because all the plants from this variety were infected and destroyed prior to be collected from the field.

Variety name	Level of resistance according to the detached infection assay	Level of resistance according to the Potato Database
*ANNABELLE	Susceptible	2
CHICAGO	Susceptible	6
*DUKE OF YORK	Susceptible	2
*HOME GUARD	Susceptible	2
REMBRANDT	Susceptible	5
SHARPE'S EXPRESS	Susceptible	2
RUSSET BURBANK	Susceptible	3
ROYAL KIDNEY	Susceptible	2
SOFIA	Susceptible	2
*INTERNATIONALKIDNEY	Intermediate	2
*ALMERA	Intermediate	2
EXCALIBUR	Intermediate	5
INNOVATOR	Intermediate	3
*JULIETTE	Intermediate	6
CARA	Intermediate	5
ORCHESTRA	Intermediate	2
RANGER RUSSET	Intermediate	3
NICOLA	Intermediate	2
MARKIES	Intermediate	5
*TOLUCA	Intermediate	8
VALOR	Intermediate	5
*PENTLAND SQUIRE	Intermediate	6
SETANTA	Intermediate	5
*SARPO SHONA	Resistant	7
*SARPO MIRA	Resistant	7

In this experiment, all varieties showed different degrees of callose deposition. The intermediate variety Innovator produced the highest amount of callose, contrary to the resistant variety Sarpo Mira, in which the degree of callose deposition was the lowest. The susceptible and intermediate varieties showed a negative correlation between callose deposition and degree of damage, although this value was not high (-0.3) (**Figure 3.7**).

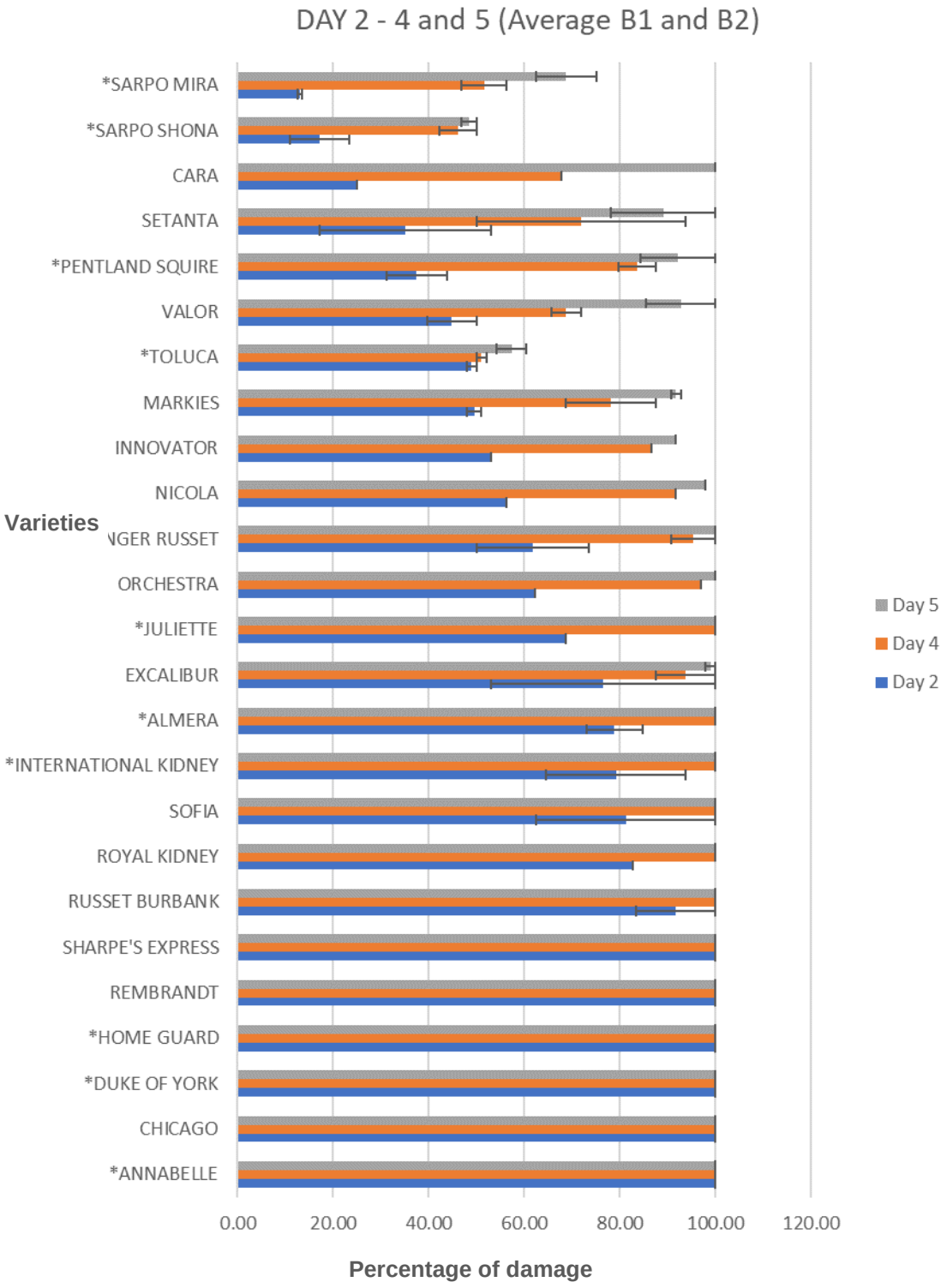


Figure 3.5 Histogram of the degree of damage of potato varieties after 2, 4- and 5-days post infection with P. infestans, strain Blue 13. This figure shows the reaction of the varieties after 2 days (blue bars), 4 days (orange bars) and 5 days (grey bars) post infection. The Y axis shows the average reaction (between block 1 and 2) per day in each variety, where 100% represents a high degree of damage in the most susceptible varieties, with the standard error by error bars. The varieties marked with an asterisk are those that were used for cell wall analysis. Duke of York was not evaluated in the detached infection assay, but its degree of damage was considered as 100% from the beginning of the infection because all the plants from this variety were infected and destroyed prior to be collected from the field.

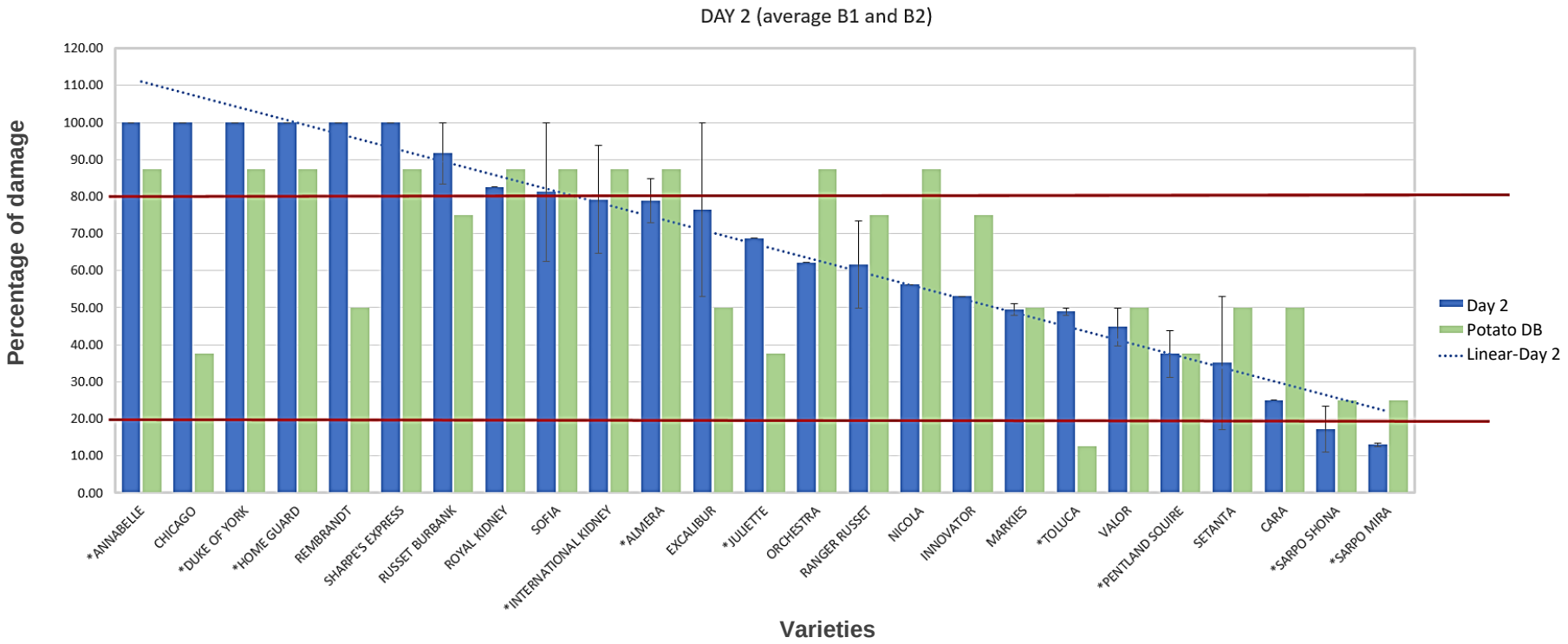
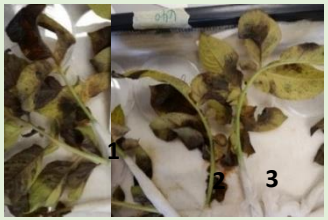
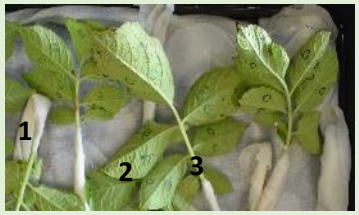


Figure 3.6 Degree of damage in potato leaves after two days of infection with *P. infestans*, and its comparison with the degree of resistance/susceptibility shown in the Potato Variety Database. The Y axis gives the level of susceptibility per variety (axis X) as a percentage and obtained experimentally (blue bars) and from the Potato database (green bars). The highest value in the axis Y is for the most susceptible while the lowest values is for the most resistant variety. The blue bar come from the result of this experiment at 2 days post-infection, which is the average reaction (between block 1 and 2) in each variety. The Potato Database provide a score from 1 to 9, where 1 is for the most susceptible and 9 for the most resistant. These values were inverted as 0 for the most resistant and 8 for the most susceptible and converted to a percentage. The red line represents the threshold separating resistant, susceptible, and intermediate responses. The varieties that have an asterisk are those that were used for cell wall analysis. Duke of York was not evaluated in the detached infection assay, but its degree of damage was considered as 100% from the beginning of the infection because all the plants from this variety were infected and destroyed prior to be collected from the field.

Table 3.4 Reaction of representative potato varieties that showed susceptible, resistant, and intermediate responses to the infection with Blue 13. The table shows status of the plant before the infection and after 2-, 4- and 5-days post-infection in some representative plants from the 3 groups: susceptible, intermediate, and resistant response to *P. infestans*. Composite leaves are labelled with numbers 1-3.

VARIETY	BEFORE INFECTION	DAY 2	DAY 4	DAY5
SUSCEPTIBLE SHARPE'S EXPRESS CODE 93			-	-
SUSCCEPTIBLE EXCALIBUR CODE 341				
INTERMEDIATE CARA CODE 440				
INTEMREDIATE TOLUCA CODE 291				

RESISTANT SARPO MIRA CODE 491				
RESISTANT SARPO SHONA CODE 390				

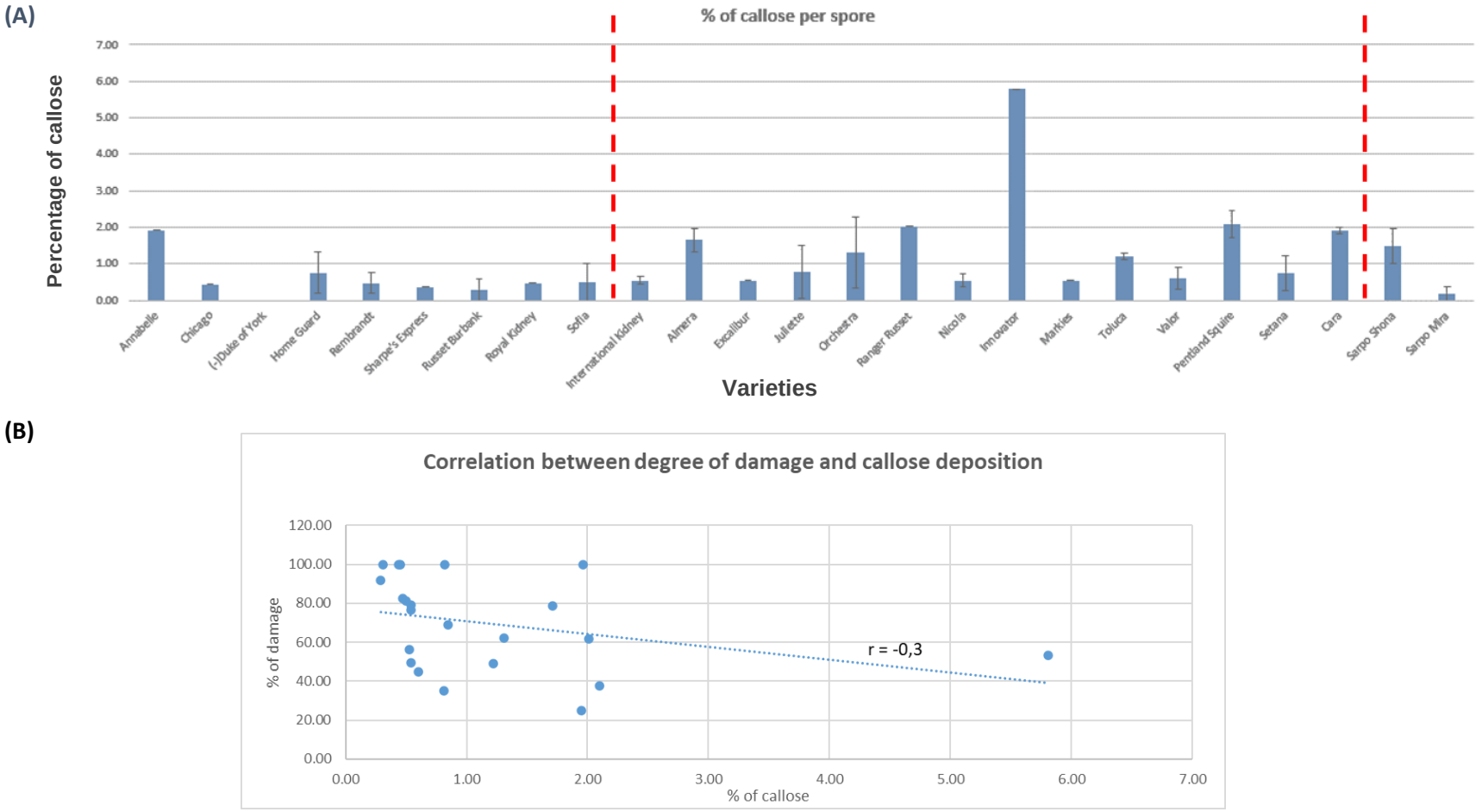


Figure 3.7 Callose deposition. (A) Percentage of callose generated per spore in potato leaflets after infection with *P. infestans*. Each bar represents the mean of the percentage of callose deposition per variety, with standard error shown by the error bars. In the case of Duke of York, there was no sample to evaluate. Vertical dotted red lines divide the susceptible (on the left), intermediate (in the middle) and resistant (on the right) varieties, as defined by the response to Blue 13 at two days post-infection. **(B)** The scatter plot shows the percentage of callose (x-axis) and damage (y-axis) found in the susceptible varieties and with intermediate response. The plot also shows the Spearmen correlation (r) between the degree of damage and callose deposition.

3.3.2. Cell wall composition analysis by CoMPP

The composition of the cell wall of the potato varieties with different degrees of resistance to *P. infestans*, according to the Potato Variety Database and the experimental result (**Table 3.3**), was analysed by CoMPP (Moller *et al*., 2007) to determine whether there is varietal variation for cell wall composition which may be related to resistance to blight.

3.3.2.1. Quality control

The consistency between technical replicates was evaluated in 14 antibodies because 3 of the 17 antibodies (LM8, LM23 and LM24) showed no signal in any sample. Pearson and Spearman correlations showed similar values for all antibodies except for LM18, although in both cases the values were consistently low for this antibody (**Table 3.5**). There was a low correlation between technical replicates for some antibodies, like LM18 and LM19, and a high correlation in others, like JIM7 and LM10 (**Table 3.5**). According to the Pearson and the Spearman correlation, from the 14 antibodies, 2 and 3 of them had a correlation lower than 0.5, respectively (red highlight, **Table 3.5**).

Table 3.5 Correlation between technical replicate 1 and 2 from CoMPP analysis of all samples. Antibodies with the lowest correlation values (< 0.50) with both or any tests (Pearson or Spearman) are shown in red.

Antibody	Pearson correlation		Spearman correlation	
	Correlation value	p-value	Correlation value	p-value
LM18	0.17	2.87E-01	0.39	1.24E-02
LM19	0.36	2.23E-02	0.38	1.43E-02
LM25	0.51	7.31E-04	0.46	2.84E-03
LM2	0.54	3.39E-04	0.53	4.22E-04
LM15	0.62	1.84E-05	0.59	5.27E-05
LM5	0.66	3.69E-06	0.67	2.53E-06
LM21	0.72	1.60E-07	0.63	1.08E-05
LM6	0.72	1.23E-07	0.69	7.41E-07
JIM5	0.74	3.54E-08	0.76	1.42E-08
LM11	0.86	2.14E-12	0.85	4.48E-12
LM1	0.86	1.99E-12	0.78	3.10E-09
JIM20	0.87	5.44E-13	0.83	4.66E-11
LM10	0.93	4.41E-18	0.91	3.06E-16
JIM7	0.93	3.97E-18	0.89	1.83E-14
LM8	NA	NA	NA	NA
LM23	NA	NA	NA	NA
LM24	NA	NA	NA	NA

To better visualize the distribution of the whole data set at the same time and observe if technical replicates form distinct groups, principal component analysis (PCA) was carried out. Different PCAs were generated and inside the graphs, each point corresponds to the sample encoded as follows: The first number (from 1 to 10) is the variety number (**Table 3.6**) and depending on the technical replicate, the following characters are T1 (technical replicate 1) or T2 (technical replicate 2). Then, depending on the system, the following letter is O (organic) or C (conventional). Finally, depending on the block, the following part of the code is B1 (block 1) or B2 (block 2). As an example, point 10T1OB1 is the sample 10 (International K.), in the technical replicate 1, growing in the organic system in block 1.

Table 3.6 Potato varieties analysed by CoMPP and their resistance category. Varieties are classified as resistant, susceptible, and intermediate according to the result of the detached infection assay at 2 days post-infection.

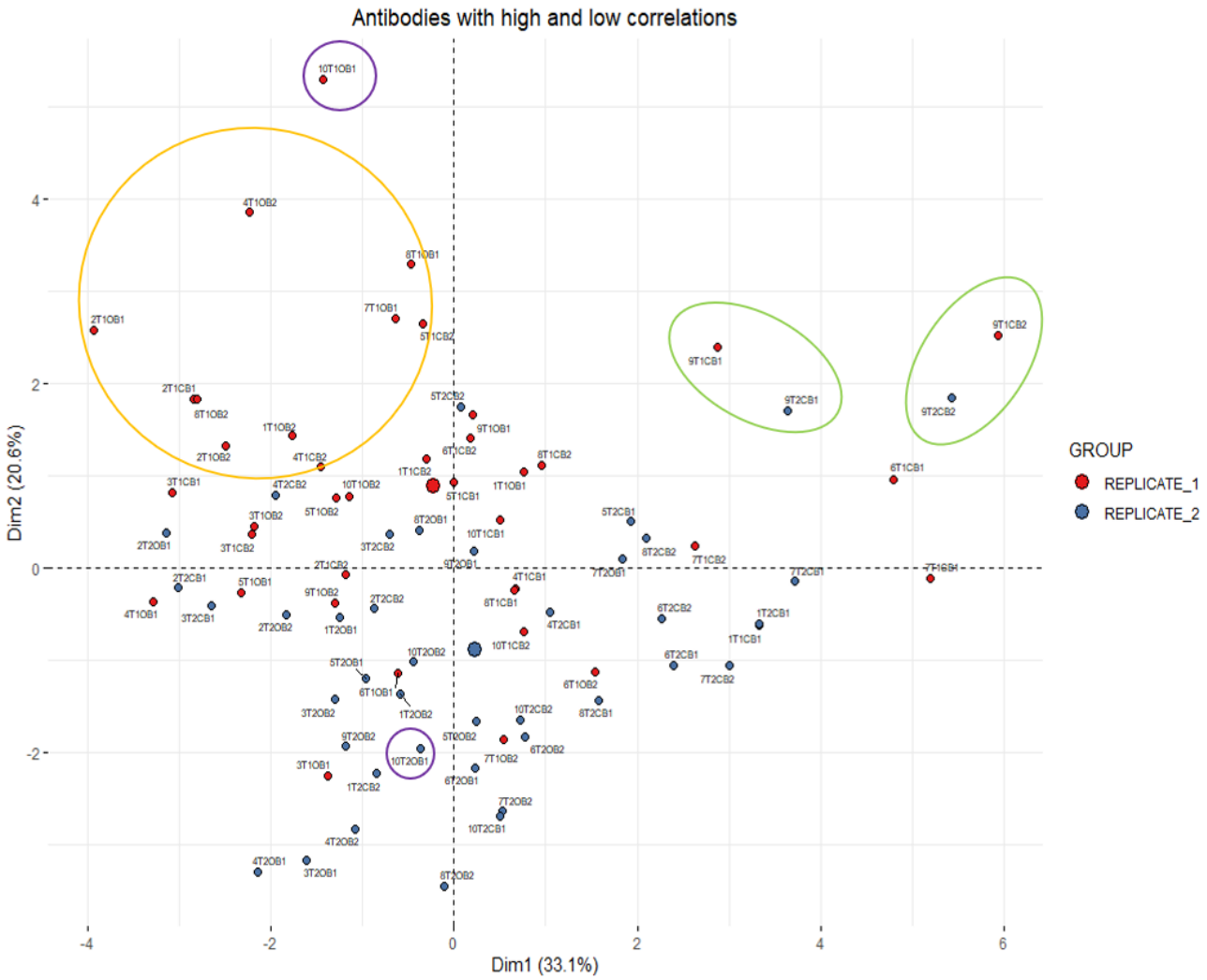
Variety code	Name	Group
1	Toluca	Intermediate
2	Sarpo Mira	Resistant
3	Sarpo Shona	Resistant
4	Juliette	Intermediate
5	Pentland Squire	Intermediate
6	Almera	Intermediate
7	Annabelle	Susceptible
8	Duke of York	Susceptible
9	Home Guard	Susceptible
10	International Kidney	Intermediate

Firstly, all the data was plotted together (technical replicates 1 and 2, organic and conventional system), with PCAs employing: (a) All the antibodies or: (b) antibodies with a correlation more than 0.5 between technical replicates 1 and 2 (**Table 3.5**). When we analyse the distribution of the replicates (**Figure 3.8**), we observe that by considering all the 14 antibodies (**Figure 3.8-A**), some points that are replicates are not close (in **Figure 3.8-A,** purple circles). Indeed, there a group of points from replicate 1 that are isolated from the rest of the others (in **Figure 3.8-A**, orange circle). When the number of variables (antibodies) is restricted by the correlation value between technical replicates (> 0.5), some points that are replicates become closer (in **Figure 3.8-B** green and purple

circles), and some points that were isolated in **Figure 3.8-A** orange circle) are now closer to their replicate (in **Figure 3.8-B** orange circles). Therefore, the distribution of the replicates in the PCA improved when data from antibodies with low correlation between technical replicated were discarded. However, when antibodies with a correlation value less than 0.6 or 0.7 were discarded (**Appendix-Figure 3.2-A, B)**, the distribution of the technical replicates did not improve. Indeed, when only antibodies with a correlation more than 0.7 are selected, some points that were close in **Figure 3.8-B** become separated (**Appendix-Figure 3.2-B**, purple and orange circle).

Antibodies with correlation between technical replicates less than a threshold of 0.5 were therefore discarded. The impact of this threshold can be seen in **Figure 3.9**, where there was a clear separation between technical replicate groups when all antibodies were used for the PCA, particularly for the organic samples (**Figure 3.9-A**), while the groups become a lot less distinct when only antibodies with correlations higher than 0.5 are included (**Figure 3.9-B, D**). Given these differences between conventional and organic samples, correlations were re-calculated between technical replicates in each system.

(A)


Antibodies with high and low correlations
Dim2 (20.6%)
Dim1 (33.1%)
GROUP
REPLICATE_1
REPLICATE_2

(B)

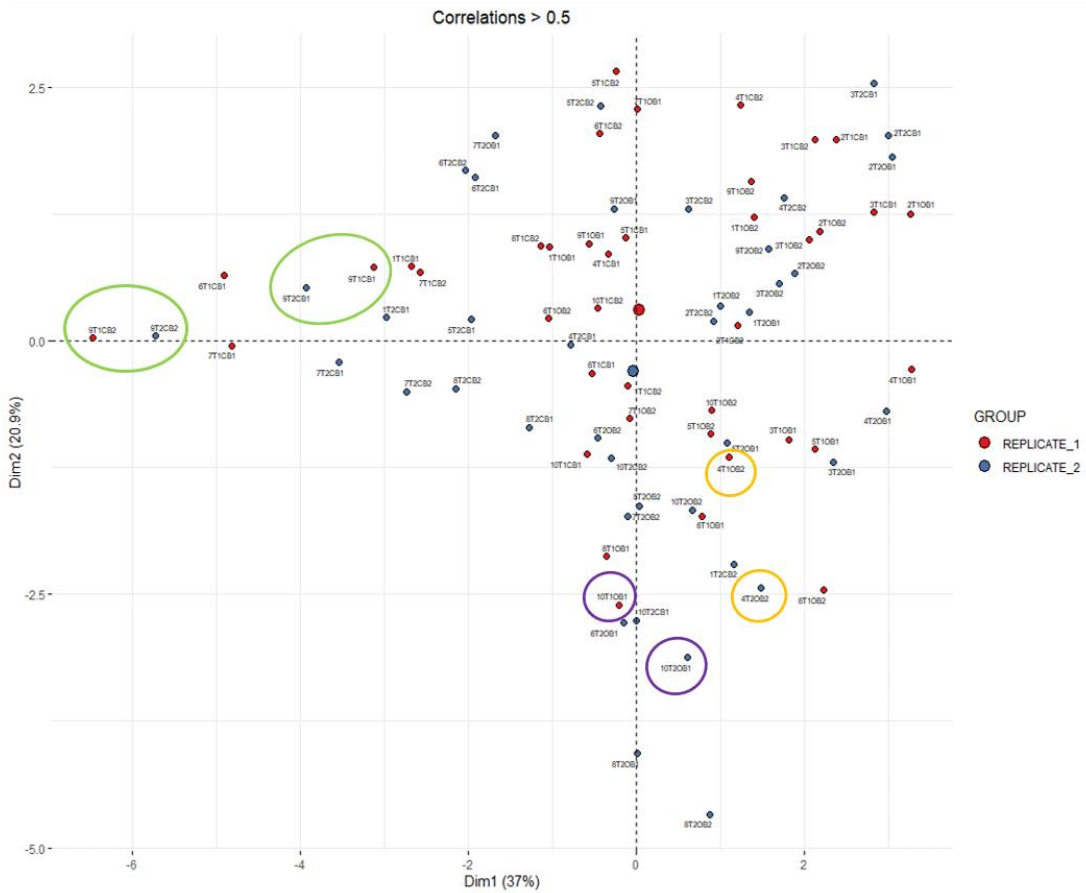


Figure 3.8 PCA showing distribution of the samples by technical replicates. In (A) all the data is represented with the PCA. The purple circles show how one individual from replicate 1 are far from its replicate 2. Orange circle shows how a group of individuals from replicate 1 form a separate group, isolated from its replicate 2. In green circles there are examples of some varieties that are close between its replicates. In (B) the data include antibodies with correlation values more than 0.5. In green and

purple circles, the figure shows how the same individuals in its different technical replicates are closer than in figure (A). In the orange circle, we observe that some points that form an isolated group in (**A**) (orange circle) are now also close to their replicates.

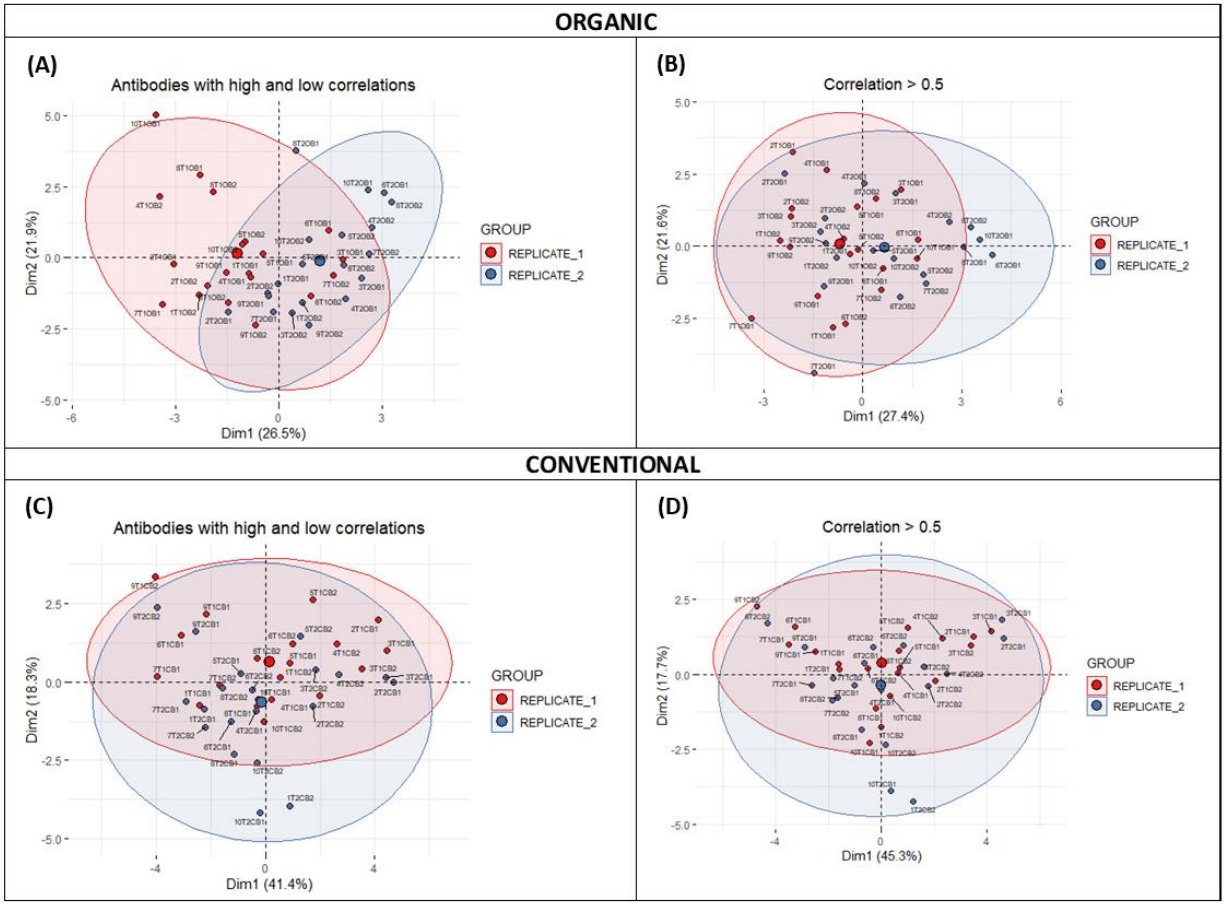


Figure 3.9 PCA of the samples divided by conventional and organic samples and grouped by technical replicates. In (**A**) and (**C**) all 14 antibodies were included in the analysis (**Table 3.5**), while in (**B**) and (**D**) the antibodies LM18 and LM19 were removed (**Table 3.5**).

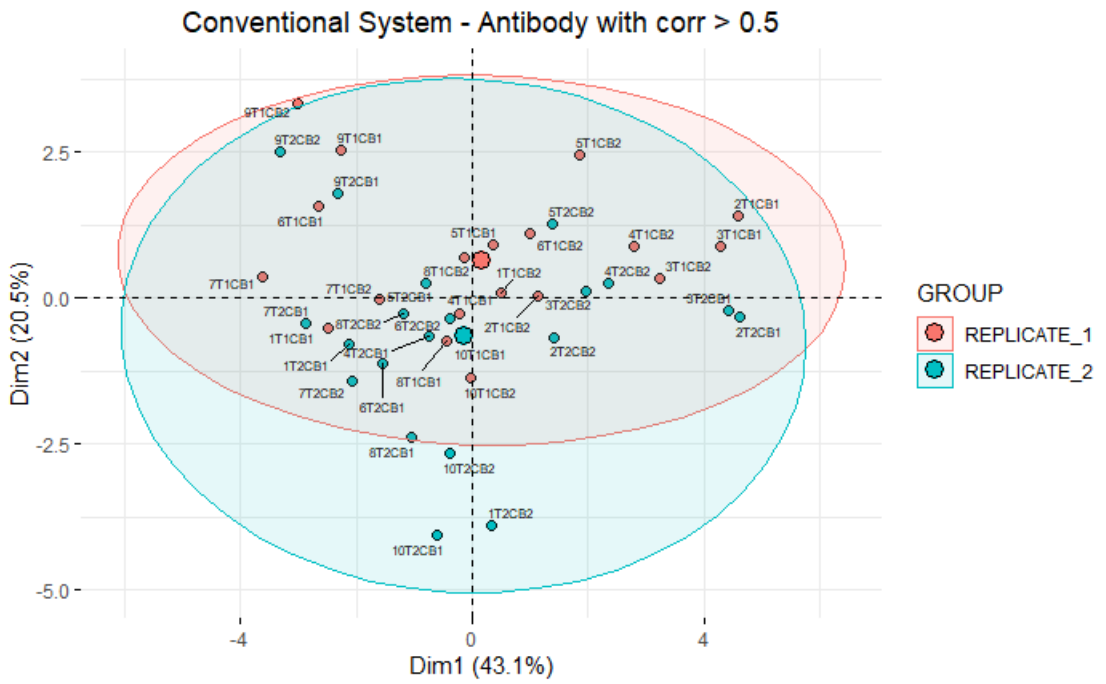
In **Table 3.5**, we observed that the antibodies LM18 and LM19 had the lowest correlation between technical replicates when samples from both farming systems were analysed as one. However, **Table 3.7** shows that the correlation for various antibodies changed, sometimes quite markedly, depending on the system. LM18 and LM19 showed correlation values higher than 0.5 in the conventional system, while the correlation in the organic system remained very low (**Table 3.7**)**.** Meanwhile, some other antibodies like LM25 or LM21 showed a low correlation only in one farming system. Antibodies with correlation between technical replicates less than a threshold of 0.5 were therefore discarded for each farming system separately for all downstream analyses.

Table 3.7 Spearman correlation between technical replicates 1 and 2 in the conventional and the organic systems. Antibodies with a correlation value less than 0.50 between technical replicates were discarded for downstream analysis, independently for each growing system (in red).

Cell wall component	Antibody	Conventional			Organic		
		Correlation value	p-value	Selected	Correlation value	p-value	Selected
Pectin	JIM5	0.9	4.62E-08	Yes	0.71	4.76E-04	Yes
	JIM7	0.66	1.40E-03	Yes	0.94	4.36E-10	Yes
	LM18	0.55	1.16E-02	Yes	0.34	1.47E-01	No
	LM19	0.73	2.55E-04	Yes	0.14	5.44E-01	No
	LM5	0.66	1.65E-03	Yes	0.71	4.71E-04	Yes
	LM6	0.72	3.75E-04	Yes	0.77	7.10E-05	Yes
Hemicellulose	LM21	0.86	1.20E-06	Yes	0.06	8.02E-01	No
	LM15	0.53	1.64E-02	Yes	0.68	8.69E-04	Yes
	LM25	0.43	6.07E-02	No	0.60	5.58E-03	Yes
	LM10	0.81	1.38E-05	Yes	0.91	2.58E-08	Yes
	LM11	0.92	7.57E-09	Yes	0.75	1.56E-04	Yes
Glycoprotein	JIM20	0.88	2.26E-07	Yes	0.78	4.43E-05	Yes
	LM2	0.33	1.61E-01	No	0.56	1.03E-02	Yes
	LM1	0.85	1.57E-06	yes	0.70	6.45E-04	yes

After discarding antibodies with low correlation values in the conventional and the organic systems, new PCAs were produced. The distribution of the samples showed that in the organic system, the data from replicate 1 does not form a distinct group from replicate 2, as observed in **Figure 3.10-A**. Similarly, in the conventional system (**Figure 3.10-B**), the technical replicates overlap extensively with each other**.**

(A)



(B)

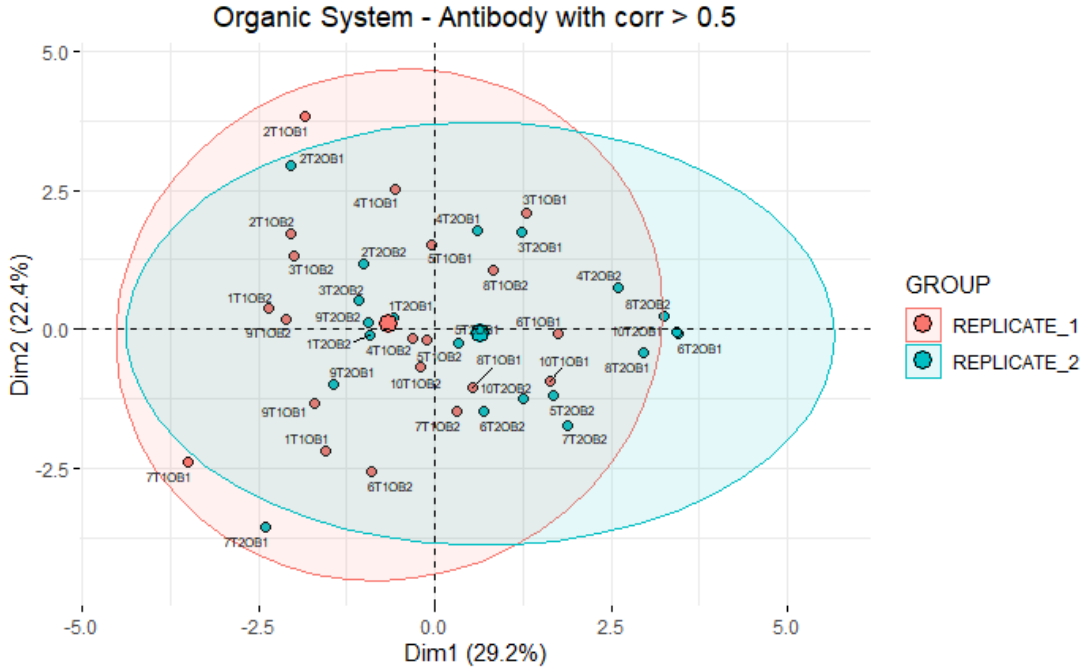


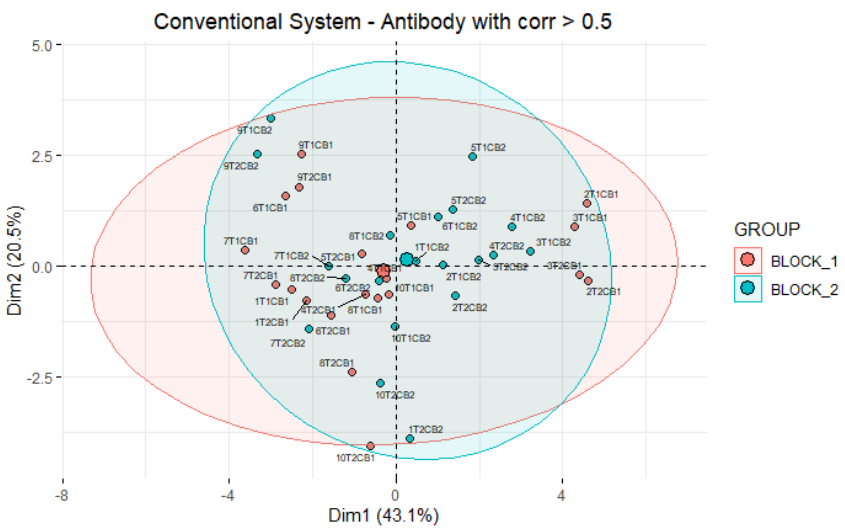
Figure 3.10 Separation of technical replicates in conventional and organic samples after removing antibodies with a correlation value between technical replicates less than 0.5 in each farming system, conventional (A) and organic (B) (Table 3.7).

3.3.2.2. Cell wall composition in two farming systems.

The two farming systems were analysed independently to observe the grouping of samples according to the replicate block, the level of resistance to *P. infestans*, or variety.

In both conventional and organic systems, samples from different blocks did not form separate groups (**Figure 3.11**). However, in both systems some samples from the same varieties growing in the same block were closer to each other than to those growing in the other block. Nevertheless, because samples from the blocks 1 and 2 grew in similar conditions and the distribution of the samples in the PCA did not show distinct groups in the two main principal components that captured most of the variation in component abundance, the two blocks were considered as biological replicates.

(A)



(B)

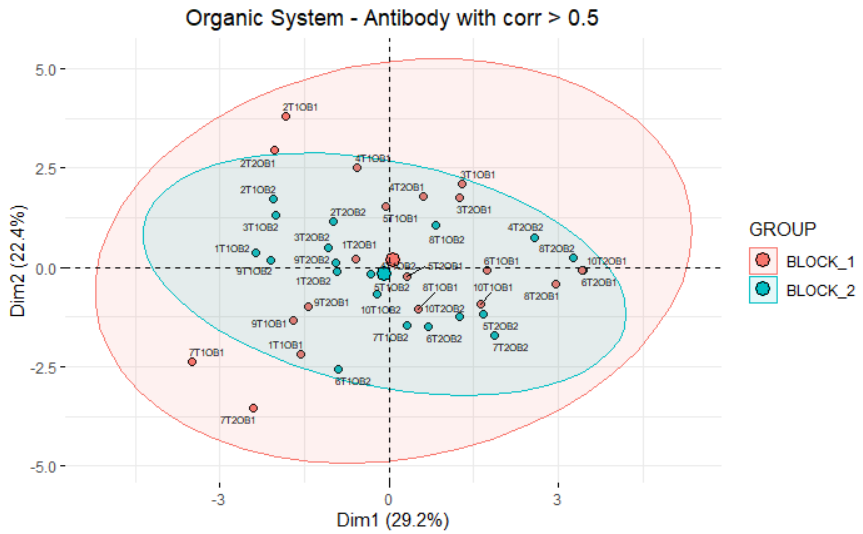


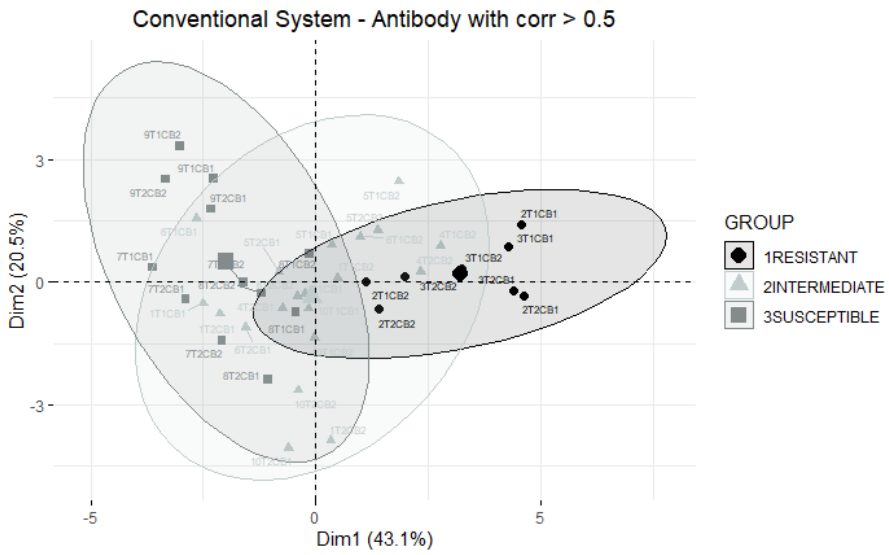
Figure 3.11 Separation of block in conventional and organic samples after removing antibodies with a correlation value between technical replicates less than 0.05 in each farming system (Table 3.7).

In both farming systems, the resistant and susceptible varieties grouped separately, with the distinction between groups clearer in the conventional than in the organic system, while varieties with intermediate response were dispersed inside the two groups. For the conventional system, the three groups were separated along the first principal component, PC1, which explained the largest (43.1%) proportion of the variance, while in the organic system this division is explained by the second principal component, PC2, explaining 22.4% of the variance (**Figure 3.12**).

The loadings in the PCA were also observed to identify the contribution of each antibody to the two principal components. In the conventional system, PC1 had strong positive loadings for the antibodies LM21, LM11 and LM10 (**Figure 3.13-A, B**), while the strong negative loading was given by LM19. These two groups could be the main antibodies responsible for the separation between susceptible and resistant groups visualized in this PC1 (**Figure 3.13**). This effect can also be observed in the heatmap with the antibodies LM11, LM10, and LM19 that clearly grouped almost all the resistant varieties and separated them from the susceptible ones (**Appendix-Figure 3.3**).

In the organic system, the loading from the PC2 divided resistant and susceptible varieties. In this component, the major positive loadings are the LM10, LM11, LM1, LM2 and JIM20, while the major negative loading involves LM5 and JIM7 (**Figure 3.13**). Similarly, in the heatmap, the antibody LM10 shows different degrees of intensity between the resistant and susceptible groups (**Appendix-Figure 3.3, Figure 3.13-C, D**). According to the loading of each antibody in the PCA, in both systems the antibodies LM10, LM11, LM1, JIM20 and LM6 had the same effect contributing to the difference between resistnat and susceptible groups, and an opposite effect with antibodies JIM7, JIM5.

(A)



(B)

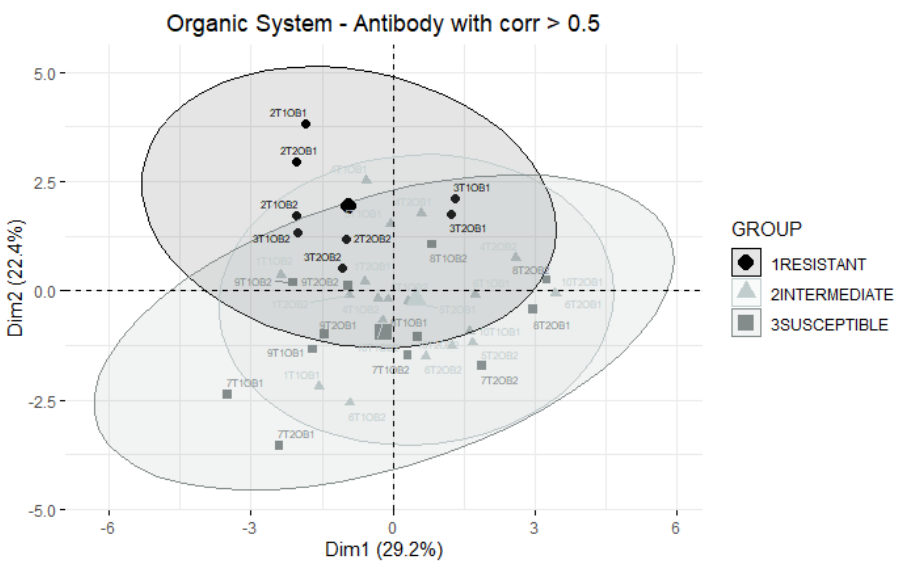
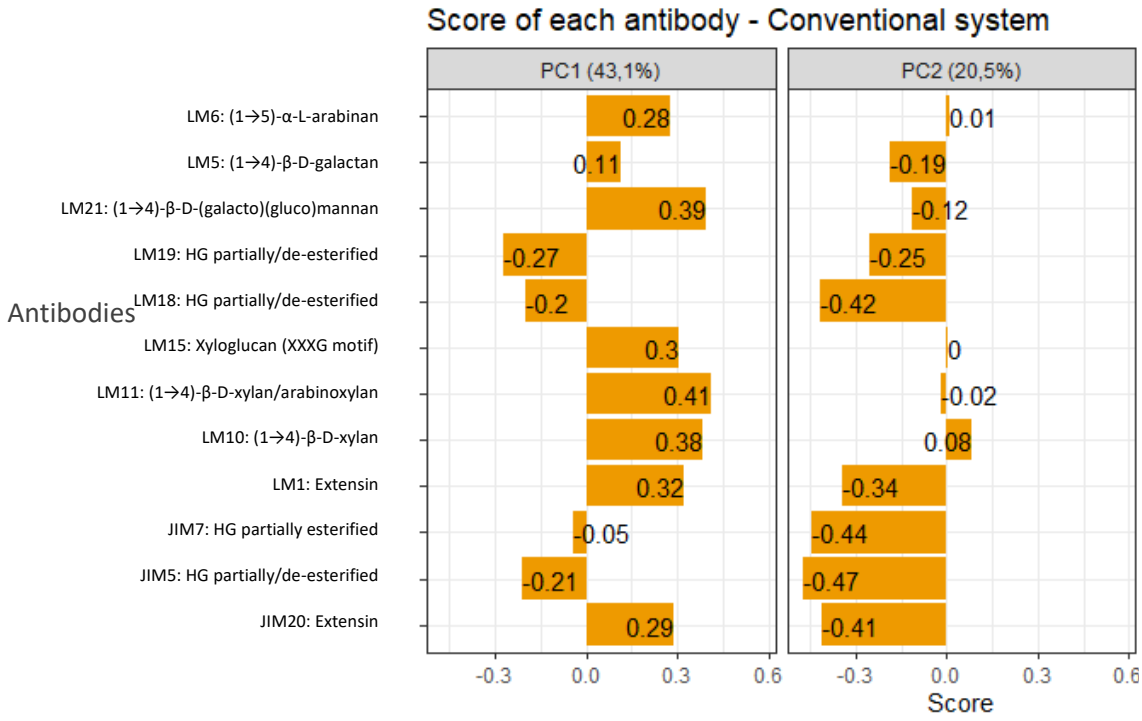


Figure 3.12 Separation of samples according to their tolerance degree (resistant, intermediate, and susceptible to *P. infestans*) in conventional and organic samples after removing antibodies with a correlation value between technical replicates less than 0.5 in each farming system (Table 3.7).

(A)



(B)

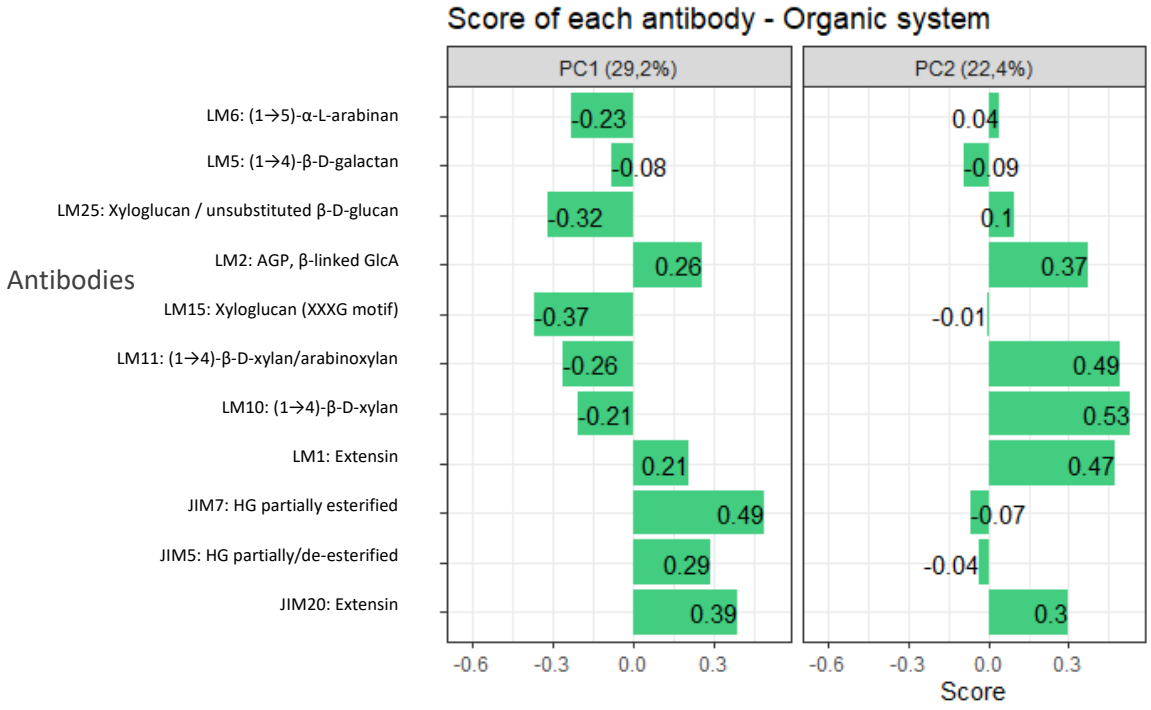


Figure 3.13 Effect of antibodies in the two principal components. In (A) and (B) the loading of each antibody is showed for the conventional and organic system, respectively.

3.3.2.3. Analysis of individual cell wall components.

To evaluate if the individual composition of pectin, hemicellulose or glycoprotein is related to the level of resistance, the data was split into these 3 main components extracted by CoMPP and into each antibody. To detect a significant difference among the three potato groups (resistant, intermediate, and susceptible) an analysis of variance was carried out. The preliminary tests for the normality assumption (Shapiro and Bartlett test) showed that most of the samples do not follow a normal distribution (**Table 3.8**, Table 3.9). Nevertheless, the analysis of variance carried out with either a parametric ANOVA or a non-parametric Kruskal-Wallis test showed consistent results (**Table 3.8**). I was decided to employ the non-parametric test for the analysis of variance for all analyses and non-parametric Wilcoxon rank-sum test for pairwise comparisons. The response among each pair of varieties for each antibody was only compared by the distribution of their abundance. The previously mentioned tests were not applied since the small sample size (4 per each variety and antibody: 2 technical replicates plus 2 blocks) makes any comparison statistically not significant.

The clearest division between the three potato groups in the PCA was observed in the conventional system, especially for the hemicellulose and glycoprotein component, while for the plants growing in the organic system, the same division was not clearly observed (**Figure 3.14**). The Kruskal-Wallis test indicated a significant difference in the abundance of hemicellulose and glycoprotein components in the conventional system, while only a significant difference in hemicellulose abundance was observed in the organic system (**Table 3.8**).

3.3.2.3.1. Pectin components

The analysis of variance based on antibodies for 6 pectin components showed there was no significant difference among the three groups (resistant, susceptible, intermediate) or in any pairwise comparisons in any farming system (**Table 3.8**), which can also be seen in the corresponding boxplot (**Figure 3.15.A**). However, significance differences were observed for some individual antibodies

(**Figure 3.16-A, Table 3.10**). In the organic system, none of the 6 antibodies were significant different between these resistant and susceptible varieties. However, in the conventional system a difference between resistant and susceptible groups was observed for the antibody LM6 that detects arabinan ((1→5)-α-L-arabinan) and two antibodies indicating a degree of de-esterification in pectin, JIM5 and LM19 (HG partially/de-esterified). In the conventional system, a higher concentration of LM6 was observed in the resistant than in the susceptible varieties, while the opposite was observed for JIM5 and LM19 (**Figure 3.16-A**). The different farming systems influenced the composition of specific pectins in specific varieties. For example, more partially/de-esterified pectin, detected with JIM5, was observed in variety Sarpo Shona in the organic compared with the conventional system, while less pectin with arabinan domain, detected with LM6, was observed in Duke of York in the organic farming system (**Figure 3.16-A**). Although there was a significant difference between resistance and susceptible varieties in the abundance of the component detected by JIM5 and LM19, by observing the distribution of the data in each variety (**Figure 3.16-A**), not all the susceptible varieties had more amount of partially/de-esterified HG than the tolerant varieties. Therefore, there was also a variety-specific impact in the composition of the cell wall.

3.3.2.3.2. Hemicellulose components

The overall abundance of the 5 hemicellulose components was significantly higher in resistant compared with susceptible varieties in both farming systems (**Table 3.8**, **Figure 3.15-B**). In general, the abundance of hemicellulose components was highest in resistnat varieties, lower in intermediate varieties, and lowest in susceptible varieties in the conventional system, but the same was not observed in the organic system, where no significant differences were observed between intermediate and susceptible varieties (**Table 3.8**, **Figure 3.15-B**). The same pattern was observed in the conventional system for individual components detected by the antibodies LM11, detecting (1→4)-β-D-xylan/arabinoxylan, and LM21, detecting (1→4)-β-D-(galacto)(gluco)mannan. Furthermore, in the conventional system, LM10 and LM15 detected significantly higher abundance of

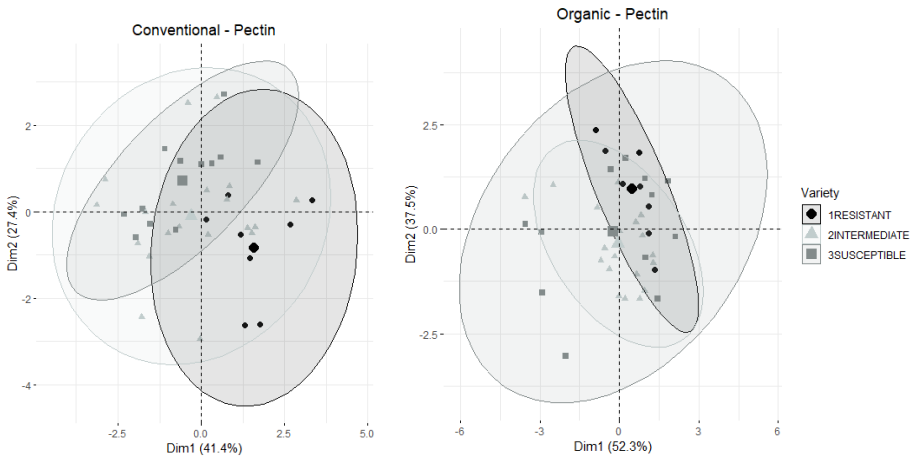
(1→4)-β-D-xylan and xyloglucan (XXXG motif) respectively, in resistant compared with susceptible varieties (**Figure 3.16-B, Table 3.10**).

In the organic system, the resistant varieties showed a significantly higher abundance of LM10 and LM11 than the susceptible. Antibody LM11 highlights differences according to the farming system. While in the conventional system, Duke of York showed a higher abundance than Annabelle and Home Guard, in the organic system Duke of York showed a comparable abundance with Annabelle, but far lower than Home Guard (**Figure 3.16-B**). If we observe each individual variety, for LM15 in the conventional system and LM11 in the organic system, the samples from one susceptible variety, Duke of York and Home Guard, respectively, showed a higher intensity than the other two susceptible varieties, and a similar intensity to the tolerant variety Sarpo Shona. Therefore, a variety-specific impact is observed with this pair of antibodies.

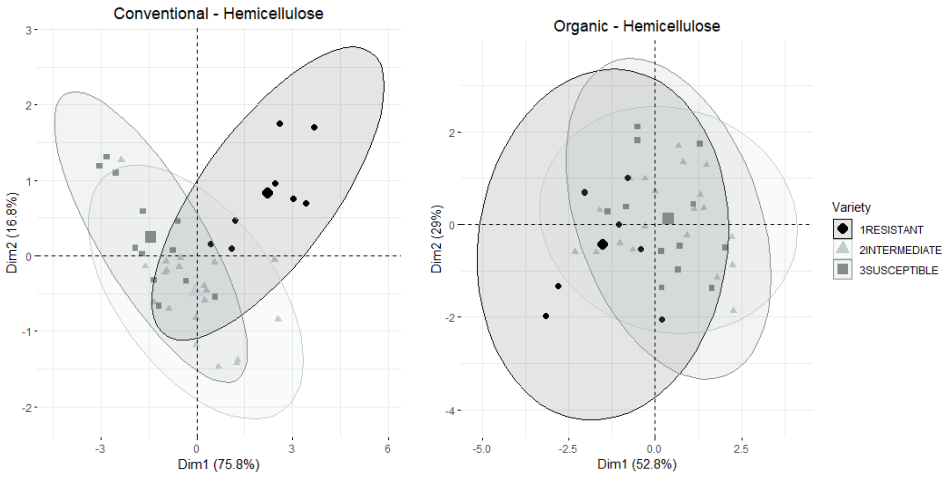
3.3.2.3.3. Glycoprotein components

The overall abundance of 3 glycoproteins components was significantly higher in resistant compared with susceptible varieties in the conventional but not the organic system (**Figure 3.15-C**, **Table 3.8**). This difference was due to antibodies JIM20 and LM1, which both detect extensins and showed higher component abundance in the tolerant varieties compared with the intermediate varieties, and in turn, the susceptible varieties (**Figure 3.16-C, Table 3.10**). In the organic system, a significant difference between the resistant and the susceptible varieties was also found with LM1. If we observe the distribution of component abundance among the susceptible varieties, Duke of York had the highest abundance for all three glycoproteins in both farming systems. For JIM20 this intensity was even higher than the resistant group in the organic system, which made the susceptible group not significantly difference than the resistant varieties.

(A)



(B)



(C)

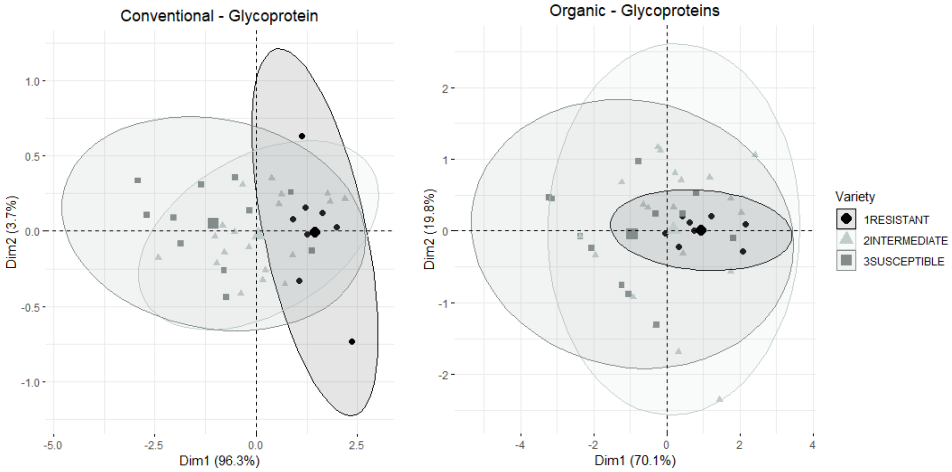


Figure 3.14 PCA plot showing the distribution of the antibody intensities per cell wall component in the conventional and organic system. The three cell wall components extracted by COMPP and valuated here are pectin (A), hemicellulose (B), and glycoproteins (C). The PCA plots shows the resistant, susceptible, and intermediate samples in different colours and shapes to observe if they form distant groups in base of the variability of the respective antibodies. The antibodies employed in the PCA are listed in **Table 3.7**.

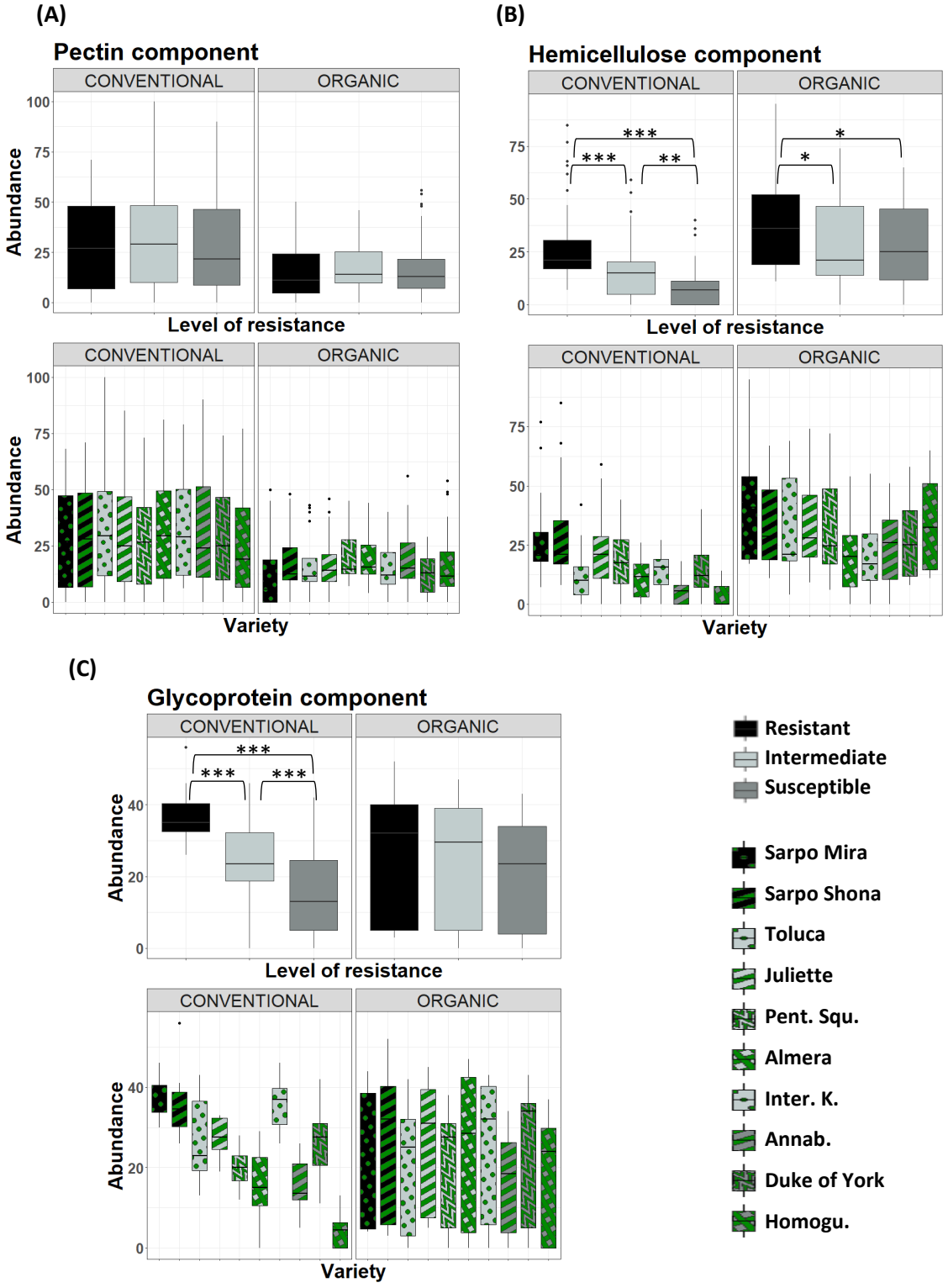
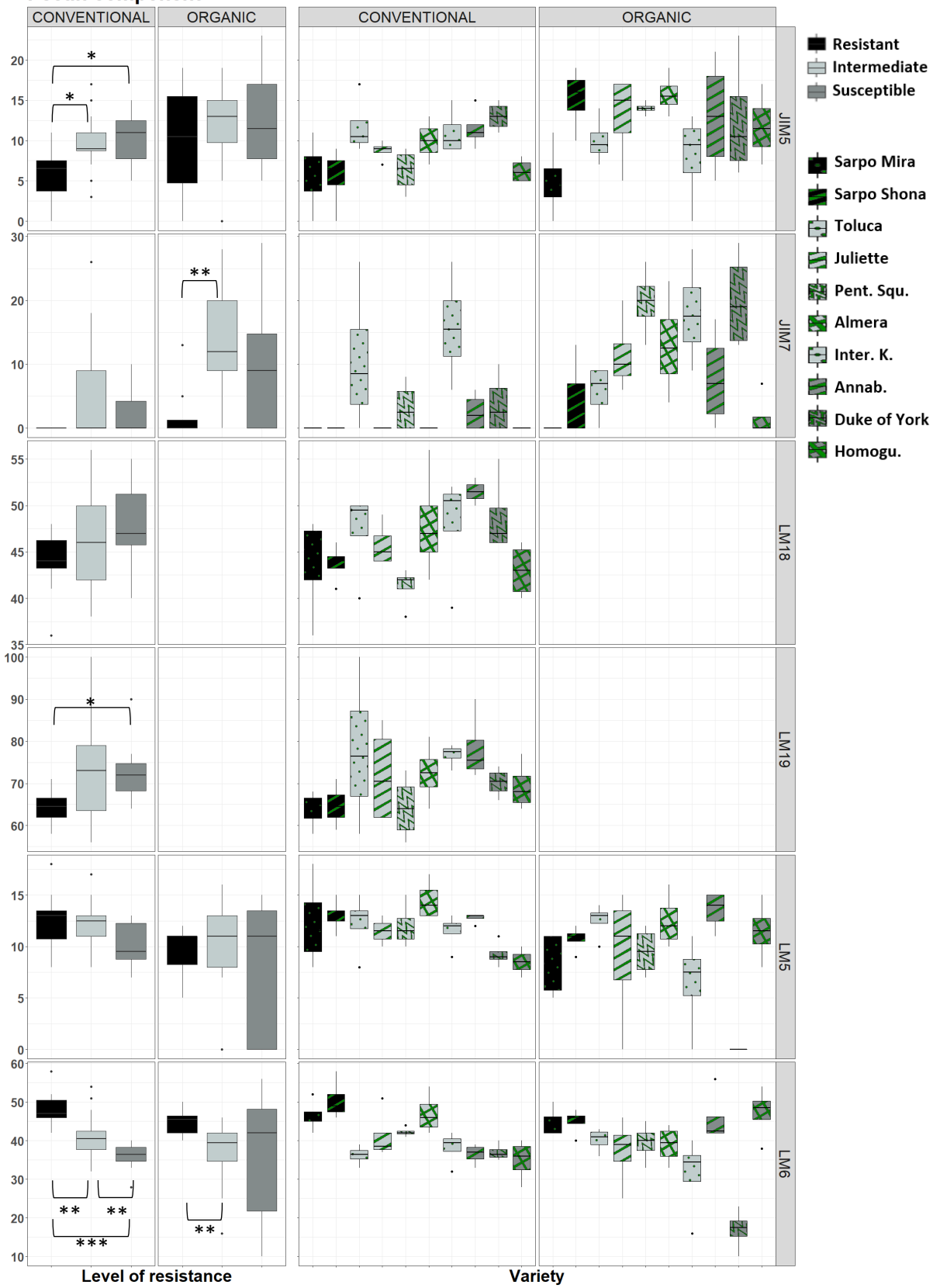


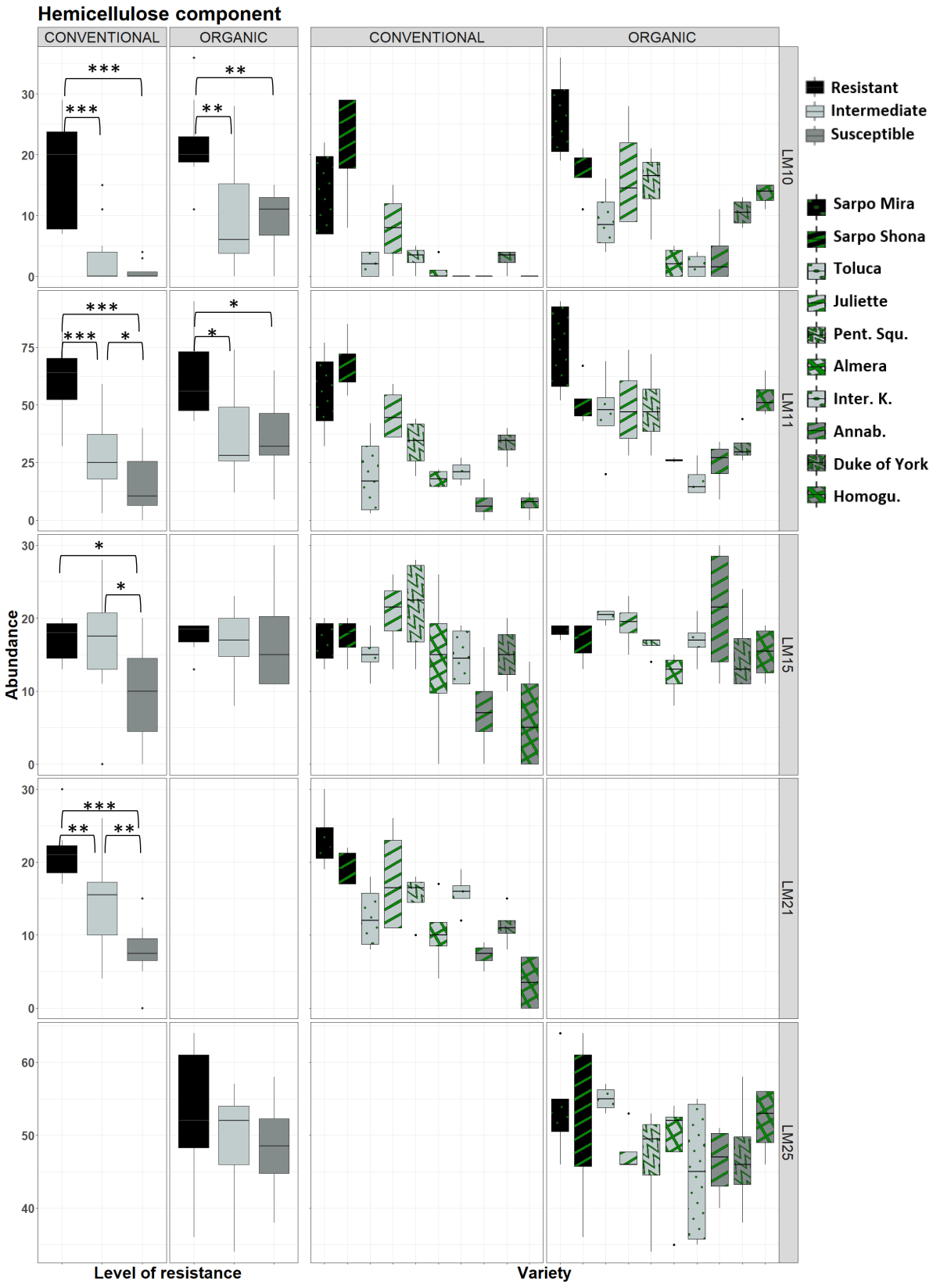
Figure 3.15 Boxplot distribution of the antibody intensities per cell wall component in the conventional and organic system. The three cell wall components extracted by COMPP and valuated here are pectin (A), hemicellulose (B), and glycoproteins (C). Asterisk indicates the significant difference (* $p<0.05$, ** $p< 0.01$, *** $p < 0.001$) between specific potato groups. The antibodies employed are listed in **Table 3.7**.

(A)

Pectin component



(B)


Hemicellulose component
CONVENTIONAL
ORGANIC
CONVENTIONAL
ORGANIC
LM10
LM11
LM15
LM21
LM25
Abundance
Level of resistance
Variety
Resistant
Intermediate
Susceptible
Sarpo Mira
Sarpo Shona
Toluca
Juliette
Pent. Squ.
Almera
Inter. K.
Annab.
Duke of York
Homogu.

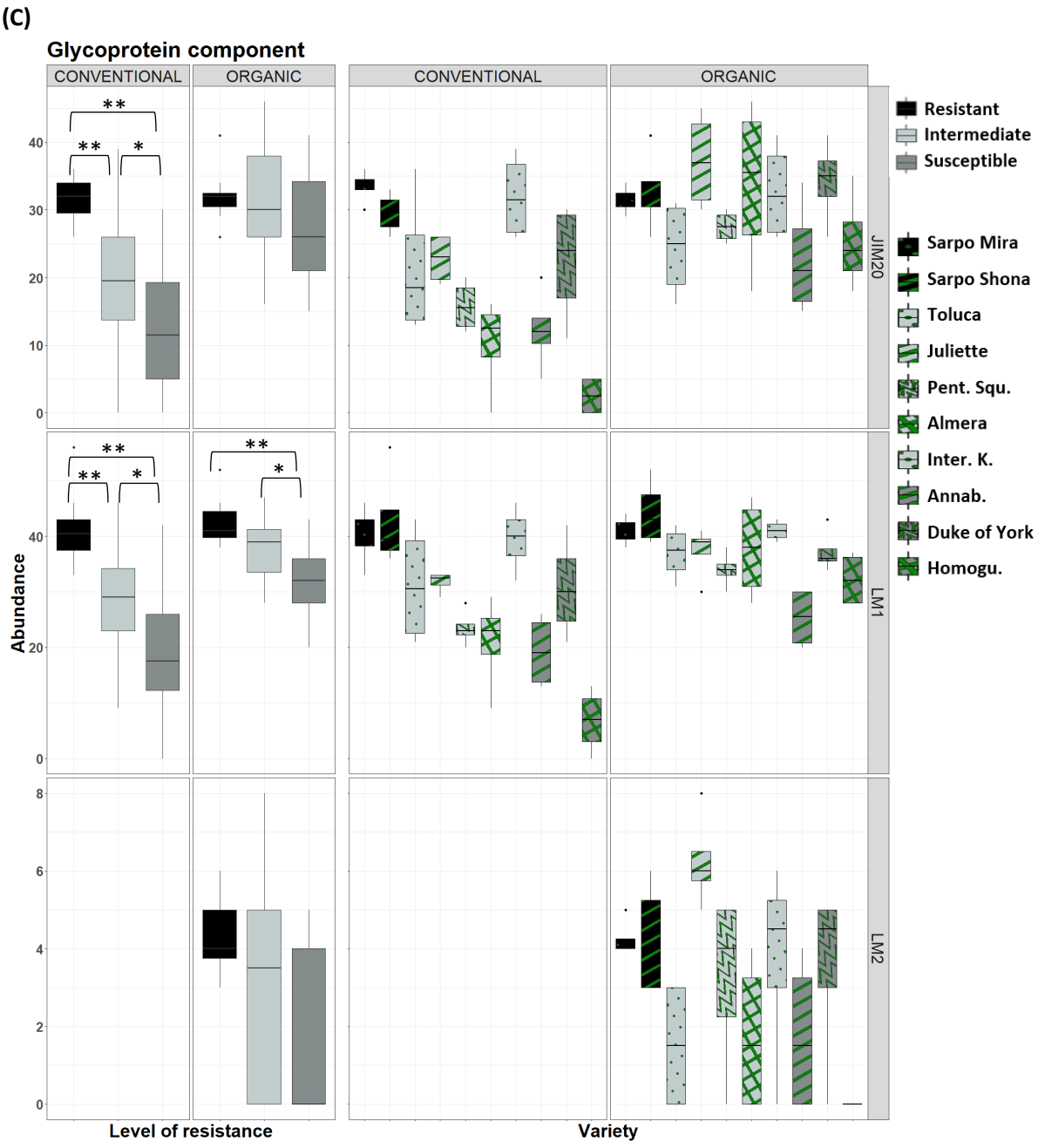


Figure 3.16 Boxplot distribution of the antibody intensities per antibody in the conventional and organic system. The distribution each antibody from each cell wall components, pectin (A), hemicellulose (B), and glycoproteins (C) is visualized here. Asterisk indicates the significant difference (* p<0.05, ** p< 0.01, *** p < 0.001) between specific potato groups. No graphs indicate that there were not intensities to evaluate for the respective antibody and the respective cultivation system, as listed in **Table 3.7**.

Table 3.8 Statistical tests for each cell wall components, pectin, hemicellulose, and glycoprotein. This table shows the preliminary tests for the normality assumption in each cell wall components, the analysis of variance among the three potato groups, and the pairwise test between potato groups in each cell wall component. The normality distribution was tested with Shapiro test, while the homoscedasticity with Bartlett test. The analysis of variance was carried out with both the non-parametric Kruskal-Wallis and the parametric ANOVA test. The pairwise test was carried out with the non-parametric Wilcoxon test and the parametric t -test. In red are the values that does not pass the Shapiro or Bartlett test (p-value < 0.05). In yellow are the samples with a significant difference (p-value < 0.05) between the three variety groups (tolerant, intermediate, susceptible) in the respective component.

Cell wall component	Shapiro test			Bartlett test	Analysis of Variance		Wilcoxon-test			t-test		
	Res	Int	Sus	Res vs Int vs Sus	Kruskal-wallis	ANOVA	Res vs Int	Res vs Sus	Sus vs Int	Res vs Int	Res vs Sus	Sus vs Int
Conventional												
Pectin	5.82E-05	6.83E-07	1.74E-05	9.72E-01	7.34E-01	8.60E-01	8.70E-01	8.70E-01	8.70E-01	8.80E-01	8.80E-01	8.80E-01
Hemicellulose	3.17E-05	3.50E-05	2.21E-06	1.80E-06	4.96E-09	3.90E-09	1.40E-04	2.00E-08	2.50E-04	5.90E-06	1.80E-09	6.30E-03
Glycoprotein	2.32E-01	9.37E-01	2.90E-01	2.04E-01	1.28E-06	9.61E-08	3.80E-04	1.40E-05	2.48E-03	4.00E-04	4.60E-08	7.30E-04
Organic												
Pectin	3.45E-05	1.23E-06	3.66E-05	7.64E-02	2.90E-01	7.96E-01	4.20E-01	4.70E-01	4.70E-01	8.90E-01	9.70E-01	8.90E-01
Hemicellulose	3.04E-03	2.38E-04	4.52E-03	3.37E-01	3.99E-02	2.32E-02	3.90E-02	3.90E-02	8.50E-01	2.50E-02	2.50E-02	9.71E-01
Glycoprotein	1.76E-03	1.69E-05	1.09E-03	7.53E-01	1.26E-01	3.20E-01	4.20E-01	1.70E-01	2.00E-01	5.30E-01	4.10E-01	4.10E-01

Table 3.9 Tests for the normality assumption for each antibody employed in CoMPP. The normality assumption was tested with the Shapiro test, while the homoscedasticity was assessed with the Bartlett's test. The analysis of variance was carried out with both the non-parametric Kruskal-Wallis and the parametric ANOVA test. In red are the values that does not pass the Shapiro or Bartlett test (p-value < 0.05). In yellow are the samples with a significant difference (p-value < 0.05) among at least one comparison between the three potato groups (tolerant, intermediate, or susceptible varieties).

Antibodies		Shapiro test			Bartlett's test	Analysis of Variance	Shapiro test			Bartlett's test	Analysis of Variance
		Res	Int	Sus	Res/Int/Sus	Kruskal-Wallis	Res	Int	Sus	Res/Int/Sus	Kruskal-Wallis
		Conventional					Organic				
Pectin											
JIM5	HG partially/de-esterified	3.56E-01	2.02E-01	3.90E-01	7.46E-01	3.05E-02	7.83E-01	1.37E-01	3.92E-01	3.91E-01	7.55E-01
JIM7	HG partially esterified	NA	8.46E-05	6.63E-04	0.00E+00	6.17E-02	9.63E-05	6.51E-01	1.12E-01	1.36E-01	5.05E-03
LM18	HG partially/de-esterified	2.38E-01	6.74E-01	8.00E-01	7.70E-01	1.95E-01	No data	No data	No data	No data	No data
LM19	HG partially/de-esterified	8.13E-01	5.32E-01	1.12E-01	3.06E-02	3.77E-02	No data	No data	No data	No data	No data
LM5	(1→4)-β-D-galactan	8.76E-01	6.32E-01	1.50E-01	4.82E-01	4.42E-02	2.37E-02	2.36E-02	8.51E-03	4.51E-02	7.76E-01
LM6	(1→5)-α-L-arabinan	4.73E-01	3.18E-01	1.96E-01	2.68E-01	8.68E-05	8.18E-01	7.47E-03	1.62E-01	1.26E-04	3.38E-02
Hemicellulose											
LM10	(1→4)-β-D-xylan	1.08E-01	4.73E-05	6.94E-05	8.97E-06	3.10E-05	3.25E-01	4.42E-02	5.46E-02	3.49E-01	2.20E-03
LM11	(1→4)-β-D-xylan/arabinoxylan	9.74E-01	7.40E-01	1.27E-01	8.70E-01	1.13E-04	9.02E-02	6.87E-02	9.15E-01	6.35E-01	1.83E-02
LM15	Xyloglucan (XXXG motif)	9.71E-02	3.04E-01	3.37E-01	7.01E-02	1.16E-02	1.61E-02	5.20E-01	3.52E-02	4.31E-03	6.78E-01
LM21	(1→4)-β-D-(galacto)(gluco)mannan	1.55E-01	7.40E-01	3.64E-01	6.65E-01	1.70E-05	No data	No data	No data	No data	No data
LM25	Xyloglucan / unsubstituted β-D-glucan	No data	No data	No data	No data	No data	5.40E-01	1.75E-03	8.08E-01	4.68E-01	4.77E-01
Glycoprotein											
JIM20	Extensin	8.06E-01	4.86E-01	2.09E-01	1.86E-02	6.27E-04	2.69E-01	3.06E-01	4.77E-01	1.47E-01	3.92E-01
LM1	Extensin	3.84E-01	7.07E-01	9.44E-01	2.80E-01	6.41E-04	1.97E-01	4.75E-01	5.40E-01	5.13E-01	1.75E-03
LM2	AGP, β-linked GlcA	No data	No data	No data	No data	No data	4.08E-01	2.42E-02	1.33E-03	5.80E-02	7.87E-02

Table 3.10 Pairwise test between potato groups for each antibody employed in CoMPP. The test was carried out with the non-parametric Wilcoxon test and the parametric t -test. In yellow are the samples with a significant difference in the respective antibody.

Cell wall components		Conventional system			Organic system		
		Wilcoxon rank sum test, p-value					
		Res vs Int	Res vs Sus	Sus vs Int	Res vs Int	Res vs Sus	Sus vs Int
Pectin							
JIM5	HG partially/de-esterified	4.00E-02	4.00E-02	5.00E-01	-	-	-
JIM7	HG partially esterified	-	-	-	3.30E-03	8.68E-02	2.34E-01
LM18	HG partially/de-esterified	-	-	-	No data	No data	No data
LM19	HG partially/de-esterified	7.10E-02	1.80E-02	9.22E-01	No data	No data	No data
LM5	(1→4)-β-D-galactan	8.15E-01	1.07E-01	5.90E-02	-	-	-
LM6	(1→5)-α-L-arabinan	4.58E-03	7.20E-04	8.37E-03	8.50E-03	4.95E-01	4.95E-01
Hemicellulose							
LM10	(1→4)-β-D-xylan	1.80E-04	1.80E-04	1.70E-01	3.00E-03	3.00E-03	7.25E-01
LM11	(1→4)-β-D-xylan/arabinoxylan	8.90E-04	8.90E-04	3.71E-02	1.90E-02	1.90E-02	8.00E-01
LM15	Xyloglucan (XXXG motif)	8.78E-01	2.40E-02	2.20E-02	-	-	-
LM21	(1→4)-β-D-(galacto)(gluco)mannan	2.84E-03	7.10E-04	1.10E-03	No data	No data	No data
LM25	Xyloglucan / unsubstituted β-D-glucan	No data	No data	No data	-	-	-
Glycoprotein							
JIM20	Extensin	8.90E-03	2.60E-03	3.35E-02	-	-	-
LM1	Extensin	7.00E-03	4.60E-03	2.63E-02	5.20E-02	3.00E-03	3.20E-02
LM2	AGP, β-linked GlcA	No data	No data	No data	-	-	-

In summary, different cell wall composition were observed between the tolerant and susceptible varieties to *P. infestans*. In some components these differences depended on the farming system where plants were growing, in other, this difference were consistent in both systems. The resistant varieties were characterized for containing more arabinan, xylan, xyloglucan, and mannan, and extensin; and less partially/de-esterified pectin (**Table 3.11**).

Table 3.11 Pre-existing composition of the cell wall in the tolerant varieties detected by CoMPP. Some of this composition depended on the farming system where plants were growing, conventional (C) or organic (O).

COMPONENT	SPECIFC COMPONENTS	ANTIBODIES	FARMING SYSTEM
Pectin	More Arabinan	LM6	C
	Less HG partially/de-esterified	JIM5 / LM19	C / C
Hemicellulose	More xylan	LM10 / LM11	C&O / C&O
	More xyloglucan	LM15	C
	More Manan	LM21	C
Glycoprotein	More extensin	LM1 / JIM20	C&O / C

3.4. Discussion

This work has shown for the first time using CoMPP that there are differences in the baseline composition of the leaf cell wall between ten potato varieties grown in the field and that some of those differences correlate with the level of resistance to late blight caused by *P. infestans*, suggesting that the "pre-existing" composition and possibly structure of the cell wall could be an important factor affecting the outcome of pathogen attack.

Using a detached leaf infection assay with *P. infestans*, the potato varieties could be classified into those with resistant, susceptible and with intermediate responses. The most resistant varieties were Sarpo Mira and Sarpo Shona. Although after five days post-infection both varieties showed few visible symptoms, Sarpo Mira presented a less visible response compared to Sarpo Shona after 2 days. It is well known there is an incompatibility between Sarpo Mira and *P. infestans*, with this commercial variety created by conventional breeding having resistance associated with at least 5 durable R genes (R3a, R3b, R4, *Rpi-Smira1* and *Rpi-Smira2*) (Rietman, 2011). Although Sarpo Mira performs well under *P. infestans* infection, it has a very limited marketplace because its agronomic traits are not highly desirable for the current market, due to its taste or cooking properties (Kessel *et al*., 2018, Nuijten *et al*., 2018). This also occurs with wild potato varieties that possess resistance to biotic factors, and so attempts are often made to introgress the corresponding resistance genes into commercial varieties to generate new varieties combining resistance with desirable traits (Gaiero, Speranza, and de Jong, 2018).

The varieties with an intermediate response to *P. infestans* infection showed a highly variable level of resistance. An interesting example is shown by Toluca. By observing the progress of the infection, the reaction of this plant was not as accentuated after 4 to 5 days post infection compared with the other "intermediate" varieties. This response provoked some confusion in its classification, although by observing the reaction on the second day post infection, there was very clear symptoms of

infection shown as brown spots around the leaves, while the two resistant varieties did not show this response. When Toluca was considered as a resistant instead of an intermediate variety, the PCA showed Toluca samples were not in the same quadrant as Sarpo Mira and Sarpo Shona, making the resistant group more dispersed and ill-defined. For this reason, Toluca was classified as an intermediate variety.

As expected, technical replicates in the CoMPP analysis in general showed higher agreement in abundance compared with biological replicates (from different blocks). However, for a minority of samples, the technical replicates had a very low correlation. As described in material and methods, samples were stored at -80°C after the AIR extraction and before the cell wall extraction. Although samples were wrapped with parafilm around their lids, some moisture entered inside the tubes causing parts of the sample to adhere or clump. This potentially led to epitopes not being exposed homogeneously and could cause the high variation between technical replicates observed in some samples. In future, it will be necessary to store the samples at room temperature after the AIR extraction. Nevertheless, the majority of the samples had a good correlation between technical replicates and could be used for the rest of the analysis.

As observed in the PCAs, samples from different blocks do not form separate groups, although for some varieties the samples from the same block tend to cluster together, meaning that a slight difference exists in the composition of the cell wall among varieties that grew in different blocks. However, this difference among same samples growing in the same blocks was not systematic. Therefore, since there were not biological replicates from within the same block, samples from the two blocks were considered as biological replicates. The cause of the observed differences could be that, in the field, the nutrients composition of the soil in each block could be subtly different. It was previously shown that differences in the environment can lead to a difference in the composition of

the cell wall (Piro *et al*., 2003). Such differences are clearly accentuated when we observe the distribution of the samples that grew in different farming systems.

As observed, the cell wall composition (pectin, hemicellulose, and glycoprotein) can vary under different farming systems, conventional and organic. These differences in the abundance of specific cell wall components may be related to differences in nutrient availability between farming systems. Although similar amount of N content was intended to apply in both farming systems, the nitrogen from the farmyard manure needs to be mineralized in form of ammonium or nitrate to be absorbed by plants. Such mineralization was observed to be no more than 70% from pig manure and 55% from hen manure of the total N contain (Bohgal *et al*., 2016, Eghball, *et al*., 2002).This illustrates variety-specific effects of the farming system on cell wall composition..

In *Vitis vinifera*, the relationship of nitrogen (N), phosphorus (P), and sulphur (S) limitation with cell wall composition was evaluated in callus tissue (Fernandes, Goulao, and Amâncio, 2016). The results showed that the number of genes with an altered expression was higher in N, followed by P and S deprivation. Deprivation of N had the greatest influence on callus morphology and cell wall composition by reducing the cellulose content and producing altered patterns of pectin methyl-esterification. This alteration was correlated with the downregulation of pectin methyl-esterases (PME) and upregulation of PME inhibitors. Other enzymes families, including glycosyl hydrolase family 9C (GH9C) and xyloglucan transglycosylase/hydrolase (XTHs), were also downregulated under N-deficiency (Fernandes, Goulao, and Amâncio, 2016). In rice, higher nitrogen fertilizer application reduces the lignin (and to a lesser extent cellulose) content of the secondary cell wall, reducing stem mechanical strength and leading to more lodging (Zhang *et al*., 2017c). It is therefore to be expected that in different farming systems, where differences in nutrient availability exist, variation in the cell wall composition will be observed. As noted in *Miscanthus*, cell wall composition is determined by genetic and environmental factors. A high level of variation was observed in 15 *Miscanthus*

genotypes in terms of their overall levels of cellulose, hemicellulose, or lignin components, with the growth environment also playing a role (Hodgson *et al*., 2010). Another study highlighted the significant genotype by environment (G x E) interactions in cell wall composition and biomass quality in *Miscanthus* genotypes evaluated in six locations across Europe over three years (van der Weijde *et al*., 2017). This is consistent with our own observations of variety-specific response to farming system.

CoMPP revealed that antibodies detecting hemicellulose (LM10 and LM11) and extensin glycoprotein (LM1) showed a significantly higher abundance in resistant compared with intermediate and susceptible varieties in both conventional and organic farming systems. Antibodies LM10 and LM11 both have (1→4)-β-D-xylan as the epitope but differ in their specificity. LM10 recognizes unsubstituted xylan or xylan with low levels of backbone substitution, while LM11 can also recognize arabinoxylans (McCartney, Marcus, and Knox, 2005). Xylanases from different organisms are classified in two principal groups, namely the glycoside hydrolase families 10 and 11 (GH10 and GH11). GH10 xylanases can act on xylan with different modifications, being less specific compared to GH11 (Lai and Liou, 2018). The important role of the xylanases in the progression of infection for several fungal pathogens have been demonstrated. For example, the silencing of different xylanases from *Magnaporthe oryzae* reduced the virulence in rice (Ngueyn *et al*., 2011). During the life cycle of the oomycete *Phytopthora parasitica*, four members of the GH10 family, including ppxn1 and ppxyn2, are expressed in different stages of the pathogen life cycle (Lai and Liou, 2018). Silencing of ppxn1 and ppxyn2 reduced the ability of the pathogen to infect tobacco and tomato plants (Lai and Liou, 2018). However, disruption of xylanase function does not always produce resistance in the host, implying a dependence on the characteristics of the pathosystem (Lai and Liou, 2018). In our potato varieties, the xylan in the hemicellulose component was significantly more abundant in the resistant varieties than in the susceptible or intermediate varieties, suggesting that xylans could create a structural barrier that is difficult to overcome by *P. infestans*, even after the secretion of xylanases.

LM1 binds extensin, specifically to a glycan epitope attached to this cell wall hydroxyproline-rich glycoprotein, HRGP (Smallwood *et al*., 1995). The role of HRGP in plant defence against pathogens has been widely reported (Esquerré-Tugayé and Mazau 1974, Esquerré-Tugayé and Lamport 1979, Esquerré-Tugayé *et al*., 1979, Deepak *et al*., 2010). For example, accumulation of extensins, including specific extensins harbouring tetra- and tri-arabinosides, was observed in the cell walls of melon plants after infection with the fungus *Colletotrichum lagenarium* as a defence response against pathogen attack (Esquerré-Tugayé and Mazau 1974). Additionally, a transgenic *Arabidopsis* line over-expressing the extensin gene *EXT1* showed less damage and bacterial load when infected with *Pseudomonas syringae* compared with the wild-type (Wei and Shirsat, 2006). The accumulation of extensins has been observed in the form of oxidatively cross-linked extensin subunits, with a role of fortifying the cell wall in response to pathogen attack (Deepak *et al*., 2010). The cross-linked network was observed to occur by intra and inter covalent cross-linking of tyrosine residues, involving extensin peroxidases (Mishler-Elmore *et al*., 2021). In *Arabidopsis,* extensin expression induced by *Xanthomonas campestris* infection was restricted to the infection site (Merkouropoulos and Shirsat, 2003). Since our resistant potato varieties had more extensin in their cell walls than the susceptible or intermediate varieties in both farming systems, this creates a stronger and effective barrier to help prevent *P. infestans* from penetrating the leaf cell wall during the earliest stages of infestation.

The further components that showed a significant difference according to the level of resistance in only the conventional system were detected with 6 antibodies. These antibodies were JIM5, LM19, and LM6 which detect pectin components, LM15 and LM21 which detect two hemicellulose components, and JIM20 which detects a glycoprotein component. It is possible that these differences were absent in the organic system due to differences in nutrient availability between the two farming systems. All of these components have been previously linked with resistance against pathogens in other pathosystems.

JIM5 and LM19 revealed a higher abundance of both partially or de-esterified homogalacturonan in susceptible compared with resistant potato varieties, with intermediate varieties showing a similar abundance to the susceptible varieties. While JIM5, LM18 and LM19 all detect partially or de-esterified homogalacturonans, they have different binding specificities, which may be because other domains, such as the degree of acetylation, that affect antibody binding (Verhertbruggen *et al*., 2009). It has been observed that the degree and pattern of methylesterification affects the resistance to pathogens (Lionetti *et al*., 2012; 2017). To some extent, cell walls with highly methyl-esterified pectin are protected against microbial enzymes including PGs (polygalacturonases) and PLs (pectate lyases) (Bellincampi, Cervone, and Lionetti, 2014). In potato, jasmonic acid modulates the degree of pectin methylesterification to protect pectin from degradation by the pectate lyases produced by the soft rot pathogen *Dickeya dadantii* (Taurino *et al*., 2014). Furthermore, higher levels of methylesterified pectin positively correlated with the resistance of potato tubers to *P. carotovorum*, of bean to *Colletotrichum lindemuthianum*, and of tomato to *Ralstonia solanacearum* (McMillan *et al*., 1993; Boudart *et al*., 1998, Wydra *et al*., 2006). It is therefore possible that the lower levels of partially or de-esterified homogalacturonans observed in the resistant varieties means there is a relatively higher level of methylesterified pectins, which could be contributing to the higher level of baseline resistance to *P. infestans*.

Another pectin component, (1→5)-α-L-arabinan, a linear side chain of pectins with 5 or 6 arabinose residues found in rhamnogalacturonans I (RG-I), was specifically recognized by LM6 (Willats, Marcus and Knox, 1998) and showed higher abundance in resistant compared with susceptible or intermediate varieties. *Arabidopsis* mur8-I mutants, with reduced RG I content and rhamnose, showed a substantial increase in penetration by the fungus *C. higginsianum* compared to the wild type (Engelsdorf, *et al*., 2017). In the current study, a high of arabinan may indicate a high level of RG-I in the potato cell wall, which could be associated with the resistance to *P. infestans* in potato. In addition, more RG-I could produce a high amount of RG-I fragments after infection. Indeed, potato

RG-I fragments was observed to elicit a defence response in tomato by increasing the amount of beta 1,3 -glucanase, chitinase, and peroxidase (Jimenez-Maldonado, *et al*., 2018).

For the two hemicellulose components, LM21 recognizes (1→4)-β-D-mannan polysaccharides in mannan, glucomannan and galactomannan. Recently, mannan oligosaccharides (MOS) were recognized as a novel DAMP which triggered a defence response in rice and tobacco (Zhang *et al*., 2019), conferring resistance against *Xanthomonas oryzae* and *Phytophthora nicotianae*. This was associated with an increase in the generation Ca^{+2} and ROS, stomatal closure, and cell death. Moreover, expression of defence-related genes was produced after treatment with MOs (Zhang *et al*., 2019). LM15 recognizes xyloglucan, the most abundant hemicellulose in dicotyledons, specifically with the XXXG motif, where X represent a glucosyl residue attached to xylose and G is an unbranched glycosil residue (Marcus *et al*., 2008). This component has also been recognized as a novel DAMP in *Vitis vinifera* and *A. thaliana* (Claverie *et al*., 2018). It produced resistance to *Botritys cinera* and *Hyaloperonospora arabidopsidis* through the activation of the MAPK cascade and the transcription of a pathogenesis related 1 (PR1) gene, PR2, a phytoalexin deficient 3 gene, and a plant defensin 1.2 gene 48 hours post-infection, although ROS production was not detected (Claverie *et al*., 2018). In the resistant potato varieties, it is possible that the higher abundance of these mannan oligosaccharides and xyloglucans could boost the potential for accumulation of these novel DAMPs in response to pathogen attack, thus contributing to the enhanced resistance to *P. infestans*. However, while OGAs are recognised by WAK receptors, the perception mechanisms for other more novel types of DAMP have not been characterised.

Beyond the specific components investigated here using CoMPP, previous studies have shown that there are widespread changes in the cell wall of leaves of potato varieties responding to *P. infestans*. For example, the integration of transcriptomic and metabolomic data identified several changes that in the cell wall composition, with resistant varieties showing increased levels of metabolite

biosynthetic genes associated with secondary cell wall thickening, including extensin, expansin, and pectinesterases; a high expression of WAK transcripts was also observed, where WAK is a cytoplasmic receptor that detects pectin fragments (DAMPs) and emits signals into the cytoplasm to trigger a resistance response in plants (Yogendra and Kushlappa, 2016). Furthermore, the plant cell wall can be also modified by the action of pathogen enzymes. Analysis of the transcriptome combined with the apoplastic proteome of resistant and susceptible potato varieties detected candidate targets of *P. infestans* effectors, including pectinesterase-2, an enzyme that releases methoxyl groups from the galacturonic acid residues of pectins (Ali *et al*., 2014).

It was previously reported that some components of the potato cell wall from tissues other than leaves are also modulated during *P. infestans* infection. Previous analyses of potato tubers have shown a change in the cell wall composition under *P. infestans* attack. After 4 days post-infection, a susceptible variety showed less content of pectin in infected discs, which was related to the production of pectinolytic enzymes produced by *P. infestans* (Friend and Knee, 1969). Specifically, galactanase has been observed to be secreted by *P infestans* in liquid culture and alteration of the cell wall at an early stage would be the dissolution of galactan from pectin (Jarvis, Threlfall, and Friend, 1981). However, galactanase from *P. infestans* also removes arabinogalactan. In our data, no difference in the abundance of galactan was detected between the susceptible and resistant varieties. The evaluated resistant leaves, however, contained more arabinan, which may act as a protective barrier in this tissue. Another important cell wall component that was abundant in tuber discs infected with *P infestans* was lignin, more in the resistant than in the susceptible varieties. Lignin was therefore considered to be an important characteristic of resistant varieties (Ampomah and Friend, 1988). Although no antibodies were used in this chapter to detect lignin, it is an important component that should be evaluated in the future. Transcriptomic analysis of potato leaf and tuber detected high upregulation of extensin genes in leaves after 24 h.p.i. in both leaf and root

(Gao and Dradeen, 2016). Extensin levels were higher in our resistant leaves, indicating this tissue has been protected since before infection by this cell wall protein.

3.4.1. Conclusions and future work

The results have shown that there are differences in the cell wall composition between potato varieties that can be correlated with the degree of resistance to infection with *P. infestans*. While the CoMPP analyses were performed in plant leaf material collected from the field, where pests and pathogens are likely to exist, this suggests that there may be baseline differences in cell wall composition that can facilitate the defence response when the pathogen appears in the field, which could be validated in future studies with potato varieties grown in controlled conditions.

Furthermore, these results build on the very limited literature for the role of the cell wall in potato defence responses (Taurino *et al*., 2014). I hypothesise that a "cell wall ideotype" for resistance to *P. infestans* would confer improved resistance through several intersecting and complementary mechanisms, including mechanical strengthening of the cell wall, resistance to degradation by pathogen-derived CWDEs and enhanced potential for production of DAMPs in response to pathogen attack. Key features could include a cell wall with a high level of methylesterified and RG I/arabinan pectins, high levels of several hemicellulose components including (1→4)-β-D-xylans, mannan oligosaccharides, and xyloglucans, and high levels of glycoproteins including extensins.

Further analyses are necessary to explore deeply if the composition and structure of the leaf cell wall could be a causal factor in the resistance of potato varieties against *P. infestans*. This could potentially contribute a robust alternative form of resistance independent from and complementing the role of R genes. It will be necessary to analyse the abundance of cell wall components in different varieties after as well as prior to infection, to dissect the static versus dynamic role of cell wall composition in the resistance response. CoMPP analysis necessitates disruption of the cell to extract cell wall components, thus preventing analysis of the cell wall structure. CoMPP may therefore be

complemented by using the same antibodies for an *in-situ* analysis of plant cell wall components to visually observe the organization of the cell wall in the different varieties and the reorganization of the cell wall in response to infection. Furthermore, mutants with altered cell wall composition or transient expression assays, e.g. in *Nicotiana benthamiana*, could be used to confirm the roles played by specific components in the resistance response.

CHAPTER 4: Transcriptomic response of potato cell wall to *P. infestans* infection.

4.1. Introduction

Among the most important biotic factors causing reduced yields in potatoes is *P. infestans.* During 2010 and 2014, the infection with this pathogen was estimated to have resulted in a yield loss of 3.24% in Northwest Europe, 8.08% in the North of India, 4.09% in West Asia and North Africa, and 4.18% in Sub-Saharan Africa (Savary *et al*., 2019). To manage the disease, from the total cost of the production, between 10 and 20% is invested in chemical pesticides (Haverkort *et al*., 2009). This pathogen is a serious problem since its mobile zoospores can invade leaves, stems, and potato tubers, and after two weeks of the initial infection, they can destroy an entire crop field (Fry *et al*., 2015). As a consequence, different strategies have been implemented to combat this disease, as more sustainable alternatives to chemical pesticides. This involves the use of resistant potato commercial varieties, like Sarpo Mira, although it is not highly desirable for the current market (Kessel *et al*., 2018; Nuijten *et al*., 2018), or the identification of resistance genes, which are usually found within non-commercial potato genotypes, to introduce them into commercial varieties.

Quantitative trait loci (QTLs) for the resistance phenotype against *P. infestans* have been identified in potato, containing R gene clusters (Tan *et al*., 2008) or defence gene clusters (Trognitz *et al*. 2002). In this last case, the clusters can be composed of genes involved in the phenylpropanoid pathway, such as phenylalanine ammonium lyase, chalcone isomerase, chalcone synthase, WRKY genes, osmotin, and a cytochrome P450 (Trognitz *et al*. 2002). Nevertheless, much effort is still focused on developing qualitative resistance through the identification of R genes. A growing number of studies have used genomic association analysis to investigate the genetic basis of Quantitative Disease Resistance (Alvarez *et al*., 2017; Juyo *et al*., 2019). Progress in breeding the next generation of potato cultivars with enhanced resistance to blight will depend on understanding the components of resistance at

many different scales (Willocquet *et al*. 2017). The plant cell wall is the first barrier that pathogens must confront to successfully infect their host. In response to the infection, plants can modify their cell wall by strengthening their structure and accumulating antimicrobials. For example, callose is a β-(1,3)-D-glucan polymer that is deposited at the site of infection as a barrier after pathogen attack, forming a papilla or cell wall apposition. Another example is the reinforcement with hydroxyproline-rich glycoprotein (HRGP) observed in the intercellular space and at papillae to strengthen the cell wall and arrest pathogen entry. The high concentration of HRGP conferred resistance in some crops, such as tomato and sorghum (Benhamou *et al*., 1991; Basavaraju *et al*., 2009; Deepak, *et al*., 2010). Change in the expression of genes involved in cell wall modification has also been reported in potato after *P. infestans* infection. After two days post-infection, a high expression of genes encoding pectinesterases and xyloglucan endotransglucosylase-hydrolases (XTHs), also genes involved in the cell wall reinforcement, such as extensin, in addition to expansin, was observed in two resistant potato genotypes (Yogendra and Kushalappa, 2016). However, a more detailed description of these changes in the potato cell wall is needed to describe how they might contribute to the resistance response against *P. infestans*.

This chapter aims to identify in potato which dynamic changes in the cell wall or signals from these changes may be produced after *P. infestans* infection and may be associated with a more resistant phenotype. To address this aim, the transcriptomic response of late blight-infected leaves will be compared between resistant and susceptible potato varieties.

4.2. Material and methods

4.2.1. Biological material

The potatoes varieties employed were Sarpo Mira, Sarpo Shona and Duke of York. Sarpo Mira and Sarpo Shona are the most resistant and Duke of York one of the most susceptible varieties to *P. infestans* among more than 100 potato varieties listed in the Agriculture and Horticulture Development Board (AHDB) Potato Variety Database (varieties.adhb.org.uk/varieties). Further, among 24 potato varieties previously tested in a detached infection assay, Sarpo Mira and Sarpo Shona were the most resistant to *P. infestans* infection (described in chapter 3, **Figure 3.6, Table 3.4**), while Duke of York showed a high susceptibility in the field in 2019 (**Figure 4.1**) The plants were grown in a greenhouse with a temperature fluctuation of 20-25°C and a day/night cycle of 16/8 hours for four months before being infected.

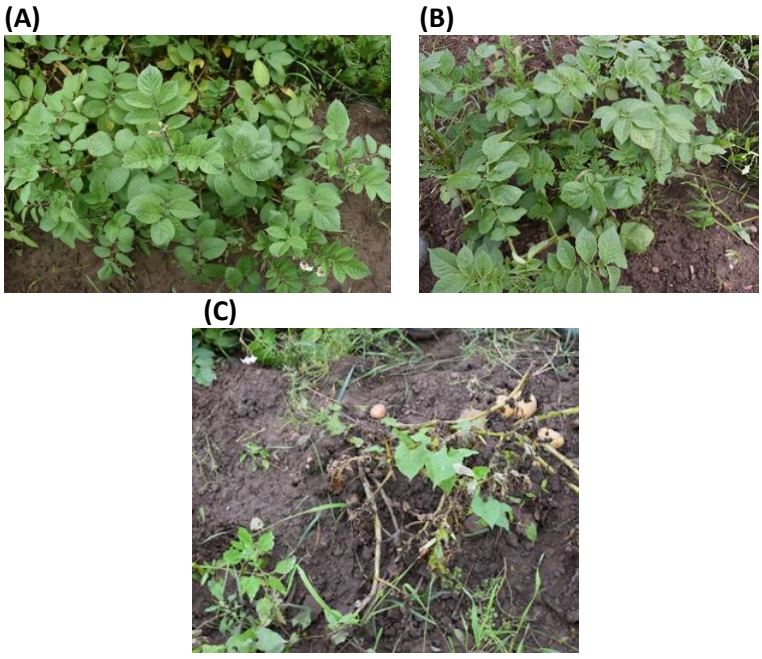


Figure 4.1 Potato varieties growing in Newcastle in 2019. Mixed infection of early and late blight was observed in the field where the three varieties Sarpo Mira (a), Sarpo Shona (b), and Duke of York (c) grew. Clearly, the susceptible variety Duke of York was more affected and almost destroyed, while the resistant varieties, Sarpo Shona and Sarpo Mira showed almost no visible symptoms.

P. infestans strain 88069 carrying the TdT10 fluorescent protein (tomato-red fluorescent) was provided by the James Hutton Institute. It was cultured in Rye B agar medium (Organic rye grain, Bactoagar, sucrose, and B-sorbitol), which contained cut potato leaves, at 18°C in the dark for 2 weeks. To ensure the reactivation of *P. infestans*, two successive cultures were made before collecting them for the infection. After this period, mycelia were collected directly from the agar plates with the help of a sterile L-shaped cell spreader and sterile distilled water, which later was placed in a 15mL falcon and incubated at 4°C in the dark for one hour to induce zoospore release. Zoospores were counted under a microscope using a Neubauer camera, and the zoospore solution was diluted to a concentration of $10x10^5$spores per mL to infect potato leaves in a detached infection assay.

4.2.2. Experimental design and detached infection assay

Composite leaves from three individual plants of each variety were cut from the plant for the detached infection assay, as in Chapter 4. A first set of composite leaves was immediately stored at -80°C without any inoculation (Control). A second set of composite leaves were inoculated with water (mock) and incubated for 24 hours (Mock-24), or 48 hours (Mock-48). A third set of composite leaves was infected by droplet-inoculation with $10x10^5$spores/mL of *P. infestans* zoospores and incubated for 24 or 48 hours. Due to the size of the leaflets, each one contained a different number of droplets, but there was a minimum of 4 droplets per leaflet. After 24h or 28h of incubation, samples were collected and frozen in liquid nitrogen. For infected leaves, cork borers (number 3) were used to collect samples from the infected (Inf.) and outside the infected (Outinf.) areas separately. Therefore, from the infected leaves there were the following samples: inside-infected area at 24 hours (Inf-24) and at 48 hours (Inf-48) post-infection and outside-infected area at 24 hours (OutInf-24) and 48 hours (OutInf-48) post-infection. Per treatment (Control, mock, infection) or region (inside and outside the infected area) there were 3 biological replicates (plants) (**Figure 4.2**). After infection, the diameters of the lesions on the infected plants of each variety were measured.

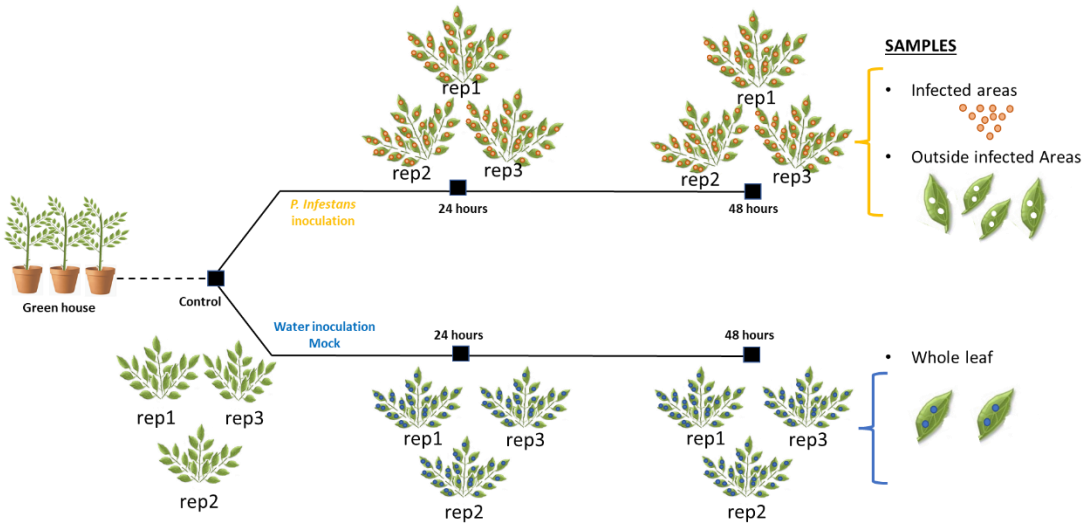


Figure 4.2 Experimental design for infection with *P. infestans*. Representation of the treatments applied to one potato variety during the detached infection assay. The same treatment was applied to the other two potato varieties.

4.2.3. Callose staining

Samples from inside the infected area of the leaves were collected at 24 hours post-infection (h.p.i) and incubated in 98% ethanol in the dark for 2 weeks to obtain decolored leaves. Samples were then double stained with aniline blue and calcofluor to visualize the accumulation of callose against the infection with *P. infestans*. The protocol was followed as described by Ton and Mauch-Mani, 2004, as in Chapter 4. The stained samples were visualized under a UV light microscope equipped with a digital camera at 20X resolution and collected with the GXCAPTURE software. Image analysis and callose measurement was performed using ImageJ 1.53. The percentage of callose formed was calculated with the following formula:

$$\%\ of\ callose = \frac{Area\ of\ callose}{Total\ image\ area} \times 100$$

The percentage of callose deposition was calculated in each image. Then all the percentages obtained from plants of same variety were averaged to obtain the percentage of callose formation per variety.

4.2.4. RNA extraction and transcriptome sequencing.

Total RNA was extracted from leaf samples using TRI®Reagent (Sigma) according to the manufacturer protocol (**Appendix-Protocol 4.1**). The RNA integrity was observed by electrophoresis on a 1% agarose gel stained with GelRed® (Biotium) and the concentration of each sample was measured with the NanoDrop ™ 1000 spectrophotometer. Samples were sent to Novogene Company (Cambridge) to determine their purity and to prepare paired-end RNA-Seq libraries for sequencing. The library preparation steps involved the isolation of mRNAs using poly-T fragments attached to magnetic beads, fragmentation, first and second strand cDNA synthesis using random hexamer primers, end repair, A-tailing in the 3' ends, sequencing adapter ligation, size selection, amplification, and purification, as specified in the provided Novogene report (**Figure 4.3**). Then, the paired-end libraries with fragments 150nt in length were sequenced with the Illumina® HiSeq HWI-ST1276 platform.

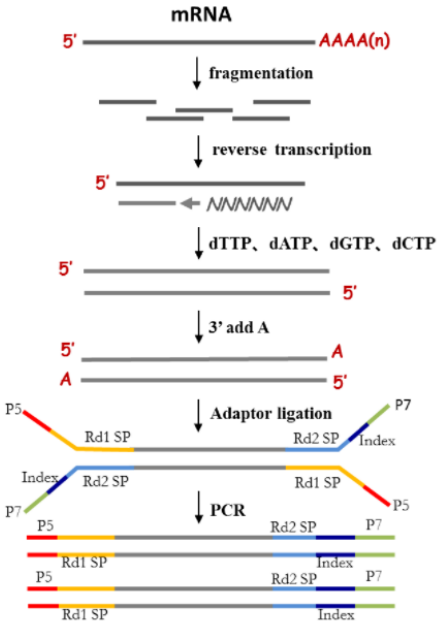


Figure 4.3 Library preparation for paired-end RNA-seq sequencing. Reproduced from the Novogene sample report.

4.2.5. Bioinformatic analysis

4.2.5.1. Read trimming and mapping

Read quality of each library was observed with FASTQC v.11.19 (bioinformatics.babraham.ac.uk/projects/fastqc/) and reads were trimmed with TrimGalore v.6.5 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Trimming involved base-quality trimming, where low quality reads with a Phred value less than 28 were discarded, and Illumina adapter trimming. Reads with length less than 20nt after the 2 previous steps were discarded. High quality trimmed reads were mapped to the reference potato genome v6.1 (Pham *et al.*, 2020) downloaded from SpudDB (http://spuddb.uga.edu/), with STAR v.2.7.2.b (Dobin *et al.*, 2013). Before mapping, index files were built for the potato genome with the option –runMode genomeGenerate and using the gff3 annotation file. For mapping, the option –quantMode TranscriptomeSAM was used to align the reads to the genome. The number of counts per gene was obtained with HTSeq v.0.11.0 (Anders *et al.*, 2015) with the htseq-count script and the options --mode=union and --nonunique=none. The first option indicates the mode of counting reads by the program, in this case the union of a set of reads that map to a gene will be considered as part of the gene. The second option specifies to not count ambiguous reads that map to several positions in the genome.

4.2.5.2. Normalization and DE analysis

Gene count normalization and differential expression analysis were carried out with the DESeq2 package v1.26.0 (Love *et al.*, 2014) using the R statistical software v3.6.3 within Rstudio v.1.2.1335. Normalization was performed with the DESeq dispersion function, and the Wald test was applied to determine a significant differential expression of each gene between different pairs of samples. Genes with a Benjamini-Hochberg adjusted P-value below 0.05 and with an absolute shrunken log 2-fold change (LFC) >= 1 were considered as differentially expressed genes (DEGs). To corroborate if biological replicates are actual replicates, sample quality control was carried out by a Principal

Component Analysis of the 1,000 genes whose normalized count showed the most variability across all the libraries using the function plotPCA from the DESeq2 package.

4.2.5.3. Gene ontology analysis

The gene ontology (GO) terms associated with the annotated genes were downloaded from SpudDB (http://spuddb.uga.edu/GO). The GO enrichment analysis of the DEGs was performed with the g:profiler2 R package v0.2.0 (Raudvere *et al.*, 2019) and the function gost(). Enriched GO terms were considered if the Bonferroni-corrected *P*-values from the hypergeometric test were lower than 0.05.

4.2.5.4. Selection of cell wall genes

To select genes related to the plant cell wall, several approaches were used and the gene lists were combined. First, DEGs inside the enriched GO terms specifically related to cell wall modification were selected. Second, DEGs inside related GO terms involving either the apoplast or the extracellular region were selected if their functional annotation suggested a direct involvement in cell wall modification. Thirdly, genes annotated with a function relating to cell wall modification were selected even if they were not annotated with any enriched wall-related GO term.

4.2.5.5. Selection of genes relating to defence against pathogens

This list of mainly receptor genes included DEGs that were part of the enriched GO terms identified in the infected area and related to the defence response to pathogens. In addition, by observing the gene function associated with each of the DEGs in the infected area, other receptor genes that were not annotated with any enriched GO term were also included. All these genes were classified as pattern recognition receptors (PRR) receptors, leucine-rich repeat receptor kinases (LRR-RKs) or leucine-rich repeats receptor-like kinases (LRR-(RLKs), receptor-like kinases (RLKs), resistant (R) genes, or receptors with an LRR domain that were co-receptors of PRR proteins, such as male discoverer 1-interacting receptor-like kinase 2 (MIK2). Genes encoding for pathogen-related genes

(PR-proteins) were also included because although they are not receptors, their expression increases after pathogen attack to degrade pathogen structures.

4.3. Results

To determine if the resistance of potato against *P. infestans* is associated with changes in the cell wall, the transcriptomic response of two resistant (Sarpo Mira and Sarpo Shona) and one susceptible (Duke of York) varieties infected with *P. infestans* were analysed. In order to visually determine cell wall change, callose deposition was also observed at an early time point of 24 hours post-infection.

4.3.1. Response to infection

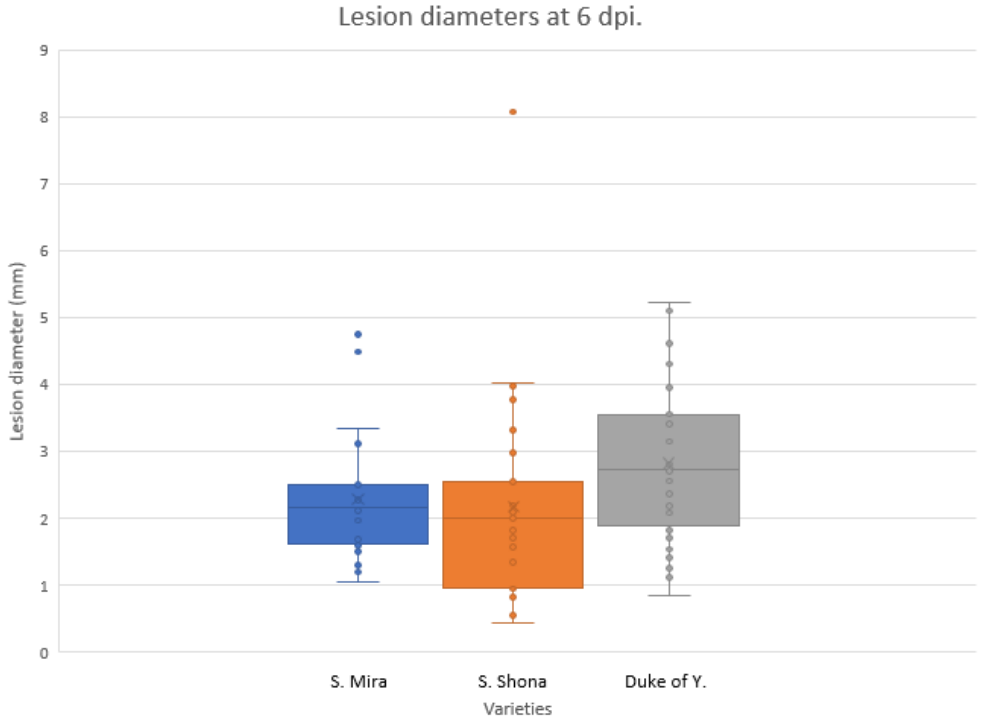
The reaction of the plant to *P. infestans* infection was observed after 24 and 48 h.p.i. in the detached infection assay. Sarpo Shona showed a clear reaction to the infection as dark necrosis spots at 24 h.p.i. (**Table 4.1B**), while in Duke of York this reaction began to be observed at 48 hours post-infection (**Table 4.1C**). For Sarpo Mira, it was difficult to determine if this reaction occurred at 24 h.p.i. since their leaves showed dark spots while they were growing in the greenhouse. However, at 48 h.p.i. this reaction was clearly observed (**Table 4.1A**).

Table 4.1 Potato leaves infected with *P. infestans* at 24 and 48 h.p.i. Reaction to the infection was observed as dark spots of necrosis in the infected areas of the leaves. The table also shows potato leaves before the infection (0 h.p.i).

0 h.p.i	24 h.p.i	48 h.p.i
(A) Reaction of Sarpo Mira		
		
(B) Reaction of Sarpo Shona.		
		
(C) Reaction of Duke of York.		
		

After 9 days post-infection with *P. infestans*, necrotic areas observed as irregular or circular brown lesions, were observed in all three varieties. The most affected variety was Duke of York, since its leaves turned yellow-brown compared with the two more resistant varieties, indicating that entire leaves were affected by the infection (**Table 4.2**).

Table 4.2 Potato leaves 9 days post-infection with *P. infestans*. The table shows the distribution of the lesion diameter in mm found in Sarpo Mira, Sarpo Shona and Duke of York. The infected composite leaves are also shown after 9 days of infection in the three potato varieties.

9 days post-infection	
Damage score	
Infected leaves	

4.3.2. Callose deposition

After 24 hours post-infection, visual inspection showed Duke of York had substantially less callose deposition compared with the two resistant varieties. Interestingly, in the detached infection assay,

Sarpo Shona showed earlier visible symptoms of infection than the susceptible Duke of York, whose reaction to the infection was observed at 48 h.p.i. For the two resistant varieties, Sarpo Mira had a higher accumulation of callose than Sarpo Shona (**Table 4.3**).

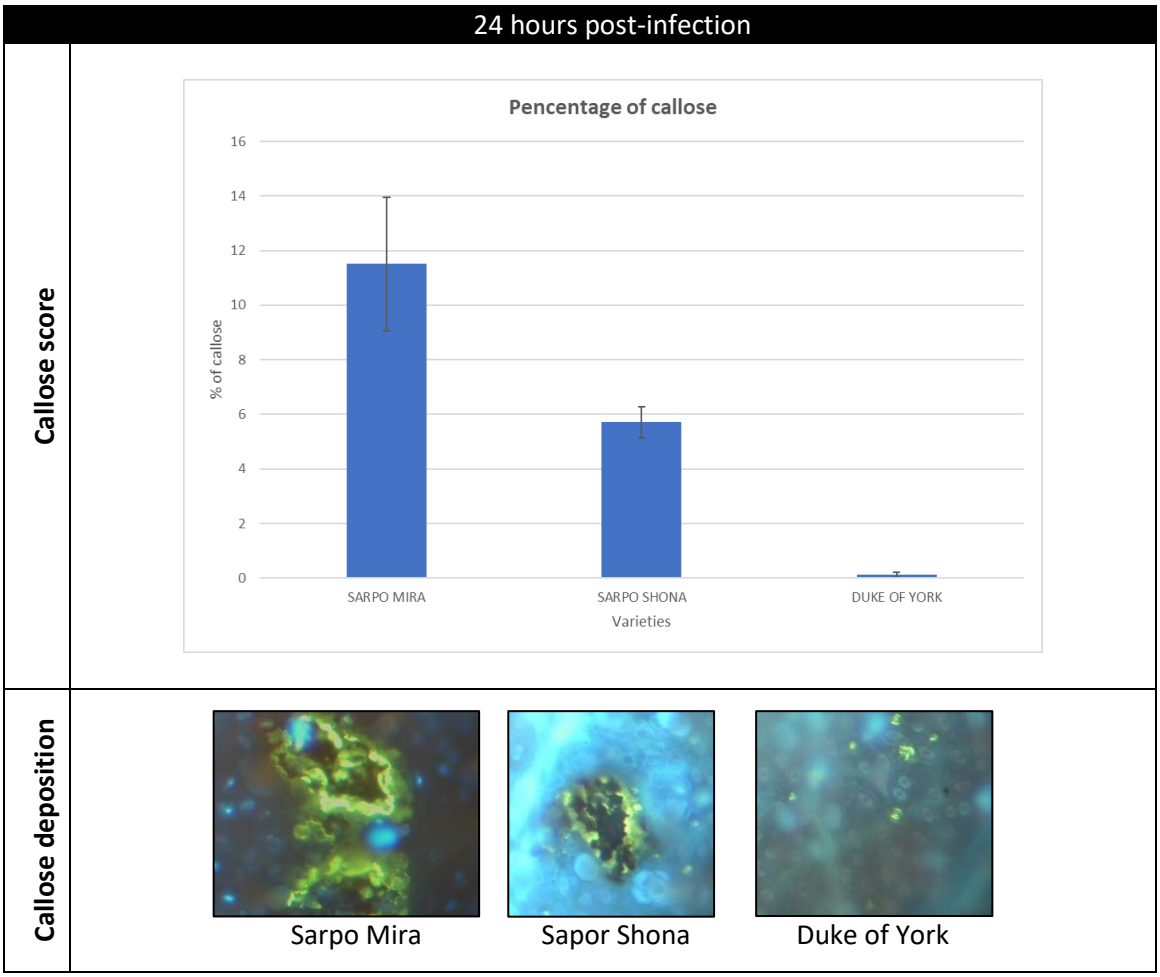


Table 4.3 Callose deposition in potato leaves at 24 h.p.i with *P. infestans.* The table shows a bar plot with the percentage of callose observed in each variety. Each bar represents the mean of the percentage of callose deposition per variety with the standard error shown by the error bars. The table also shows the callose deposition observed as green areas under a 20X resolution in each variety.

4.3.3. RNA quality for sequencing library preparation

Samples from 48 hours post-infection were selected to for RNA-sequencing because the signal of necrosis caused by the infection was observed in all three potato varieties at this time point, while changes in the cell wall at this time point in response to *P. infestans* infection have been previously reported (Yogendra and Kushalappa, 2016). These included the control, mock-48, Inf-48 and OutInf-

48 samples (**Figure 4.2**). Three biological replicates were collected for each inoculation and variety, giving a total of 36 samples to be sequenced from the three varieties (**Table 4.4**).

The quality of extracted RNA for each sample was observed by agarose gel and with the Agilent 2100 bioanalyzer at Novogene. The agarose gels revealed the 28S and 18S rRNA bands of each sample, indicating the extracted RNA was not degraded (**Figure 4.4**). The RNA Integrity Number (RIN) of the 36 samples were all around or above the quality threshold of 8 (7.5-9.4) (**Table 4.4**), and thus all samples were adequate for library construction and the RNA-sequencing.

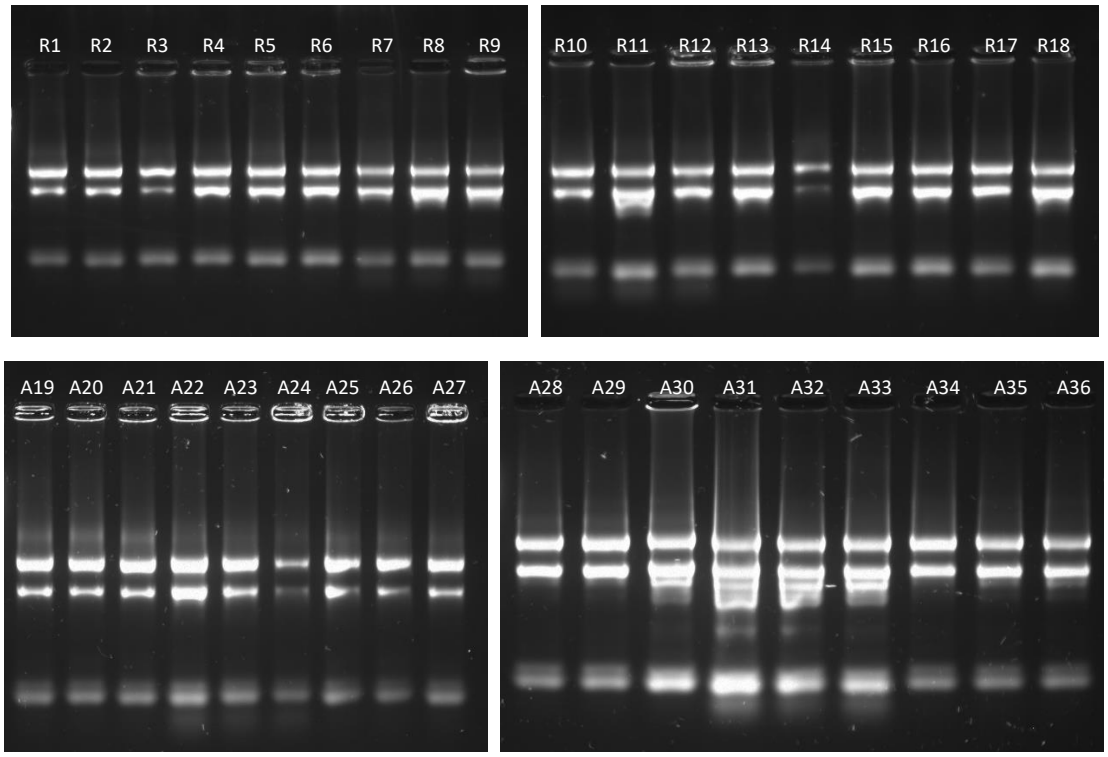


Figure 4.4 RNA integrity. Electrophoresis of the RNA samples in 1% agarose gel. The sample names in the gel correspond to the library names shown in **Table 4.4**.

Table 4.4 RIN values of the extracted RNA from the 36 samples. This table also shows the RNA concentration of each sample. The minimum RNA Integrity Number (RIN) obtained from all the samples was 7.5.

Variety	Treatment	Replicate	Library name	Concentration (ng/ul)	RIN
Sarpo Mira	Control	1	R1	90	9
Sarpo Mira	Control	2	R2	55	8.9
Sarpo Mira	Control	3	R3	77	8.9
Sarpo Mira	Outside of Infected area	1	R4	98	9.4
Sarpo Mira	Outside of Infected area	2	R5	58	9.4
Sarpo Mira	Outside of Infected area	3	R6	51	9.1
Duke	Control	1	R7	49	7.9
Duke	Control	2	R8	56	7.9
Duke	Control	3	R9	64	7.8
Duke	Outside of Infected area	1	R10	62	8.8
Duke	Outside of Infected area	2	R11	88	8.3
Duke	Outside of Infected area	3	R12	69	8.2
Sarpo Shona	Control	1	R13	88	8.7
Sarpo Shona	Control	2	R14	82	8.8
Sarpo Shona	Control	3	R15	94	8.4
Sarpo Shona	Outside of Infected area	1	R16	72	8.7
Sarpo Shona	Outside of Infected area	2	R17	89	9.2
Sarpo Shona	Outside of Infected area	3	R18	83	8.7
Sarpo Mira	Infected area	1	A19	47	8.4
Sarpo Mira	Infected area	2	A20	58	8.1
Sarpo Mira	Infected area	3	A21	72	9
Duke	Infected area	1	A22	68	8.8
Duke	Infected area	2	A23	74	8
Duke	Infected area	3	A24	88	9.2
Sarpo Shona	Infected area	1	A25	61	8.2
Sarpo Shona	Infected area	2	A26	67	8.5
Sarpo Shona	Infected area	3	A27	60	7.9
Sarpo Mira	Mock	1	A28	49	8.2
Sarpo Mira	Mock	2	A29	54	8.8
Sarpo Mira	Mock	3	A30	49	8.8
Duke	Mock	1	A31	54	7.7
Duke	Mock	2	A32	62	7.5
Duke	Mock	3	A33	57	7.9
Sarpo Shona	Mock	1	A34	51	8.1
Sarpo Shona	Mock	2	A35	70	8.7
Sarpo Shona	Mock	3	A36	55	8.3

4.3.4. Sample QC and distribution

From the 36 RNA-seq libraries, the minimum Phred quality value of the reads was 26 (**Appendix-Figure 4.1**), which indicated that all reads were acceptable for further analysis. However, to have high confidence reads, only those with a Phred value above 28 were kept. Most of the reads in each library were retained and employed in the following analysis, with the minimum percentage of reads

kept after trimming and mapping to the reference potato genome being 84.76% (**Appendix-Table 4.1**).

After read count normalization with DESeq2, principal component analysis (PCA) was carried out on the top 1,000 genes showing the most variation across samples. The two principal components (PCs) explained most of the variation across the samples, with 51% and 26% of variance explained by PC1 and PC2, respectively. The proximity of biological replicate samples indicated a very low intra-replicate variance and therefore the response of each replicate was very similar within each treatment and variety. PC1 captured the major variation according to treatment, with control samples distributed wide apart from the mock and the infected samples. This indicated more similarity in the transcriptomic response between the mock and infected samples compared with the control samples. Inoculated samples (with water or pathogen) were exposed to the same conditions during the period of treatment, involving incubation for 48 hours in the dark after mechanical stress caused by cutting each composite leaf from the plant, with the primary difference then being infection with either water or *P. infestans* zoospores. PC2 very clearly distinguishes samples from the two resistant varieties, Sarpo Mira and Sarpo Shona, versus the susceptible variety, Duke of York, indicating substantial differences in the transcriptome of more resistant varieties compared with the susceptible variety across all treatments (**Figure 4.5**).

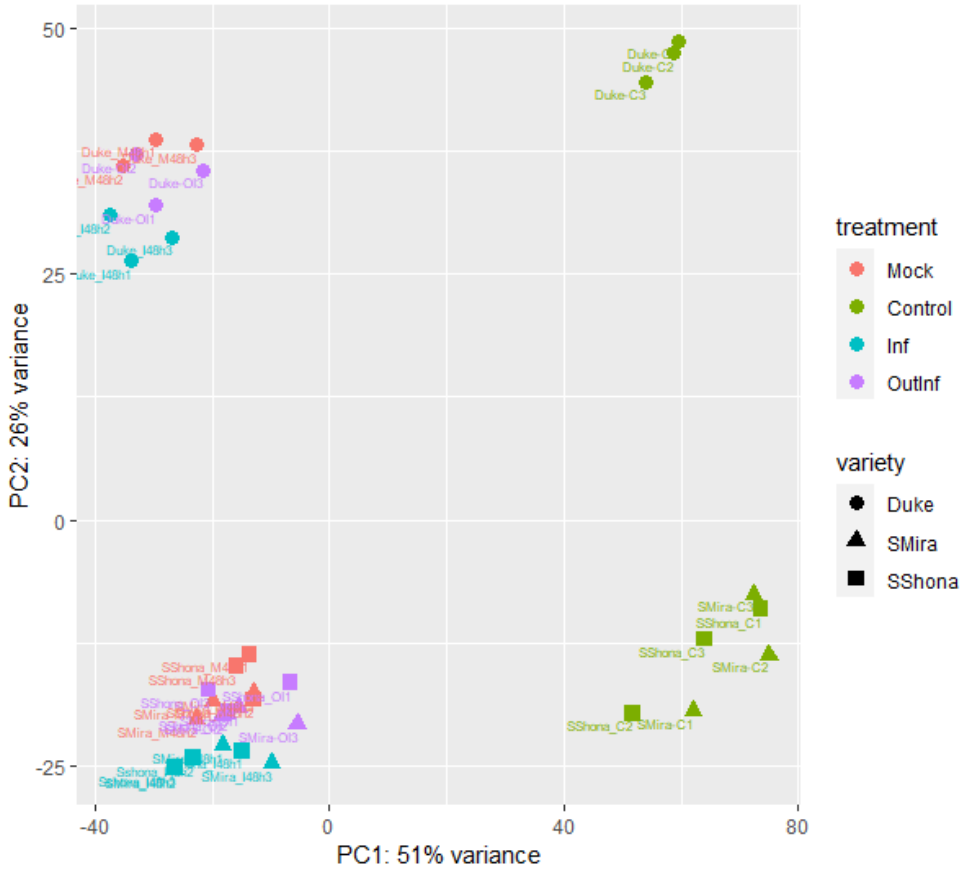


Figure 4.5 Sample distribution according to the PCA plot.

4.3.5. Differential expression analysis

4.3.5.1. DEGs in the infected leaves and mock samples.

To identify DEGs after 48 hours post-infection, expression within and outside the infected areas was compared with the expression in the mock samples (Inf-48 vs Mock-48 and OutInf-48 vs Mock-48, respectively). The reaction of the mock samples was also observed by comparing the expression of mock with the control samples (Mock-48 vs control).

At 48 hours post-infection, there was a much lower number of DEGs (up- or down- regulated) in the infected or outside the infected areas than in the mock for all three varieties (**Figure 4.6**). This suggested the incubation conditions affected the plant much more than the infection itself, which was also reflected in the distribution of the samples in the PCA plot, where control samples were

very far from the mock and infected samples (**Figure 4.5**). By comparing the response of the 3 varieties inside the infected area, the two resistant varieties had a lower number of DEGs (in Sarpo Mira 448 and 160 DEGs, and in Sarpo Shona 510 and 303, for up and down regulated genes, respectively) than the susceptible Duke of York (536 and 647 up and downregulated DEGs, respectively) (**Figure 4.6**), with this difference being particularly apparent for downregulated genes. This can also be observed in the distribution of genes in the volcano plots in **Figure 4.7**. The same pattern was also seen (and to an even greater extent) in the number of DEGs between mock and control (**Figure 4.6**). Therefore, fewer changes in gene expression were observed in the resistant varieties 48 hours post-infection than in response to the mechanical injury and absence of light during the detached infection assay.

In contrast, the number of upregulated DEGs outside the infected area was higher in both resistant varieties than in Duke of York (**Figure 4.6**). Moreover, the percentage of common DEGs between the infected and outside the infected areas was also higher in both resistant varieties (**Figure 4.8**-**A**), though the same did not occur for downregulated genes. This could indicate that, at this time point, while the infection affected transcription less in resistant varieties, they could emit a stronger signal of infection to the neighbouring cells, by upregulating their genes.

Consistent with the PCA (**Figure 4.5**), a similar transcriptomic response between the resistant varieties can be observed. The percentages of common DEGs between Sarpo Mira and Sarpo Shona (12% up and 3.5% downregulated genes in the infected area, and 20.3% and 3%, respectively, outside infected area) were higher than the percentages of common DEGs between each resistant variety and Duke in almost all comparisons. The percentages of common DEGs between Sarpo Mira and Duke in the infected area were 2.6% and 1.3%, and outside the infected area were 0.9% and 0.2%, for up and down regulated genes respectively; while for Sarpo Shona and Duke these values were 6% and 4.5% in the infected area, and 2.3% and 0.9% outside the infected area, for up and

down-regulated DEGs respectively (**Figure 4.8-B, C**). The number of unique DEGs in each variety was always higher than the number of genes in common among varieties, showing there were more variety-specific genes differentially expressed both within and outside the infected areas (**Figure 4.8-B, C**).

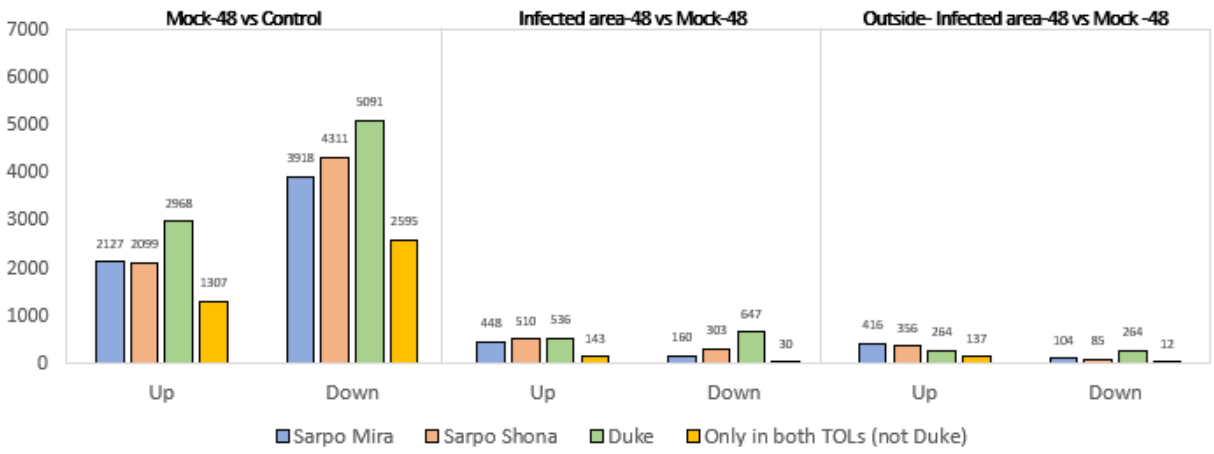


Figure 4.6 Number of DEGs (abs(log2foldchange) > 1 and padj. < 0.05) in each variety responding to the different treatments (control, mock, infection).

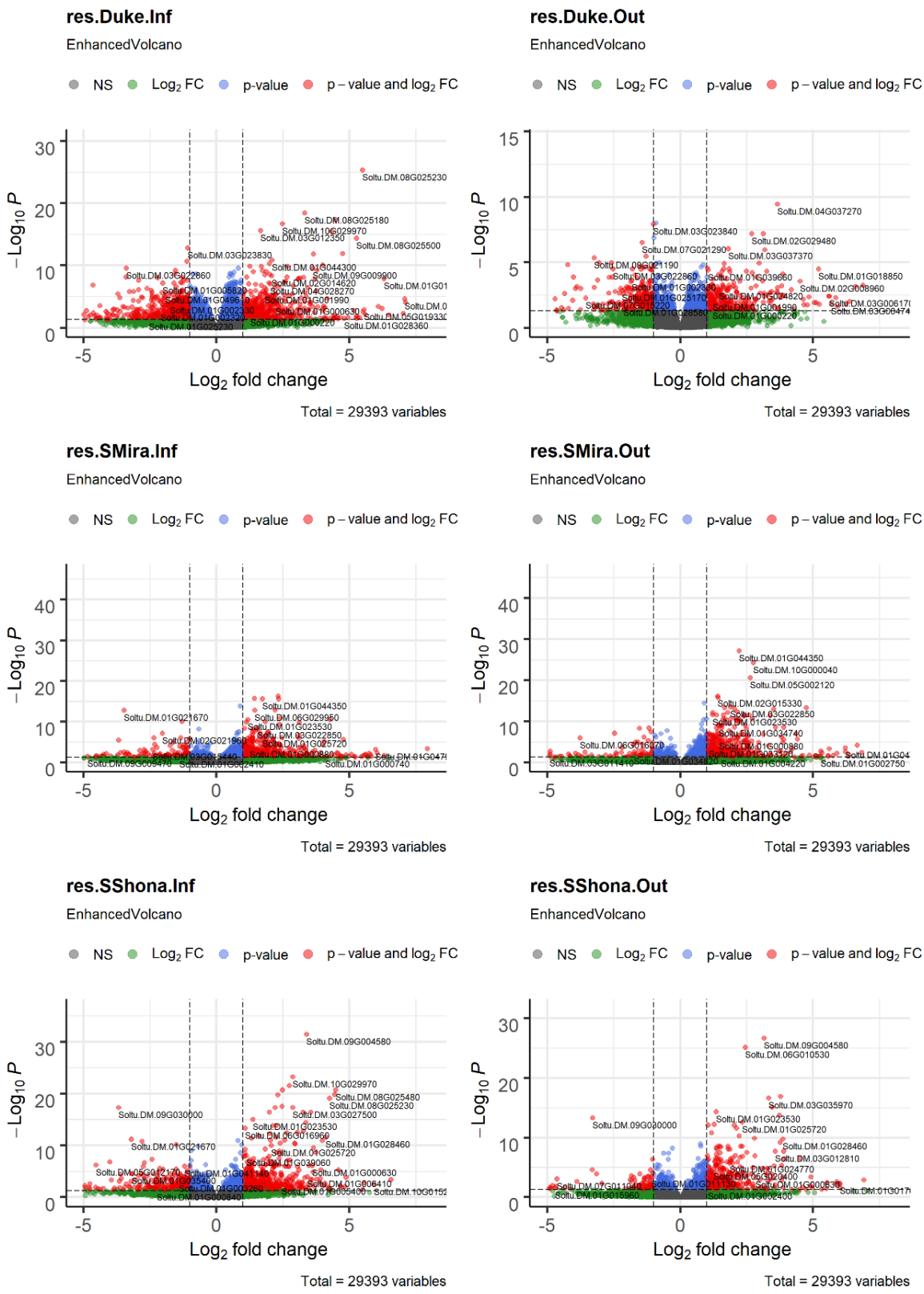


Figure 4.7 Gene expression distribution in volcano plots for each variety, inside and outside the infected area. Vertical dashed lines show the threshold for the log2foldchange (-1 and 1). Horizontal dashed line shows the threshold for the adj. p-value (0.05) expressed in -Log10. Therefore, red points are the genes that pass the threshold of padj. < 0.05 and abs(log2foldchange) >1.

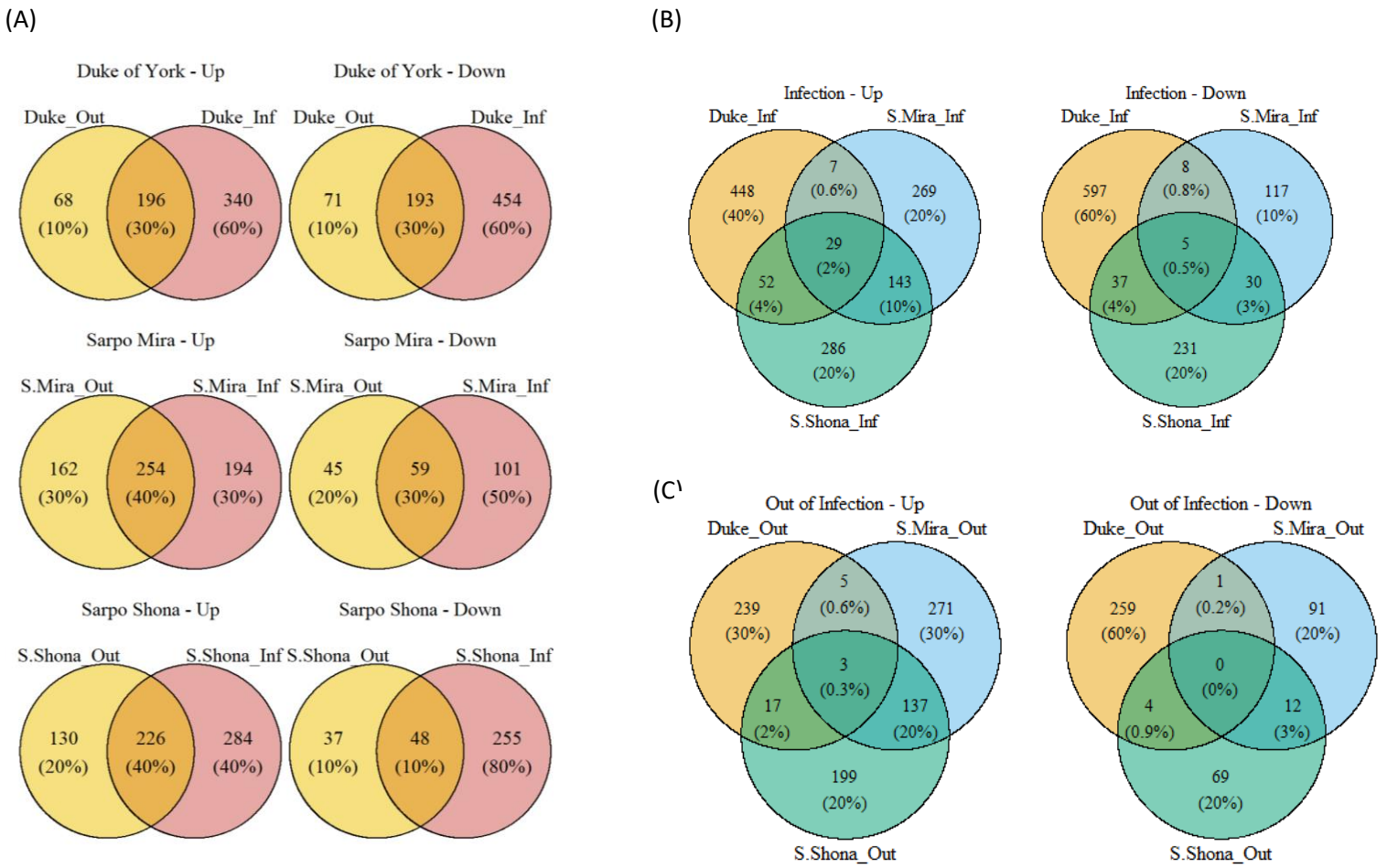


Figure 4.8 Venn diagram of the DEGs (abs(log2foldchange) > 1 and padj. < 0.05) in each variety from the comparison of gene expression between the infected area or outside the infected area vs the mock. The Venn diagrams show the DEGs unique or in common between the infected area and outside the infected area in each variety **(A)** or among the different varieties in the infected area **(B)** or outside the infected area **(C)**.

The 20 most up-regulated DEGs in the infected area in both resistant varieties, but not significantly upregulated in Duke of York, were observed. This group included genes related to the modification of the cell wall components, with two encoding pectin lyase-like superfamily proteins (*Soltu.DM.01G027350, Soltu.DM.02G011180*), one expansin-like B1 (*Soltu.DM.08G001190*), and one laccase (*Soltu.DM.04G028320*). With the exception of *Soltu.DM.01G027350*, there was no significant difference between the control and mock (Mock-48 vs Control) in both resistant varieties, suggesting that the change in expression occurred specifically in response to infection. Among the 20 most downregulated DEGs in common between only the resistant varieties, there was a gene coding for a xyloglucan endotransglucosylase/hydrolase. Its change in expression was not only caused by the stress of the infection, but also by the stress of the incubation in Sarpo Shona (DEG between Mock-48 vs control), but it was exclusive to the infection in Sarpo Mira (**Table 4.5**). These 4 up-regulated genes related to the cell wall showed no significant difference outside the infected area (OutInf-48 vs Mock-48), except for *Soltu.DM.01G027350* in Sarpo Shona, which indicates that the signal related to these genes may not propagate to other surrounding uninfected areas (**Table 4.5**). The downregulation of xyloglucan endotransglucosylase/hydrolase was significant in both resistant varieties, both inside and outside the infected areas. The resistant plants might be propagating this signal of change in the composition of xyloglucans caused by the infection to the nearby regions within the infected leaves (**Table 4.5**).

By observing the DEGs in common between the infected and outside the infected area within each variety (**Figure 4.8-A**), changes in the expression of genes involved in the modification of the cell wall were found. Within the 254 upregulated DEGs in Sarpo Mira there was a plant invertase/pectin methylesterase inhibitor superfamily, genes encoding UDP-Glycosyltransferase superfamily proteins, genes encoding enzymes involved in the phenylpropanoid/lignin biosynthesis pathway (4-coumarate:CoA ligase and cinnamate-4-

hydroxylase) and one gene encoding for a glycine-rich cell wall structural protein. While in the 226 upregulated genes in Sarpo Shona another enzyme involved in lignin biosynthesis was observed (cinnamyl alcohol dehydrogenase). Also, in both resistant varieties, there were downregulation of plant invertase/pectin methylesterase inhibitor superfamily protein and xyloglucan endotransglucosylase/hydrolase (XTH) genes. In Duke of York, change in the expression of genes encoding XTHs was also observed, but in the group of upregulated genes, in addition to a hydroxyproline-rich glycoprotein family protein, and genes for the synthesis of lignin (cinnamoyl Coa reductase), as in both resistant varieties. Further, the 3 varieties overexpressed a pectin lyase-like superfamily protein gene, but the expression was by far higher in the two resistant compared to the susceptible variety (**Table 4.6**).

In summary, at 48 h.p.i there were more transcriptomic changes in the susceptible variety than in either of the two resistant varieties. However, the resistant varieties showed more shared DEGs. Additionally, in each of the three varieties, the group of genes differentially expressed outside and within the infected areas included genes that modify the cell wall. However, the top 20 DEGs also showed that some of the changes in the expression of genes related to the cell wall were specific to the resistant varieties.

Table 4.5 Common DEGs between Sarpo Mira and Sarpo Shona but not Duke of York. In this table are the 20 most up and down-regulated DEGs in the infected area (Inf-48 vs Mock-48) in common between the two resistant varieties, with their respective log2FC (LFC) and adjusted p-value (padj.). Also shown is the LFC and padj. value of these genes from the comparisons between [Mock-48 vs control] and between [OutInf-48 vs Mock-48]. padj. values > 0.05 are shown in grey. Genes relating to the cell wall are shown in red.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Function	Sarpo Mira						Sarpo Shona						Duke					
		Mock-48 vs Control		Inf-48 vs Mock-48		OutInf-48 vs Mock-48		Mock-48 vs Control		Inf-48 vs Mock-48		OutInf-48 vs Mock-48		Mock-48 vs Control		Inf-48 vs Mock-48		OutInf-48 vs Mock-48	
		LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj
Upregulated DEGs from the comparison of Inf48 vs mock 48.																			
Soltu.DM.01G027350	Pectin lyase-like superfamily protein	-5.78	3.28E-03	5.68	3.22E-02	5.29	5.87E-02	-4.80	1.69E-02	5.49	2.97E-02	6.02	2.28E-02	6.03	7.46E-03	0.61	8.68E-01	0.72	8.75E-01
Soltu.DM.10G015280	Cupredoxin superfamily protein	-5.04	4.94E-03	5.61	1.58E-02	4.01	1.58E-01	-4.32	1.23E-02	5.93	3.63E-03	5.88	6.24E-03	4.08	3.46E-02	-0.08	9.80E-01	0.37	9.32E-01
Soltu.DM.06G016360	terpene synthase	-3.77	3.95E-03	4.58	5.11E-03	1.89	4.68E-01	-2.37	8.65E-02	4.19	9.40E-03	1.24	7.04E-01	3.19	1.42E-02	-0.18	9.52E-01	-2.95	1.21E-01
Soltu.DM.05G008950	similar to RCD one	-2.04	9.78E-04	4.27	2.93E-11	4.74	3.88E-14	-2.60	1.44E-05	1.71	3.15E-02	1.94	1.88E-02	2.09	5.08E-04	0.11	9.29E-01	-0.05	9.80E-01
Soltu.DM.08G001190	expansin-like B1	-1.65	2.97E-01	4.00	3.48E-02	1.46	6.62E-01	-1.24	4.62E-01	4.25	1.78E-02	2.98	1.97E-01	2.59	6.76E-02	-1.36	5.25E-01	-2.28	3.07E-01
Soltu.DM.01G040930	terpene synthase	0.90	4.99E-01	3.92	2.96E-03	1.77	3.81E-01	-1.37	2.91E-01	4.87	1.44E-04	0.37	9.32E-01	0.50	7.22E-01	0.83	6.41E-01	-0.22	9.43E-01
Soltu.DM.01G048780	allene oxide synthase	-1.05	3.20E-01	3.71	8.33E-04	-0.22	9.49E-01	-0.15	9.09E-01	4.58	5.08E-06	0.70	7.86E-01	0.22	8.66E-01	0.62	6.94E-01	-1.29	4.26E-01
Soltu.DM.04G028320	laccase	-1.61	1.20E-01	3.62	2.25E-03	1.20	5.69E-01	-1.22	2.53E-01	2.64	3.49E-02	1.22	5.50E-01	2.98	1.64E-03	-1.43	2.87E-01	-2.49	6.53E-02
Soltu.DM.06G016370	Terpenoid cyclases/Protein prenyltransferases superfamily protein	-3.23	2.27E-03	3.44	1.20E-02	1.10	6.65E-01	-3.18	4.20E-03	3.36	1.53E-02	0.56	8.80E-01	2.30	5.36E-02	-1.01	5.69E-01	-1.65	3.90E-01
Soltu.DM.02G011180	Pectin lyase-like superfamily protein	-1.81	8.11E-02	3.40	5.48E-03	-1.48	5.03E-01	-1.17	2.91E-01	3.24	6.69E-03	1.38	4.89E-01	-0.62	6.38E-01	0.80	6.54E-01	-0.56	8.35E-01
Soltu.DM.09G000670	phosphate transporter 4;2	-1.56	6.75E-02	3.37	4.14E-04	1.34	3.81E-01	-0.92	3.03E-01	2.16	3.69E-02	0.78	6.90E-01	1.55	5.72E-02	-0.71	5.76E-01	-1.63	1.76E-01
Soltu.DM.02G018060	Protein of unknown function (DUF_B2219) domain containing protein	0.13	9.14E-01	3.35	1.14E-03	0.36	8.98E-01	0.34	7.58E-01	3.53	3.03E-04	1.16	5.06E-01	2.19	9.59E-03	-0.77	5.69E-01	-1.34	3.40E-01
Soltu.DM.01G034180	1-amino-cyclopropane-1-carboxylate synthase	-1.83	1.24E-02	3.28	6.62E-05	1.31	3.07E-01	-2.33	1.03E-03	2.44	5.04E-03	1.46	2.13E-01	4.17	9.17E-10	-0.82	4.44E-01	-1.08	3.65E-01
Soltu.DM.06G030710	LOB domain-containing protein	-4.78	3.18E-11	3.18	4.69E-04	2.61	7.73E-03	-2.83	2.67E-05	2.13	1.45E-02	1.99	4.11E-02	-0.43	5.96E-01	-1.14	2.17E-01	-0.72	5.76E-01
Soltu.DM.06G019870	plant natriuretic peptide A	-3.37	2.14E-03	3.14	3.52E-02	1.81	3.92E-01	-4.39	1.63E-04	4.01	5.67E-03	3.19	6.75E-02	-1.20	2.98E-01	1.19	4.59E-01	-1.24	5.49E-01
Soltu.DM.10G001460	plastid movement impaired1	-3.77	7.75E-18	3.12	1.62E-10	3.52	1.04E-13	-3.38	8.32E-15	1.71	1.94E-03	1.98	2.58E-04	-2.64	1.66E-09	0.08	9.37E-01	-0.07	9.62E-01
Soltu.DM.05G013350	alpha/beta-Hydrolases superfamily protein	-5.45	3.97E-24	3.11	1.08E-06	3.58	2.53E-09	-4.96	4.59E-20	3.04	1.12E-06	3.75	6.59E-10	-0.27	7.05E-01	0.44	6.45E-01	0.88	3.58E-01

Soltu.DM.01G047870	Cupredoxin superfamily protein	-5.55	6.01E-09	3.10	1.93E-02	2.90	3.54E-02	-3.88	5.96E-05	3.57	2.57E-03	3.81	1.51E-03	0.73	5.39E-01	-0.21	9.19E-01	0.25	9.25E-01
Soltu.DM.07G013650	cytochrome P450, family 716, subfamily A, polypeptide	-2.14	8.71E-03	3.01	2.16E-03	-0.63	7.87E-01	-0.91	3.07E-01	2.90	1.94E-03	-0.04	9.88E-01	-1.15	2.27E-01	0.97	4.79E-01	-1.37	4.54E-01
Soltu.DM.04G022640	ferulic acid 5-hydroxylase	-3.90	1.28E-06	2.80	9.42E-03	0.78	7.24E-01	-2.66	1.43E-03	3.03	2.41E-03	1.58	2.85E-01	-1.11	1.85E-01	0.37	8.00E-01	0.11	9.64E-01
Downregulated DEGs from the comparison of Inf48 vs mock 48.																			
Soltu.DM.02G023870	Gibberellin-regulated family protein	-2.10	2.83E-02	-6.00	2.38E-03	-2.59	9.94E-02	-1.34	1.77E-01	-6.75	2.21E-04	-1.75	2.95E-01	-3.71	5.71E-05	1.80	1.58E-01	2.29	9.62E-02
Soltu.DM.12G004670	xylem cysteine peptidase	-1.32	3.28E-01	-5.14	3.02E-02	-0.34	9.39E-01	-0.83	5.51E-01	-5.64	8.62E-03	-4.48	8.16E-02	-4.29	1.15E-04	0.17	9.50E-01	0.04	9.91E-01
Soltu.DM.02G026970	beta HLH protein	-3.65	1.82E-04	-4.54	4.53E-02	0.39	9.17E-01	-0.06	9.60E-01	-2.68	4.86E-02	-2.33	1.40E-01	-1.36	1.43E-01	-1.61	1.78E-01	-1.15	4.67E-01
Soltu.DM.02G030450	GAST1 protein homolog	-3.19	3.63E-02	-4.30	3.09E-02	-1.26	7.40E-01	-2.20	1.64E-01	-4.95	5.46E-03	-1.22	7.51E-01	-6.33	6.24E-06	-0.69	7.99E-01	1.84	4.78E-01
Soltu.DM.06G016070	alcohol dehydrogenase	2.08	2.89E-03	-3.68	2.94E-06	-3.77	9.74E-07	0.94	2.18E-01	-1.96	2.79E-02	-3.15	1.22E-04	-3.12	3.02E-06	0.39	7.58E-01	-0.04	9.87E-01
Soltu.DM.02G016290	alpha/beta-Hydrolases superfamily protein	-3.46	6.76E-05	-3.48	2.38E-03	-1.54	3.57E-01	-4.72	3.49E-08	-5.58	1.01E-03	-1.06	6.13E-01	-7.16	1.57E-17	-1.74	1.71E-01	0.99	5.59E-01
Soltu.DM.05G027280	SNF1-related protein kinase 2.5	3.09	3.69E-04	-3.36	2.96E-03	-4.90	1.98E-03	3.01	2.66E-07	-1.83	1.57E-02	-1.28	1.88E-01	1.79	2.38E-03	-0.52	5.95E-01	-0.65	5.60E-01
Soltu.DM.05G027280	Protein kinase superfamily protein	3.09	3.69E-04	-3.36	2.96E-03	-4.90	1.98E-03	3.01	2.66E-07	-1.83	1.57E-02	-1.28	1.88E-01	1.79	2.38E-03	-0.52	5.95E-01	-0.65	5.60E-01
Soltu.DM.04G002770	MLP-like protein	1.66	1.01E-01	-2.75	3.75E-02	-0.58	8.33E-01	1.77	6.61E-02	-3.66	7.73E-04	0.30	9.28E-01	-1.04	3.01E-01	-2.06	8.14E-02	0.96	5.82E-01
Soltu.DM.02G028040	hypothetical protein	-1.84	1.57E-02	-2.75	6.66E-03	-2.13	5.51E-02	-2.87	7.07E-05	-2.35	1.43E-02	-1.22	3.85E-01	-4.46	2.06E-10	-0.26	8.62E-01	0.55	7.25E-01
Soltu.DM.06G001110	indoleacetic acid-induced protein	-0.10	9.26E-01	-2.59	6.86E-03	-1.43	2.67E-01	-0.35	7.07E-01	-3.04	4.23E-04	-0.40	8.62E-01	-3.23	4.63E-06	-0.40	7.61E-01	0.49	7.60E-01
Soltu.DM.09G030000	xyloglucan endotransglucosylase/hydrolase	-0.15	7.91E-01	-2.35	8.68E-07	-2.47	7.89E-08	2.04	8.56E-07	-3.69	4.32E-18	-3.30	5.18E-14	-1.81	1.32E-05	-0.98	7.41E-02	-0.59	4.27E-01
Soltu.DM.02G014100	Drug/metabolite transporter superfamily protein	4.46	1.43E-08	-2.13	6.79E-03	-1.02	3.50E-01	4.83	4.65E-06	-2.23	6.23E-03	-1.29	2.07E-01	0.64	2.97E-01	1.97	2.03E-03	1.87	8.95E-03
Soltu.DM.02G009940	Protein of unknown function (DUF1637)	2.45	7.32E-04	-2.12	2.99E-02	-2.41	1.05E-02	3.39	2.77E-06	-2.00	2.91E-02	-2.08	3.76E-02	-1.41	5.07E-02	-0.46	7.08E-01	-0.42	7.94E-01
Soltu.DM.08G006600	cation/hydrogen exchanger	4.09	2.67E-10	-1.86	1.58E-02	-0.61	6.49E-01	3.58	4.84E-10	-1.62	2.66E-02	-1.00	3.21E-01	4.38	5.08E-10	-0.98	2.18E-01	-0.36	7.80E-01
Soltu.DM.07G001430	Plant protein of unknown function (DUF828) with plant pleckstrin homology-like region	0.01	9.92E-01	-1.83	2.61E-02	0.26	8.84E-01	-0.03	9.65E-01	-2.33	1.83E-03	-0.02	9.91E-01	-2.81	1.53E-07	0.18	8.76E-01	1.16	1.87E-01
Soltu.DM.01G045480	interferon-related developmental regulator family protein / IFRD protein family	-0.15	8.34E-01	-1.75	1.62E-02	-1.21	1.62E-01	-1.27	1.51E-02	-2.25	1.02E-03	-1.29	1.30E-01	-1.53	2.42E-03	-0.56	4.97E-01	0.20	8.81E-01

Soltu.DM.08G003230	Long-chain fatty alcohol dehydrogenase family protein	1.06	6.29E-02	-1.62	2.52E-02	-1.20	1.66E-01	0.74	2.10E-01	-1.48	3.47E-02	-1.54	4.24E-02	-0.75	1.83E-01	0.72	3.40E-01	0.45	6.70E-01
Soltu.DM.10G028270	Leucine-rich repeat protein kinase family protein	0.87	1.02E-01	-1.62	1.35E-02	-0.38	7.76E-01	1.15	2.23E-02	-2.33	2.89E-05	-0.02	9.89E-01	-1.70	3.78E-04	0.96	1.35E-01	1.64	9.29E-03
Soltu.DM.04G037290	SKU5 similar	-5.17	2.23E-28	-1.61	2.11E-02	-1.02	2.52E-01	-5.03	3.83E-27	-1.99	1.22E-03	-1.14	1.60E-01	-5.61	1.90E-33	-0.58	4.56E-01	0.39	7.06E-01

Table 4.6 Common DEGs between the infected and outside the infected areas in three varieties. These DEGs are involved in cell wall modification and shown with their respective log2FC (LFC) and adjusted p-value (padj.).

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Function	Inf-48 vs Mock-48		OutInf-48 vs Mock-48		Athaliana Gene ID	Athaliana Gene Name
		LFC	padj.	LFC	padj.		
Sarpo Mira - Upregulated DEGs from the comparison of Inf48 vs mock 48.							
Soltu.DM.02G010210	Pectin lyase-like superfamily protein	7.93	3.82E-04	6.83	3.52E-03	AT2G43870	AT2G43870
Soltu.DM.01G039680	Plant invertase/pectin methylesterase inhibitor superfamily	2.21	2.57E-03	1.96	1.16E-02	AT3G05620	PME22
Soltu.DM.01G034740	UDP-Glycosyltransferase superfamily protein	1.27	4.47E-07	1.44	1.35E-09	AT4G01070	UGT72B1
Soltu.DM.02G006810	UDP-Glycosyltransferase superfamily protein	4.43	1.45E-02	5.29	1.47E-03	AT2G18570	UGT72D1
Soltu.DM.06G024540	4-coumarate:CoA ligase	1.46	3.89E-04	1.41	5.28E-04	AT3G21240	4CL2
Soltu.DM.06G032850	cinnamate-4-hydroxylase	1.29	1.76E-03	1.03	2.25E-02	AT2G30490	CYP73A5
Soltu.DM.06G032860	cinnamate-4-hydroxylase	1.37	4.47E-03	1.09	4.37E-02	AT2G30490	CYP73A5
Soltu.DM.07G017630	Glycine-rich cell wall structural protein	1.49	2.24E-03	1.23	2.04E-02	NA	NA
Sarpo Mira - Downregulated DEGs from the comparison of Inf48 vs mock 48.							
Soltu.DM.03G015480	Plant invertase/pectin methylesterase inhibitor superfamily protein	-2.33	3.78E-05	-1.52	1.84E-02	AT5G62360	AT5G62360
Soltu.DM.09G030000	xyloglucan endotransglucosylase/hydrolase	-2.35	8.68E-07	-2.47	7.89E-08	AT4G14130	XTH15
Soltu.DM.12G006530	Rhamnogalacturonate lyase family protein	-1.75	7.21E-05	-1.77	3.49E-05	AT1G09890	AT1G09890
Sarpo Shona- Upregulated DEGs from the comparison of Inf48 vs mock 48.							
Soltu.DM.01G027350	Pectin lyase-like superfamily protein	5.49	2.97E-02	6.02	2.28E-02	AT3G07970	QRT2
Soltu.DM.01G046930	cinnamyl alcohol dehydrogenase	1.21	7.32E-04	1.03	9.63E-03	AT3G19450	CAD4
Soltu.DM.04G011090	UDP-glycosyltransferase 73B4	1.31	2.95E-02	1.40	2.90E-02	AT4G34135	UGT73B2
Soltu.DM.04G011110	UDP-glycosyltransferase 73B4	2.20	1.00E-03	2.05	4.16E-03	AT2G15490	UGT73B4
Soltu.DM.10G024530	UDP-glucosyl transferase 73C6	1.36	6.99E-06	1.47	1.15E-06	AT2G36780	UGT73C3
Sarpo Shona- Downregulated DEGs from the comparison of Inf48 vs mock 48.							
Soltu.DM.01G050190	glycosyl hydrolase 9B18	-3.16	3.05E-05	-3.31	2.44E-05	AT4G39010	AtGH9B18
Soltu.DM.03G015480	Plant invertase/pectin methylesterase inhibitor superfamily protein	-2.71	2.03E-07	-1.84	1.96E-03	AT5G62360	AT5G62360
Soltu.DM.08G001850	Plant invertase/pectin methylesterase inhibitor superfamily protein	-2.13	7.03E-04	-1.49	4.73E-02	AT3G62820	AT3G62820
Soltu.DM.09G030000	xyloglucan endotransglucosylase/hydrolase	-3.69	4.32E-18	-3.30	5.18E-14	AT4G14130	XTH15
Soltu.DM.05G002580	galacturonosyltransferase-like	-1.16	2.56E-02	-1.37	9.47E-03	AT1G13250	GATL3
Soltu.DM.05G008440	polygalacturonase	-1.61	1.52E-03	-1.85	2.60E-04	AT1G70370	PGL3
Soltu.DM.08G003110	Pectinacetylesterase family protein	-1.20	2.65E-02	-1.76	4.44E-04	AT4G19420	AT4G19420
Duke- Upregulated DEGs from the comparison of Inf48 vs mock 48.							
Soltu.DM.09G028450	Pectin lyase-like superfamily protein	2.78	2.49E-04	1.86	4.82E-02	AT4G13710	AT4G13710
Soltu.DM.06G024250	cinnamoyl coa reductase	1.22	1.30E-02	1.25	2.20E-02	AT1G15950	CCR1
Soltu.DM.02G007340	hydroxyproline-rich glycoprotein family protein	1.49	6.17E-03	1.88	8.07E-04	AT5G65660	AT5G65660
Soltu.DM.03G002910	xyloglucan endotransglucosylase/hydrolase	1.87	4.15E-02	2.46	9.39E-03	AT1G11545	XTH8
Soltu.DM.05G008140	xyloglucan:xyloglucosyl transferase	2.88	1.52E-07	3.13	6.47E-08	AT1G10550	XTH33
Soltu.DM.03G015040	xyloglucan endotransglycosylase	2.91	4.61E-03	3.39	2.03E-03	AT4G25810	XTH23
Soltu.DM.03G015050	xyloglucan endotransglycosylase	3.15	6.82E-04	3.86	7.51E-05	AT4G25810	XTH23
Soltu.DM.03G015060	xyloglucan endotransglycosylase	2.83	7.54E-03	3.66	8.85E-04	AT4G25810	XTH23

Soltu.DM.03G015070	xyloglucan endotransglycosylase	2.83	6.32E-03	3.45	1.71E-03	AT4G25810	XTH23
Soltu.DM.08G025180	extensin-like protein	3.32	3.61E-19	1.41	2.67E-03	AT4G12510	AT4G12510
Soltu.DM.08G029560	cellulose synthase like G3	2.55	4.63E-06	2.48	4.56E-05	AT4G23990	CSLG3
Soltu.DM.10G020910	UDP-Glycosyltransferase superfamily protein	1.62	1.55E-05	1.09	1.47E-02	AT2G36970	UGT86A1
Soltu.DM.03G011980	UDP-glucosyl transferase 85A7	2.45	5.59E-03	2.20	3.03E-02	AT1G22340	UGT85A7
Soltu.DM.03G012350	Glycosyl hydrolase family protein	1.66	2.51E-16	1.01	2.51E-05	AT1G02640	BXL2
Soltu.DM.S002220	Glycosyl hydrolase superfamily protein	2.75	6.90E-06	1.78	1.64E-02	AT4G16260	AT4G16260
Soltu.DM.07G016430	glycosyl hydrolase 9B8	3.23	4.39E-02	3.70	3.38E-02	AT2G32990	AtGH9B8
Duke- Downregulated DEGs from the comparison of Inf48 vs mock 48.							
Soltu.DM.11G005440	UDP-Glycosyltransferase superfamily protein	-1.96	1.03E-02	-2.11	1.16E-02	AT5G49690	UGT91C1
Soltu.DM.11G005760	UDP-Glycosyltransferase superfamily protein	-2.52	3.74E-02	-2.77	4.16E-02	AT5G65550	UGT91B1
Soltu.DM.03G028830	UDP-glucosyl transferase 89B1	-1.09	2.29E-04	-1.10	7.36E-04	AT1G73880	UGT89B1
Soltu.DM.04G011320	UDP-glucosyl transferase 73B5	-1.17	1.51E-02	-1.43	5.49E-03	AT2G15480	UGT73B5
Soltu.DM.06G030490	glycosyltransferase family protein	-1.52	2.07E-02	-1.47	4.81E-02	AT5G60700	AT5G60700

4.3.5.2. Consistent transcriptional differences between resistant and susceptible varieties

The expression of genes in Sarpo Mira and Sarpo Shona was compared with the expression in Duke in each treatment (Control, Mock-48, Inf-48, and OutInf-48) to identify genes whose expression was consistently higher or lower in the resistant varieties compared with the susceptible Duke of York, across all treatments.

In control or mock inoculation treatments, there were more genes with lower expression in either or both resistant varieties compared to Duke of York (e.g. in the control samples: 3,329 and 3,833 upregulated vs 4,013 and 4,093 downregulated in Sarpo Mira and Sarpo Shona, respectively). In contrast, after infection both within and outside the infected areas, the number of genes with higher expression in either or both resistant varieties was more than the number of genes with lower expression (e.g. in the infected area: 2,772 and 2,552 upregulated vs 2,279 and 2,337 downregulated in Sarpo Mira and Sarpo Shona, respectively).

Across all the treatments, more differences in gene expression were observed in the control than in any treatment. Consistently across all treatments, 685 genes had a higher and 431 had a lower expression in both Sarpo Mira and Sarpo Shona than in Duke of York (**Figure 4.9**). From these 685 genes, the top 20 genes with higher expression in both resistant varieties compared with Duke of York included genes involved in the modification of the cell wall composition, such as a glycosyl hydrolase superfamily protein (*Soltu.DM.01G005230*) and a beta-1,3-glucanase (*Soltu.DM.01G005190*). Also, one R gene with an LRR and NB-ARC domain (*Soltu.DM.02G001820*) was included in this group. The gene with the highest expression in both resistant varieties was a basic chitinase (*Soltu.DM.10G018010*) with a log2-fold change of more than 15 in all conditions, in comparison with the susceptible variety (**Table 4.7**). It is possible that these genes with a constitutively higher or lower expression in the resistant varieties across all treatment conditions

could help them to better fight the infection since they allow these varieties to be better armed to defend themselves against *P. infestans*.

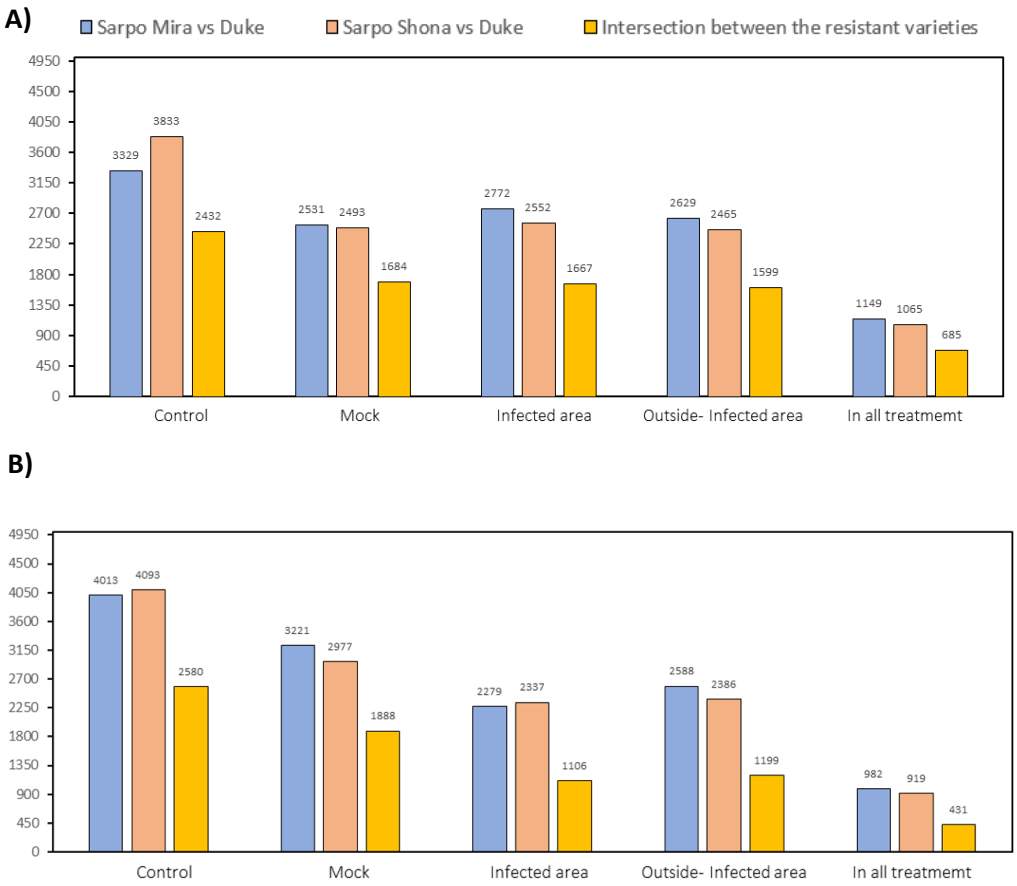


Figure 4.9 Genes whose expression was significantly higher (A) or lower (B) in either or both of the resistant varieties, for each treatment, compared with Duke of York.

Table 4.7 Top 20 genes with a higher expression in both resistant varieties than in Duke of York across all treatments. The table shows the log2FC (LFC) of each gene that had a significant adj. p-value less than 0.05. In red are the cell wall-related genes.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Function	Sarpo Mira vs Duke				Sarpo Shona vs Duke				Sarpo Mira			Sarpo Shona			Duke		
		Control	Mock	Infected area	Out-infected area	Control	Mock	Infected area	Out-infected area	Mock-48 vs Control	Inf-48 vs Mock-48	OutInf-48 vs Mock-48	Mock-48 vs Control	Inf-48 vs Mock-48	OutInf-48 vs Mock-48	Mock-48 vs Control	Inf-48 vs Mock-48	OutInf-48 vs Mock-48
Soltu.DM.10G018010	basic chitinase	15.65	16.76	15.47	17.26	15.26	16.55	15.56	17.20	2.07	-	-	2.24	-	-	-	-	-
Soltu.DM.07G001820	ribonuclease	14.26	9.06	8.37	9.66	13.23	9.90	9.68	11.00	-5.20	-	-	-3.33	-	-	-	-	-
Soltu.DM.07G028150	translocase outer membrane 20-2	13.29	8.75	8.83	12.01	13.07	8.65	8.91	12.12	-	-	-	-	-	-	4.44	-	-
Soltu.DM.11G026800	RNA-binding (RRM/RBD/RNP motifs) family protein	12.12	8.14	10.76	9.35	11.83	8.15	10.72	9.35	-	-	-	-	-	-	3.66	-	-
Soltu.DM.12G024140	hypothetical protein	10.97	9.67	9.35	9.02	9.69	8.50	8.18	7.29	-1.30	-	-	-1.19	-	-	-	-	-
Soltu.DM.02G001820	LRR and NB-ARC domains-containing disease resistance protein	10.91	11.74	12.12	12.10	10.49	11.36	11.18	11.03	0.83	-	-	0.87	-	-	-	-	-
Soltu.DM.07G028120	hypothetical protein	10.76	10.54	10.39	10.46	10.71	10.12	9.92	10.09	-	-	-	-0.60	-	-	-	-	-
Soltu.DM.12G009260	Protein kinase family protein with leucine-rich repeat domain	10.43	5.03	3.94	4.63	10.19	4.08	3.26	3.28	-	-	-	-1.25	-	-	4.86	1.80	-
Soltu.DM.04G031630	Polynucleotidyl transferase, ribonuclease H-like superfamily protein	10.37	10.53	10.46	10.15	10.67	10.84	10.51	10.44	-	-	-	-	-	-	-	-	-
Soltu.DM.08G023700	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	10.18	8.02	9.39	9.15	10.00	9.15	10.49	10.22	-1.20	-	-	-	-	-	-	-	-
Soltu.DM.03G026620	Raffinose synthase family protein	10.05	10.21	9.53	9.92	9.78	9.67	9.62	9.79	-	-	-	-	-	-	-	-	-
Soltu.DM.11G004670	cytochrome P450, family 71, subfamily B, polypeptide	9.96	7.42	5.86	5.08	9.38	6.62	6.08	5.09	-	-	-	-1.80	2.77	-	-	-	-
Soltu.DM.01G005230	Glycosyl hydrolase superfamily protein	9.84	9.86	8.95	9.52	7.29	7.35	6.67	7.19	0.97	-	-	1.01	-	-	-	-	-
Soltu.DM.03G014140	Transmembrane amino acid transporter family protein	9.69	2.56	6.01	4.57	9.08	4.10	7.70	6.17	-5.86	-	-	-3.71	-	-	-	-3.07	-
Soltu.DM.01G005190	beta-1,3-glucanase	9.61	12.19	8.32	10.45	8.10	11.29	7.70	9.99	-	-	-	1.33	-	-	-	-	-

Soltu.DM.11G004690	cytochrome P450, family 71, subfamily B, polypeptide	9.59	4.96	9.82	7.06	9.08	4.30	9.92	6.22	-2.21	2.56	-	-2.36	3.32	-	-	-	-
Soltu.DM.05G017400	hypothetical protein	9.51	9.78	9.47	9.36	9.32	9.59	9.69	9.86	-	-	-	-	-	-	-	-	-
Soltu.DM.08G029450	Protein of unknown function (DUF295)	9.46	9.24	8.33	8.68	10.27	9.39	9.07	9.37	-	-	-	-0.87	-	-	-	-	-
Soltu.DM.03G017080	Cytidine/deoxycytidylate deaminase family protein	9.28	8.54	8.65	8.70	6.69	5.55	5.74	6.08	-	-	-	-	-	-	-	-	-
Soltu.DM.01G037280	hypothetical protein	9.28	10.77	10.88	10.60	9.62	10.68	10.76	10.67	-	-	-	-	-	-	-	-	-

4.3.6. Gene ontology analysis

The gene ontology (GO) analysis was carried out in each variety and independently for up and downregulated DEGs.

4.3.6.1. DEGs that respond to the infection in each variety.

4.3.6.1.1. DEGs inside the infected area

The infected area (Inf-48 vs Mock-48) was enriched with more GO terms for the biological process (BP) in the susceptible variety Duke (16 for down and 14 for up-regulated genes) than in Sarpo Mira (2 and 9, respectively) or Sarpo Shona (0 and 9, respectively). The two resistant varieties had only two enriched GO terms in common, which were related to the synthesis of lignin, namely the "lignin biosynthesis process" (GO:0009809), also enriched in Duke, and the "phenylpropanoid biosynthetic process" (GO:0009699).

There were many GO terms enriched in only 1 of the 3 varieties. Enriched GO terms specific to Sarpo Mira included GO terms related to response to light (“response to light stimulus” -GO:0009416, photosynthesis light harvesting in photosystem I -GO0009768, and “response to high light intensity” -GO:0009644) and related to the circadian rhythm (“circadian regulation of gene expression” -GO:0032922 and “circadian rhythm” -GO:0007623), among others. (**Figure 4.10-A**).

Enriched GO terms specific for Sarpo Shona include a term related to the reaction against the infection (“response to defence” -GO:0006952) and a term related to the modification of cutin in the cell wall (“cutin transport” -GO:0080051). In addition, the "coumarin biosynthetic process" -GO:0009805, the "ethylene-activated signalling pathway" -GO:0009873, and, with a high number of DEGs, the "regulation of transcription, DNA-templated" -GO:0006355.

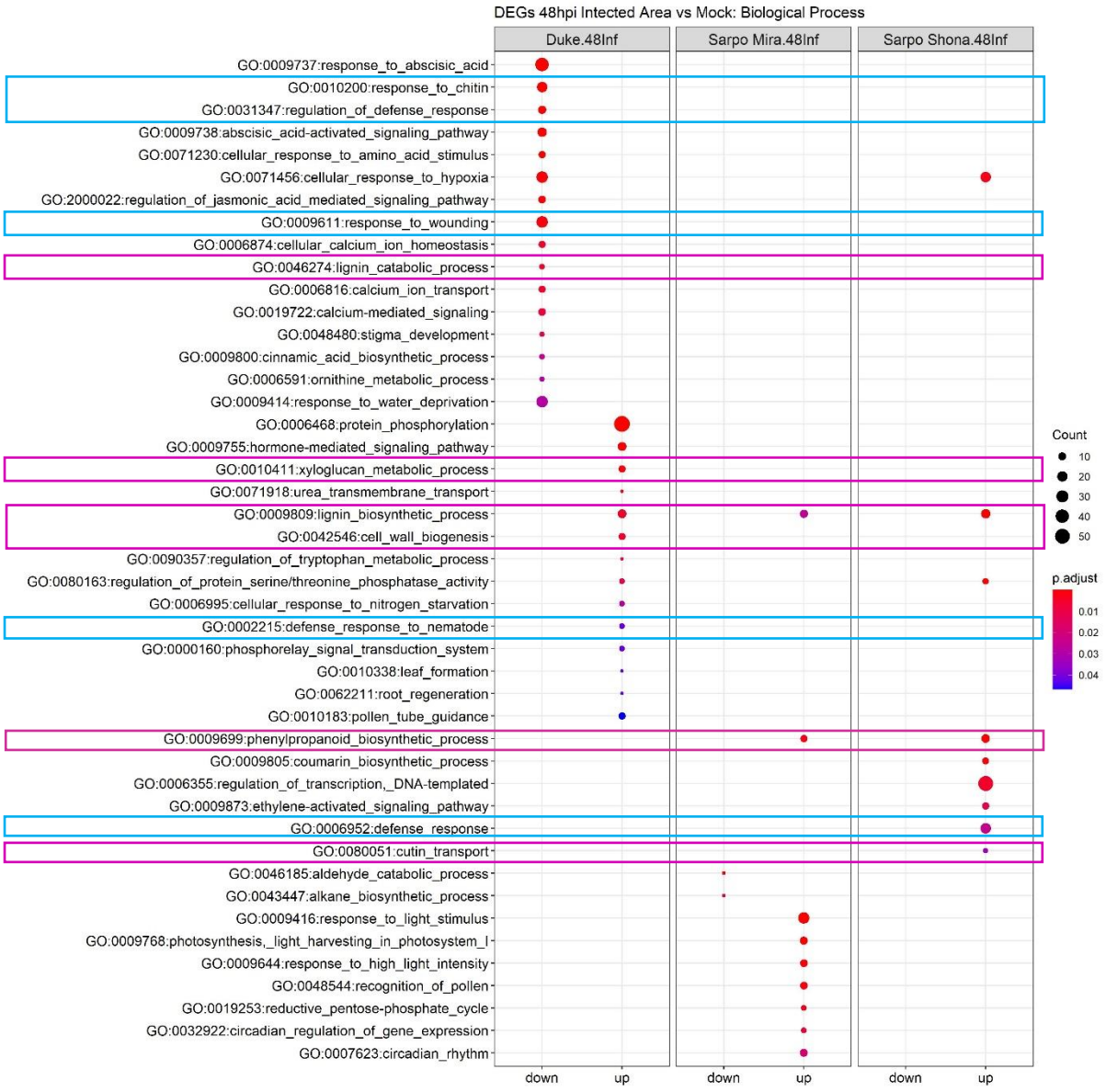
Duke of York showed more enriched GO terms than either resistant variety related to the response to pathogen infection, including three for the downregulated DEGs (“response to chitin” -GO:0010200, “regulation of defence response” -GO:0031347, and “response to wounding” -

GO:0009611) and one for upregulated DEGs (“response to nematode” -GO:0002215). Also, three terms enriched only in Duke were related to the cell wall (“lignin catabolic process”-GO:0046274, “xyloglucan metabolic process”-GO:0010411, and “cell wall biogenesis”-GO:0042546). The "protein phosphorylation” -GO:0006468 term was also enriched only in the Duke of York, with a particularly high number of DEGs (**Figure 4.10-A**).

In the category of cellular component, enriched terms specific to the cell wall were identified in Duke of York (“plant-type cell wall” -GO:0009505) and related to the apoplast region in Duke of York and Sarpo Mira (“apoplast” -GO:0048046), although these enriched GO terms contain downregulated genes and upregulated genes in each variety, respectively. GO terms related to the extracellular region were observed in all three varieties (“extracellular region” -GO:0005576) (**Figure 4.10-B**). In the category of molecular function, enriched GO terms involved in changes in cell wall components were observed in Duke (for downregulated genes “phenylalanine ammonia lyase activity” -GO:0045548 and for upregulated genes “xyloglucan:xyloglucosyl transferase” -GO:0016762) and in Sarpo Shona (“Cellulase activity”-GO:0008810) (**Figure 4.10-C**).

Therefore, according to the GO enrichment analysis, changes in the expression of genes that can modify the cell wall composition of leaves are observed in the three varieties inside the infected area. While an enrichment of genes related to the composition of the hemicellulose xyloglucan was specific to Duke, and an enrichment of genes related to the cutin transport was specific to Sarpo Shona, the enrichment of genes related to the synthesis of lignin was a common response among the three varieties. On the other hand, enriched GO terms related to defence response against pathogens were also observed in the Duke of York and Sarpo Shona. Interestingly, while in the susceptible variety this enrichment was mostly observed for downregulated genes, in the resistant Sarpo Shona it was for upregulated genes.

(A)


DEGs 48hpi Intected Area vs Mock: Biological Process
Duke.48Inf
Sarpo Mira.48Inf
Sarpo Shona.48Inf
GO:0009737:response_to_abscisic_acid
GO:0010200:response_to_chitin
GO:0031347:regulation_of_defense_response
GO:0009738:abscisic_acid-activated_signaling_pathway
GO:0071230:cellular_response_to_amino_acid_stimulus
GO:0071456:cellular_response_to_hypoxia
GO:2000022:regulation_of_jasmonic_acid_mediated_signaling_pathway
GO:0009611:response_to_wounding
GO:0006874:cellular_calcium_ion_homeostasis
GO:0046274:lignin_catabolic_process
GO:0006816:calcium_ion_transport
GO:0019722:calcium-mediated_signaling
GO:0048480:stigma_development
GO:0009800:cinnamic_acid_biosynthetic_process
GO:0006591:ornithine_metabolic_process
GO:0009414:response_to_water_deprivation
GO:0006468:protein_phosphorylation
GO:0009755:hormone-mediated_signaling_pathway
GO:0010411:xyloglucan_metabolic_process
GO:0071918:urea_transmembrane_transport
GO:0009809:lignin_biosynthetic_process
GO:0042546:cell_wall_biogenesis
GO:0090357:regulation_of_tryptophan_metabolic_process
GO:0080163:regulation_of_protein_serine/threonine_phosphatase_activity
GO:0006995:cellular_response_to_nitrogen_starvation
GO:0002215:defense_response_to_nematode
GO:0000160:phosphorelay_signal_transduction_system
GO:0010338:leaf_formation
GO:0062211:root_regeneration
GO:0010183:pollen_tube_guidance
GO:0009699:phenylpropanoid_biosynthetic_process
GO:0009805:coumarin_biosynthetic_process
GO:0006355:regulation_of_transcription,_DNA-templated
GO:0009873:ethylene-activated_signaling_pathway
GO:0006952:defense_response
GO:0080051:cutin_transport
GO:0046185:aldehyde_catabolic_process
GO:0043447:alkane_biosynthetic_process
GO:0009416:response_to_light_stimulus
GO:0009768:photosynthesis,_light_harvesting_in_photosystem_I
GO:0009644:response_to_high_light_intensity
GO:0048544:recognition_of_pollen
GO:0019253:reductive_pentose-phosphate_cycle
GO:0032922:circadian_regulation_of_gene_expression
GO:0007623:circadian_rhythm
down
up
down
up
down
up
Count
10
20
30
40
50
p.adjust
0.01
0.02
0.03
0.04

(B)

(C)

Figure 4.10 Enriched GO terms in each variety in the infected area. The GO enrichment analysis was carried out in each variety and independently for up and down-regulated DEGs. The graphs show the result in the biological process (A), cellular component (B), and molecular function (C). In purple and blue boxes are the enriched GO terms related to the cell wall synthesis/modification and to the defence response to pathogen, respectively.

4.3.6.1.2. DEGs outside the infected area

In contrast to inside the infected area, in the BP category, the upregulated genes were enriched with more terms in both resistant varieties (16 terms in Sarpo Mira and 14 in Sarpo Shona) compared with Duke of York (7 GO terms). However, the susceptible variety showed more enriched GO terms than the resistant varieties for the downregulated genes (2 GO terms in Sarpo Mira, 1 in Sarpo Shona, and 11 in Duke) (**Figure 4.11**). This was consistent with the number of DEGs previously observed in this region in **Figure 4.6**, where a smaller number of upregulated DEGs and a higher number of downregulated DEGs were observed in Duke than in the two resistant varieties. Outside the infected area, there were more enriched terms in common between both resistant varieties (5 enriched GO terms) than in the infected area (2 enriched GO terms). These 5 terms were “water transport”-GO:006833, “response to high light intensity”-0009644, “response to karrikin”-GO:80167, and 2 GO terms related to the circadian rhythm (GO:0032922 and GO:0007623), none of which were enriched in the susceptible Duke of York (**Figure 4.11**).

In addition, enriched GO terms in common within and outside of the infected areas were detected. In Sarpo Mira there were 7 enriched terms in common. These terms include 2 additional GO terms related to the response to light (“response to high light intensity” -GO:0009644 and “response to light stimulus" -GO:0009416), which were specific to this variety. The other 5 terms were related to circadian rhythm ("circadian rhythm" -GO:0007623 and "Circadian regulation of gene expression" -GO:0032922), photosynthesis ("Photosynthesis, light harvesting in photosystem I" -GO:0009768), "Reductive pentose-phosphate cycle" -GO:0019253) and the “Recognition to pollen” -GO:0048544 (**Figure 4.10-A**). In Sarpo Shona, there were three enriched terms in common. These GO terms were “regulation of transcription DNA-templated” -GO:0006355, “cutin transport” -GO:0080051, and "cellular response to hypoxia" -GO:0071456 (**Figure 4.10-B**). For Duke of York, all its 17 GO terms enriched outside the infected area were also enriched inside the infected area. These included 2 GO terms related to the cell wall for upregulated genes (“cell wall biogenesis” -GO:0042546 and “xyloglucan metabolic process” -GO:0010411) and 3 GO terms related to response to pathogen

infection for downregulated genes ("regulation of defense response" -GO:0031347, "response to chitin" -GO:0009611, "response to wounding" -GO:0009611). Although the term "protein phosphorylation" -GO:0006468 was enriched in both areas, it was enriched for upregulated genes in the infected area, but for downregulated genes outside the infected area (**Figure 4.10-C, Figure 4.12**).

Consequently, it is still observed that some transcriptomic changes induced by the infection may be propagated to surrounding areas, including changes in expression of genes relating to the cell wall components. Although these specific changes were not observed in Sarpo Mira, they were observed in Sarpo Shona and Duke, for cutin and xyloglucan, respectively. In addition, several of the GO terms enriched in both resistant varieties outside the infected area were responding to the change in light and circadian rhythm.

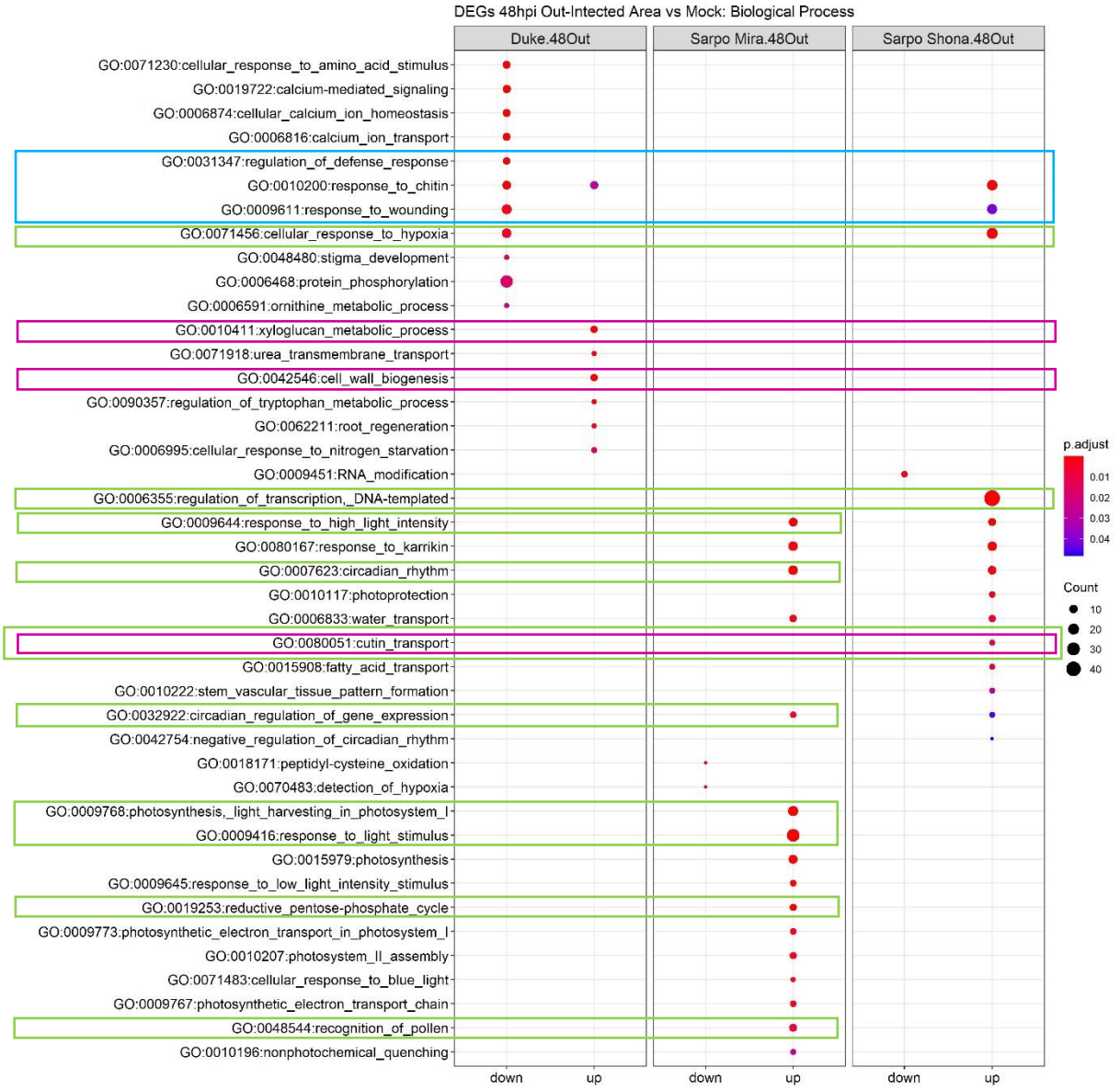
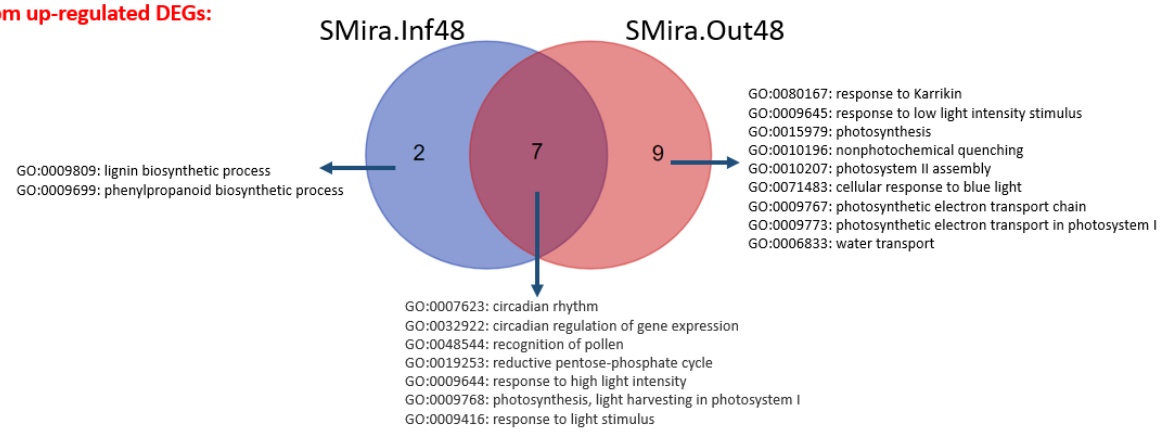


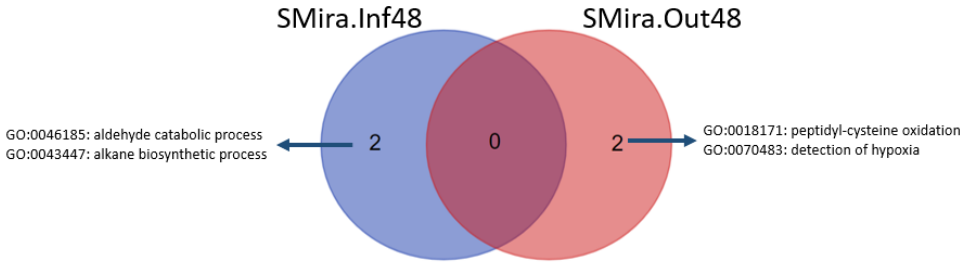
Figure 4.11 Enriched GO term in each variety outside the infected area. The enriched GO analysis was carried out in each variety and independently for up and down-regulated DEGs. The graph shows the enriched GO terms for biological process**.** In purple and blue are the enriched GO term related to the cell wall synthesis and in the defence response to pathogen, respectively. In green are enriched GO terms in common between inside and outside of the infected areas, for either Sarpo Mira or Sarpo Shona.

(A)

From up-regulated DEGs:

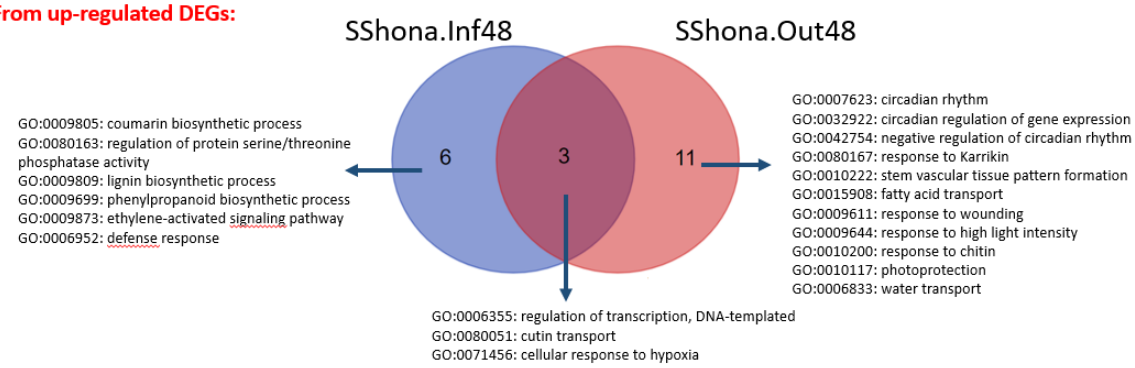


From down-regulated DEGs:

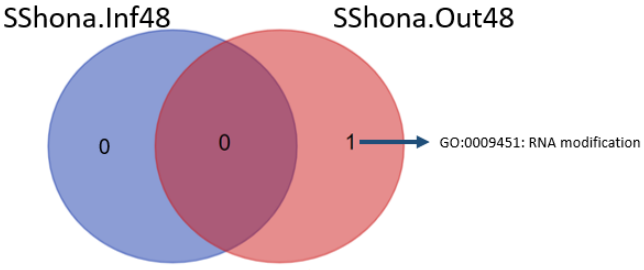


(B)

From up-regulated DEGs:



From down-regulated DEGs:



(C)

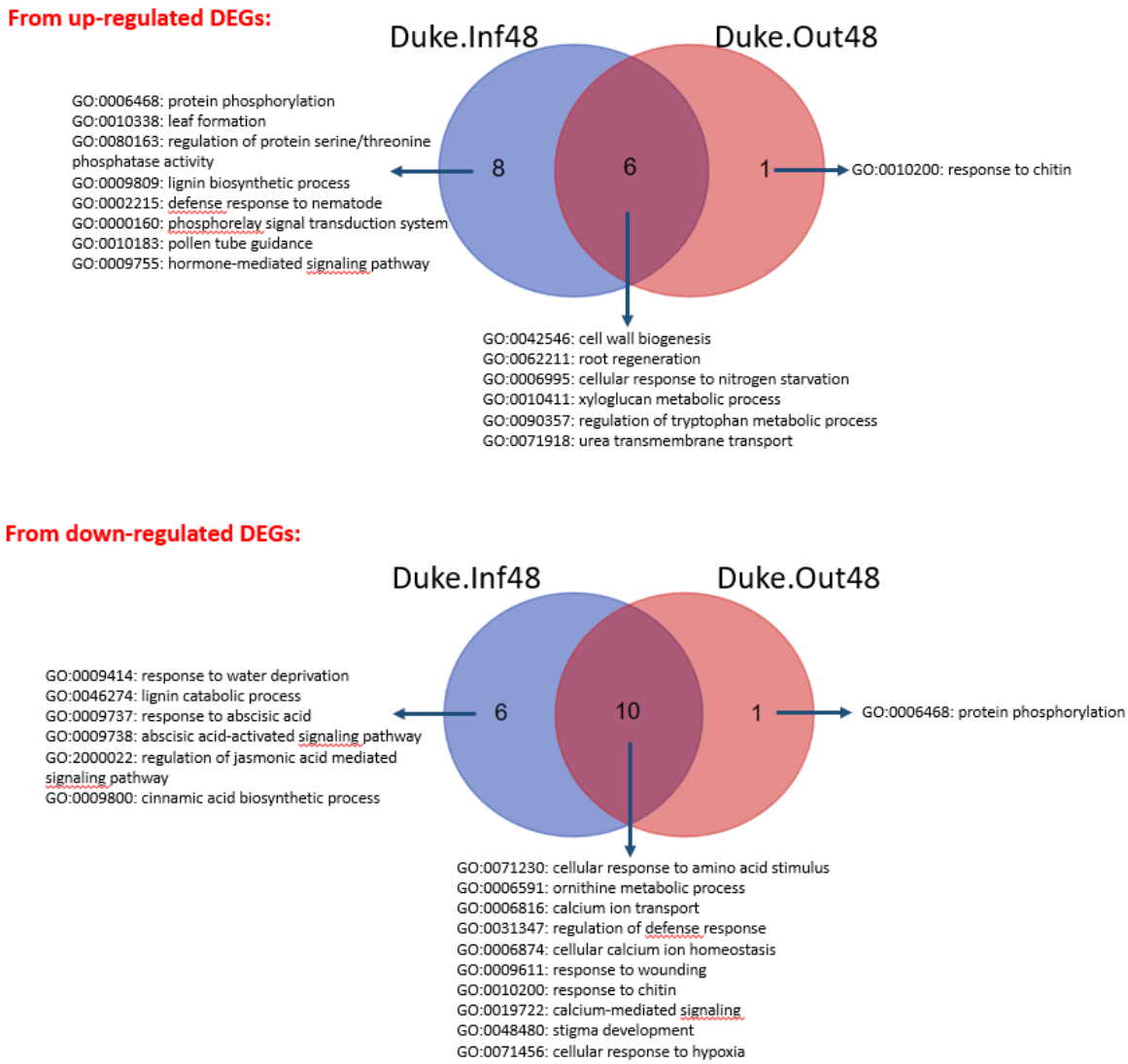


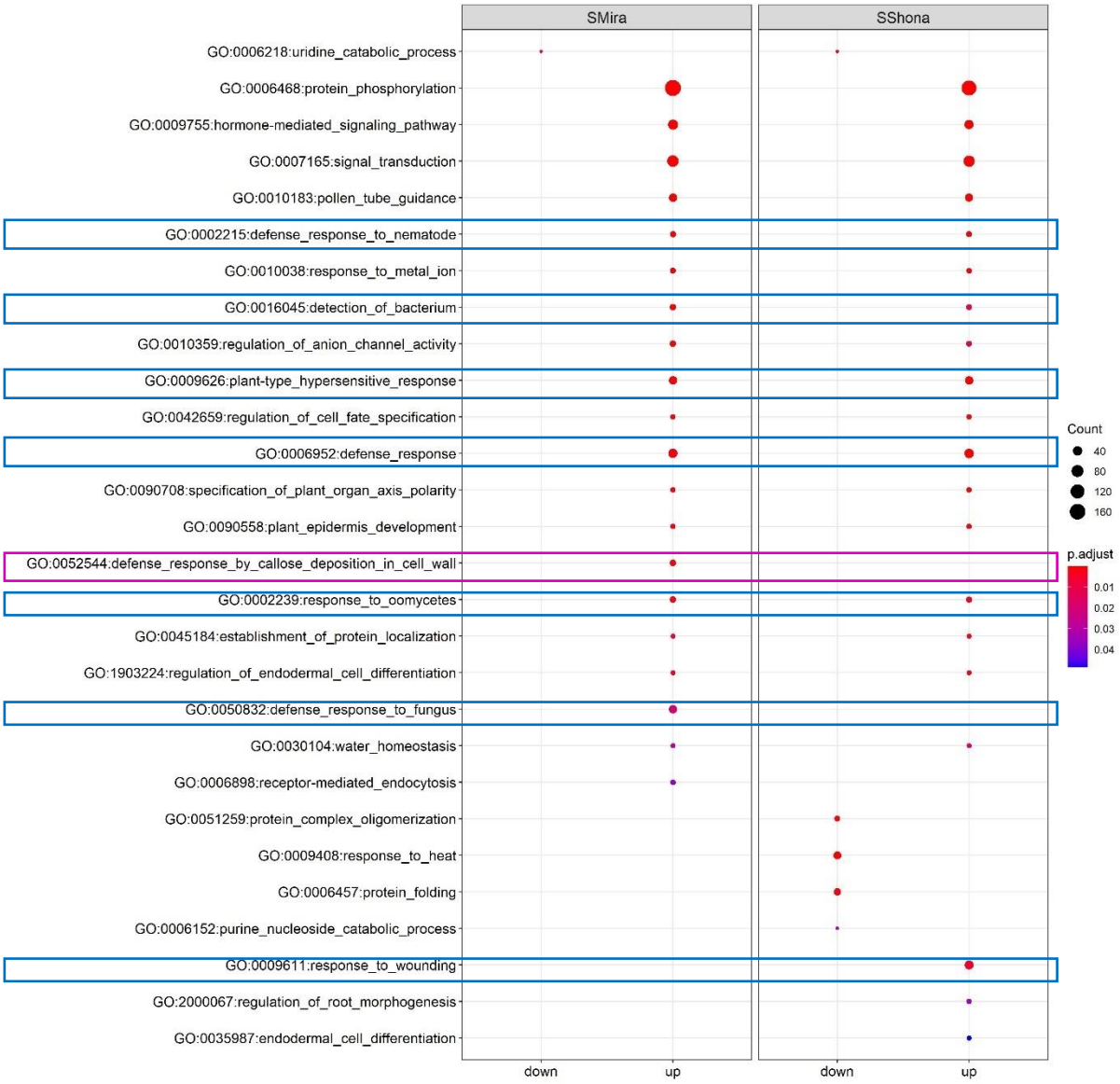
Figure 4.12 Enriched GO terms in common between inside and outside the infected area in each variety. The enriched GO terms are for each group of upregulated or downregulated DEGs in Sarpo Mira (A), Sarpo Shona (B), and Duke of York (C).

4.3.6.2. Genes consistently differentially expressed between resistant and susceptible varieties.

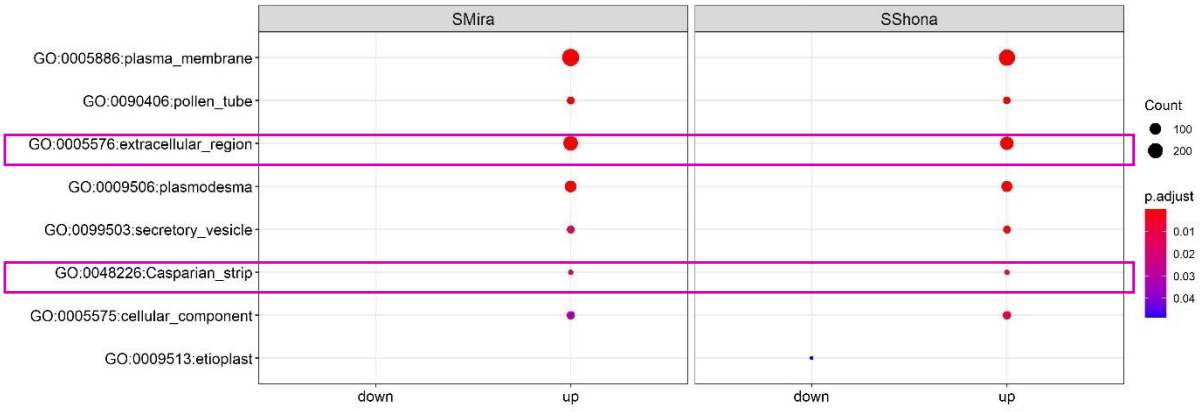
Enrichment analysis was carried out with genes with higher or lower expression during all treatments in Sarpo Mira (1149 genes and 982 genes, respectively) or Sarpo Shona (1065 genes and 919 genes, respectively) than in Duke of York (**Figure 4.9**). Many of these genes were enriched in terms related to the pathogen response and to cell wall modification, or more broadly related to the extracellular region (**Figure 4.13**).

For biological process, among 17 enriched GO terms in common between the two resistant varieties, 5 of them were related to the response to pathogen ("defense response to nematode" -GO:0002215, "detection of bacterium" -GO:0016045, "plant-type hypersensitive response" -GO:0009626, "defense response" -GO:0006952, and "response to oomycete" -GO:0002239), all of them containing genes with higher expression in the resistant varieties than in Duke. The only enriched GO term related to the cell wall modification was observed in Sarpo Mira ("defense response by callose deposition in cell wall"-GO0052544) (**Figure 4.13-A**). In the cellular component and the molecular function categories, more terms involved or possibly involved in the cell wall were found. Both resistant varieties were enriched with genes related to the "extracellular region" -GO:0005576, to the "casparian strip" -GO:0048226 (cellular component (**Figure 4.13-B**)), and with "glucan-endo-1,3-beta-D-glucosidase activity" (molecular function (**Figure 4.13-C**)). In addition, only Sarpo Mira was enriched for upregulated genes with a "UDP-glycosyltransferase activity" (molecular function (**Figure 4.13-C**)).

(A)


SMira
SShona
GO:0006218:uridine_catabolic_process
GO:0006468:protein_phosphorylation
GO:0009755:hormone-mediated_signaling_pathway
GO:0007165:signal_transduction
GO:0010183:pollen_tube_guidance
GO:0002215:defense_response_to_nematode
GO:0010038:response_to_metal_ion
GO:0016045:detection_of_bacterium
GO:0010359:regulation_of_anion_channel_activity
GO:0009626:plant-type_hypersensitive_response
GO:0042659:regulation_of_cell_fate_specification
GO:0006952:defense_response
GO:0090708:specification_of_plant_organ_axis_polarity
GO:0090558:plant_epidermis_development
GO:0052544:defense_response_by_callose_deposition_in_cell_wall
GO:0002239:response_to_oomycetes
GO:0045184:establishment_of_protein_localization
GO:1903224:regulation_of_endodermal_cell_differentiation
GO:0050832:defense_response_to_fungus
GO:0030104:water_homeostasis
GO:0006898:receptor-mediated_endocytosis
GO:0051259:protein_complex_oligomerization
GO:0009408:response_to_heat
GO:0006457:protein_folding
GO:0006152:purine_nucleoside_catabolic_process
GO:0009611:response_to_wounding
GO:2000067:regulation_of_root_morphogenesis
GO:0035987:endodermal_cell_differentiation
down
up
down
up
Count
40
80
120
160
p.adjust
0.01
0.02
0.03
0.04

(B)



(C)

Figure 4.13 Gene ontology of DEGs expressed higher or lower in any resistant than in Duke during all the treatments and region tissues. The group of DEGs with higher or lower expression are labelled as “up” or “down”, respectively. The graphs show the results the enriched GO terms separated by their biological process (A), their corresponding cellular component (B), and their molecular function (C). Purple square are the GO terms playing a role in the cell wall, apoplast or extracellular region, while blue square are the GO terms related to the response against a pathogen.

4.3.7. Genes relating to cell wall composition.

According to the GO enrichment analysis, the infection alters the composition of the cell wall, mainly in the hemicellulose, lignin, and cutin components. However, by observing the top 20 most up or down-regulated genes in both resistant varieties, other genes relating to components of the cell wall also undergo modifications, such as pectins and proteins (**Table 4.5**). To gain a better understanding of the role of different types of cell wall component in the response to infection, genes directly involved in the modification of any cell wall component were selected from up or down regulated DEGs observed inside the infected area in any of the three varieties. These included genes involved in the production of lignin, cellulose, hemicellulose, pectin, cutin, and cell wall proteins (**Figure 4.14**). As expected, based on the enrichment analysis (**Figure 4.10**-**A**), most of these genes were related to the production of lignin and to the modification of hemicellulose components.

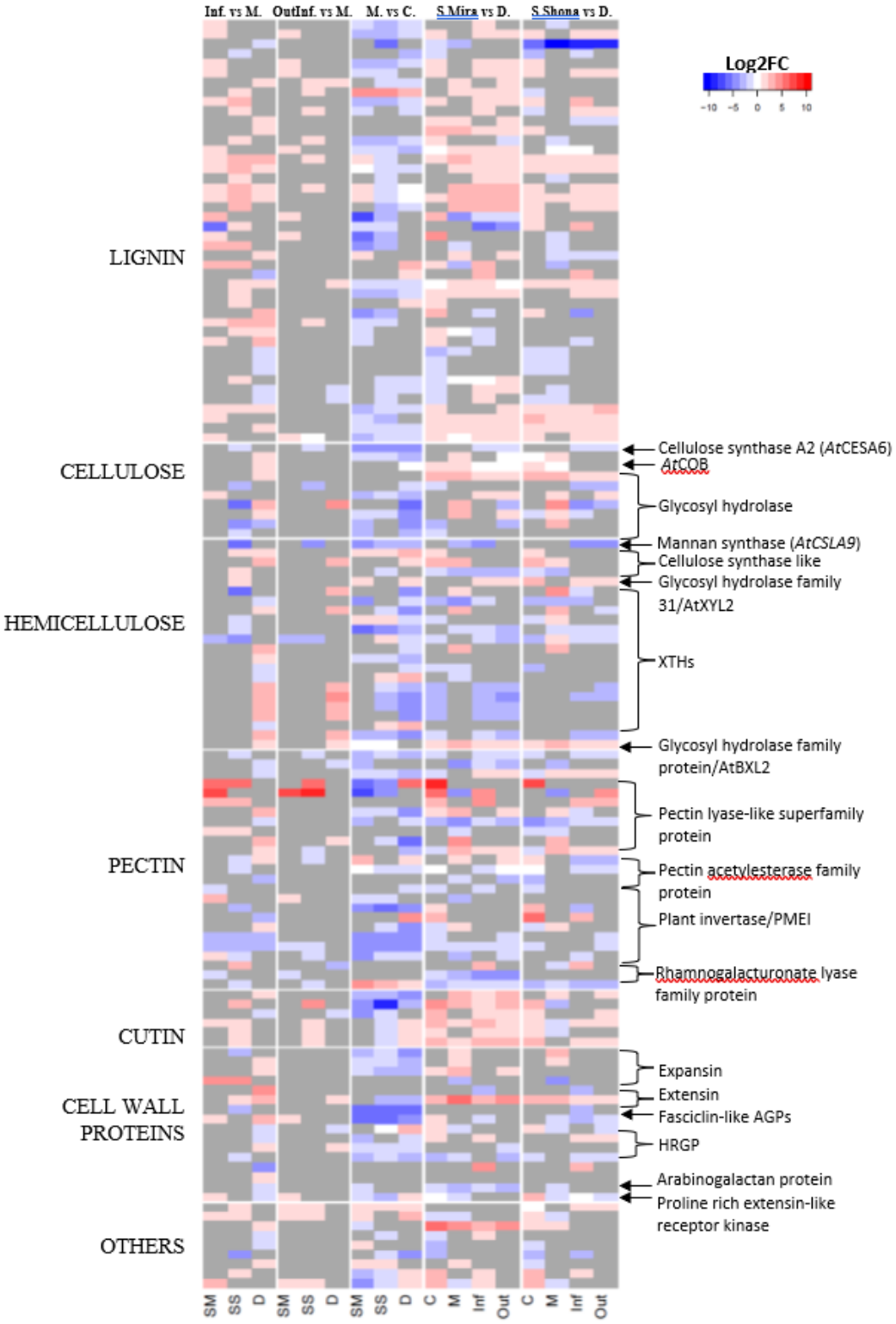


Figure 4.14 Heatmap of the DEGs related to the cell wall detected in the infected area 48 hours post-infection (Inf48 vs mock48: Inf. vs M.) in any of the 3 varieties (SM: Sarpo Mira, SS: Sarpo Shona, and D: Duke). Also shown is the expression of these genes outside the infected area (OutInf. vs M.) and in the mock (M. vs C.) and the comparison between each resistant variety and Duke (S.Mira vs D, or S.Shona vs D). In red are the DEGs whose expression was upregulated or whose expression was higher in the resistant variety. In blue are the DEGs whose expression was downregulated or whose expression was lower in the resistant variety. In grey are genes without significant differential expression.

4.3.7.1. Lignin

As observed in the GO enrichment analysis, a common response among the three varieties was the upregulation of DEGs related to lignin biosynthesis (GO:0009809) (**Figure 4.10**). Although within this term, Duke and Sarpo Mira had 11 DEGs, while Sarpo Shona had 16 (**Appendix-Table 4.2**), there was another group of DEGs related also to lignin synthesis that was not included in that GO term. These additional DEGs meant that the two resistant varieties had more upregulated genes related to lignin biosynthesis (19 and 20, respectively) than Duke (15) (**Figure 4.14**). Some of them had a significant difference both within and outside the infected area, including 7 upregulated genes in Sarpo Mira and 7 upregulated genes in Sarpo Shona (**Figure 4.14**), while only 2 were up- and 2 down- regulated in both areas in Duke of York. Indeed, Duke had more downregulated genes inside the infected area for the lignin biosynthesis process (7 genes), in comparison to Sarpo Mira (1 gene) and Sarpo Shona (1 gene) (**Figure 4.14**). Also, the GO term "lignin catabolic process" -GO:0046274 was only enriched in Duke for downregulated genes and contained DEGs encoding phenylalanine ammonia lyases (PALs), the first enzymes in the phenylpropanoid pathway that leads to monolignol biosynthesis (**Figure 4.10**).

By comparing the expression of each resistant variety with Duke, some of these genes had a higher expression in both resistant varieties, mainly in Sarpo Mira, than in Duke in the infected and outside the infected area, and even in the uninfected tissues (control and mock). Therefore, the resistant varieties are not only expressing lignin-related genes at a higher level in response to infection, but also in the absence of any infection, which could lead to more changes of lignin deposition in the cell wall. Many genes were consistently upregulated after any treatment (in mock, in the infected, and outside the infected area, In Sarpo Mira: *Soltu.DM.12G009430, Soltu.DM.10G029960, Soltu.DM.10G029970, Soltu.DM.10G030050, Soltu.DM.03G021440, Soltu.DM.02G019030, Soltu.DM.02G028550, Soltu.DM.10G014940*; while in Sarpo Shona: *Soltu.DM.10G029970,*

Soltu.DM.10G029990, Soltu.DM.10G030050, Soltu.DM.03G021440, Soltu.DM.05G026870, Soltu.DM.02G028550, Soltu.DM.10G014940).

4.3.7.2. Cellulose

Ten cellulose-related genes were identified as DEGs among the three varieties, two of them involved in the synthesis of cellulose (*Soltu.DM.04G027320* -Cellulose synthase A2 (*AtCESA6*), *Soltu.DM.02G009140* -COBRA-like extracellular glycosyl-phosphatatidil inositiol-anchored protein family (*AtCOB*)), and eight with a function of cellulase or endoglucanase (*Soltu.DM.11G003810, Soltu.DM.01G005250, Soltu.DM.01G050190, Soltu.DM.02G023970, Soltu.DM.07G016430, Soltu.DM.08G028990, Soltu.DM.12G008400, Soltu.DM.09G023670*). From these ten genes, Sarpo Mira only upregulated one cellulase, while Sarpo Shona downregulated 5 genes, 1 involved in the synthesis and 4 in the degradation of cellulose. Duke upregulated 1 gene for the synthesis of cellulose and 4 cellulases, but also downregulated another cellulase. Then, Sarpo Shona might be preventing the degradation of the cellulose component by downregulating more cellulases, while in Duke the response is not clear and in Sarpo Mira the response was minimal (**Figure 4.14**).

4.3.7.3. Hemicellulose

In at least one variety, 22 DEGs related to hemicellulose synthesis/modification changed their expression after the infection (Inf-48 vs Mock-48). According to the enrichment analysis, (**Figure 4.10-A**), Duke showed a greater response than Sarpo Mira or Sarpo Shona and this response was specifically for upregulation of xyloglucan related genes. In Duke, most of these genes encode for xyloglucan endotransglucosylase/hydrolases (XTHs) (8 up- and 4 down- regulated), which are involved in cell wall modification, by both disassembly or re-ligating the union between cellulose and xyloglucan. Furthermore, genes encoding XTHs had a higher expression at 48 h.p.i in Duke than in both resistant varieties ([S.Mira vs D] and [S.Shona vs D] in **Figure 4.14**). Also, this variety upregulated 2 cellulose synthase-like (CSL) genes, which are involved in hemicellulose backbone

synthesis, and 1 glycosyl hydrolase gene, involved in xylan remodelling (in *A. thaliana*: AtBXL2) (**Figure 4.14**). This suggests that Duke tends to modify its hemicellulose component by changing its state of looseness or xylan composition.

The response of the two resistant varieties was markedly different to Duke. Sarpo Mira only responded by downregulating one XTH, while Sarpo Shona downregulated five genes for xyloglucan modification and backbone synthesis, and one mannan synthase (*AtCSL9*); and upregulated one cellulose synthase-like and one glycosyl hydrolase (*AtXYL2*), this last one involved in releasing xylose from the cell wall (**Figure 4.14**).

4.3.7.4. Pectin

The majority of upregulated genes relating to pectin were pectin-lyase like superfamily proteins, more for Sarpo Mira and Sarpo Shona, but less for Duke of York (**Table 4.8**). Indeed, *Soltu.DM.01G027350*, one of the most up-regulated gene in this group, was up-regulated in both resistant varieties only (**Table 4.5**). By comparing the expression of each resistant variety with Duke of York, some of these genes showed higher, while other a lower expression, during the different treatments. Meanwhile, two polygalacturonases (PGs) had consistently lower expression in the resistant varieties compared with Duke of York although one of them did not significantly change its expression at 48 h.p.i. (**Table 4.8**).

On the other hand, genes coding for plant invertase/pectin methylesterase inhibitors (PMEIs) were mostly downregulated in the three varieties. As pectin lyases degrade pectin, resistant varieties may generate more pectin fragments (**Table 4.8**). By inhibiting PMEI enzymes that modulate the expression of pectin methylesterase (PME), less methylesterified pectin could be present at 48 h.p.i.

Additional modification could be probably observed in the at 48 h.p.i. in the pectin component of the wall, related to acetylation and rhamnogalacturonan content. Genes encoding pectin acetylesterase (PAE) family proteins were downregulated in Sarpo Shona, while in Duke of York upregulation and

downregulation of these genes was observed. Genes for rhamnogalacturonate lyase family protein were downregulated in Sarpo Mira, but upregulated in Sarpo Shona. While less acetylated pectin could be a variety-specific resistant phenotype, degradation of rhamnogalacturonan is less clear.

4.3.7.5. Cutin

All the DEGs genes related to this cell wall component were involved in cutin transport. As revealed in the enrichment analysis (**Figure 4.10**-A), Sarpo Shona upregulated more genes (5 DEGs) than Sarpo Mira (1 DEG) and Duke (1 DEG). Although the response of cutin-related genes was not common between the two resistant varieties, it might offer a specific type of resistance in Sarpo Shona. However, the same cutin related genes were expressed at a significantly higher level in both resistant varieties than in Duke in control leaves.

4.3.7.6. Cell wall proteins

The three varieties upregulated and downregulated these genes to different degrees, with no clear pattern related to resistance or susceptibility. However, both resistant varieties highly upregulated one gene that encodes an "expansin-like B1"(EXLB1)-*Soltu.DM.08G001190*, which was also one of the 20 most upregulated genes in common between them, but not DE in Duke of York (**Table 4.5**). Variety-specific response was observed in Sarpo Mira with the upregulation of a proline-rich extensin-like receptor kinase (PERK), and in Sarpo Shona with the downregulation of a Fasciclin-like arabinogalactan.

Duke also showed up-regulation of expansin genes (*Soltu.DM.06G004100, Soltu.DM.06G009010*) and extensin genes (*Soltu.DM.04G026420, Soltu.DM.08G025180*), and within their downregulated genes were 2 hydroxyproline-rich glycoprotein (HGRP) family proteins (*Soltu.DM.01G007280, Soltu.DM.01G045040*), one (PERK) (*Soltu.DM.05G002190*), and one arabinogalactan protein (*Soltu.DM.04G030280*).

Among the genes with a consistently higher or lower expression in the resistant varieties than the susceptible Duke of York, most of them did not have a significant difference in the infected or outside the infected areas compared with mock samples in the resistant varieties, but some of them did in Duke (**Figure 4.15**). In this group, most of the genes related to callose synthesis had a higher expression in Sarpo Mira and most of the glycosyl hydrolases in both resistant varieties.

Figure 4.15 Heatmap of the DEG related to the cell wall with consistently higher or lower expression in any of the two resistant varieties than in Duke during all treatments. The heatmap shows the log2-foldchange from the comparison between Sarpo Mira or Sarpo Shona with Duke (S.Mira vs D. and S.Shona vs D., respectively) in any treatment (C: control, M: Mock, Inf: Infected area, and Out: Outside infected area). Also shown the log2-foldchange of these genes from control to the infected area and out-infected area at 48 hours post-infections (Inf. vs M. and OutInf. vs M., respectively), and from control to mock (M. vs C.) in any of the three varieties (SM: Sarpo Mira, SS: Sarpo Shona, and D: Duke of York). The red and blue colours indicate a significant positive or negative log2-foldchange, respectively, while grey indicates a no significant log2-foldchange.

Table 4.8 Genes from pectin lyase-like superfamily proteins and polygalacturonase enzymes differentially expressed inside the infected area at 48.p.i. or whose expression were consistently higher or lower in the resistant varieties than in Duke of York. Also shows the log2-FC from mock to outside the infected area at 48 h.p.i (OutInf. area vs Mock). SM: Sarpo Mira, SS: Sarpo Shona, D: Duke of York. C: control, M: Mock, Inf: Infected region at 48 h.p.i, and OutInf.: Outside the infected region at 48 h.p.i. Red and blue indicate a higher or lower log2-foldchange. No values indicates that there was not a significant difference in the respective comparison.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Function	Inf. vs Mock			OutInf. vs Mock			Mock vs Control			Sarpo Mira vs Duke				Sarpo Shona vs Duke			
		SM	SS	D	SM	SS	D	SM	SS	D	C	M	Inf	OutInf	C	M	Inf	OutInf
		LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC	LFC
Soltu.DM.12G026520	Pectin lyase-like superfamily protein							-5.15			1.91			-2.97	-3.30	-3.42	-3.19	-2.55
Soltu.DM.08G024910	Pectin lyase-like superfamily protein									-3.05	-5.84	-2.96	-2.29	-2.80	-6.83	-2.45	-3.36	
Soltu.DM.01G027350	Pectin lyase-like superfamily protein	5.68	5.49			6.02		-5.78	-4.80	6.03	8.39				7.11			
Soltu.DM.02G010210	Pectin lyase-like superfamily protein	7.93			6.83	8.23		-7.62	-4.56		5.84	-4.74	3.81			-4.90		4.39
Soltu.DM.02G011180	Pectin lyase-like superfamily protein	3.40	3.24								2.59		3.99				3.46	2.97
Soltu.DM.02G033640	Pectin lyase-like superfamily protein			3.15				-2.18	-1.74	-3.82	1.13	2.77		1.14		1.79	-1.46	
Soltu.DM.07G014640	Pectin lyase-like superfamily protein			-1.97				-3.13		-1.20	-3.18	-5.11	-1.88	-2.77	-3.83	-3.36	-1.70	-2.05
Soltu.DM.08G028230	Pectin lyase-like superfamily protein	1.46	1.09									-1.44			-0.89	-1.34		
Soltu.DM.09G028450	Pectin lyase-like superfamily protein			2.78			1.86		-1.48	-5.25		3.87				2.81		
Soltu.DM.05G008440	Polygalacturonase		-1.61			-1.85		4.04	3.00	1.86	-3.24	-1.06	-1.43	-1.36	-2.56	-1.42	-2.80	-2.80
Soltu.DM.06G028510	Polygalacturonase								-2.99		-5.14	-4.33	-4.77	-5.05	-5.75	-8.71	-9.25	-9.35

4.3.8. DEGs relating to defence against pathogens

In this group of genes relating to the defence response against biotic stress, the two resistant varieties showed fewer DEGs in the infected area than Duke. This pattern was also observed outside the infected area (**Figure 4.16**). However, if we compare the expression of these genes in each resistant variety and Duke, most of them had a higher expression in the two resistant varieties than in the susceptible, particularly for the group of PR and PRR proteins (**Figure 4.16**).

Among the DEGs encoding PRR proteins were WAK receptors, which recognize pectin fragments called OGAs generated after pathogen infection. Of the 6 WAK receptors that were DE in any of the three varieties in the infected area, none of them were DE in Sarpo Mira, 3 were DE in Sarpo Shona, and 3 in Duke. However, although some of the WAK receptors did not show a significant change after 48 h.p.i. in the resistant varieties, 4 out of the 6 WAKs had a higher expression in these varieties than in Duke at this time point inside the infected area (In Sarpo Mira: *Soltu.DM.11G025150, Soltu.DM.10G019470, Soltu.DM.09G000290, Soltu.DM.09G009220*, in Sarpo Shona: *Soltu.DM.11G025150, Soltu.DM.10G019470, Soltu.DM.09G008370, Soltu.DM.09G009220*). Indeed, from the 6 WAKs, 3 (*Soltu.DM.11G025150, Soltu.DM.10G019470, Soltu.DM.09G000290*) and 2 (*Soltu.DM.11G025150, Soltu.DM.10G019470*) genes had a significantly higher expression in Sarpo Mira or Sarpo Shona, respectively, across all treatments (Control, Mock-48, Inf-48, and OutInf-48) compared with Duke (**Table 4.9**).

Among the R genes, of the 6 genes that upregulated their expression in Sarpo Mira in the infected area, 2 (*Soltu.DM.02G003760* and *Soltu.DM.06G011640*) were also upregulated the outside infected area, and 1 (*Soltu.DM.04G001850*) had a higher expression than in Duke across all the treatments. Another 8 R genes had a higher expression in Sarpo Mira than in Duke, although were not significantly different inside the infected area (**Table 4.10**).

Of the 11 R genes in Sarpo Shona that upregulate their expression in the infected area, 6 of them were also upregulated outside the infected area, and another 2 had a higher expression than Duke in all the treatments. In addition, 9 R genes in Sarpo Shona had a higher expression level than Duke in all the conditions, but without being DE inside the infected area. Two of them had a very high and almost constant expression with more than 9 log2FC compared with Duke (*Soltu.DM.01G003400, Soltu.DM.04G001710*). (**Table 4.10**).

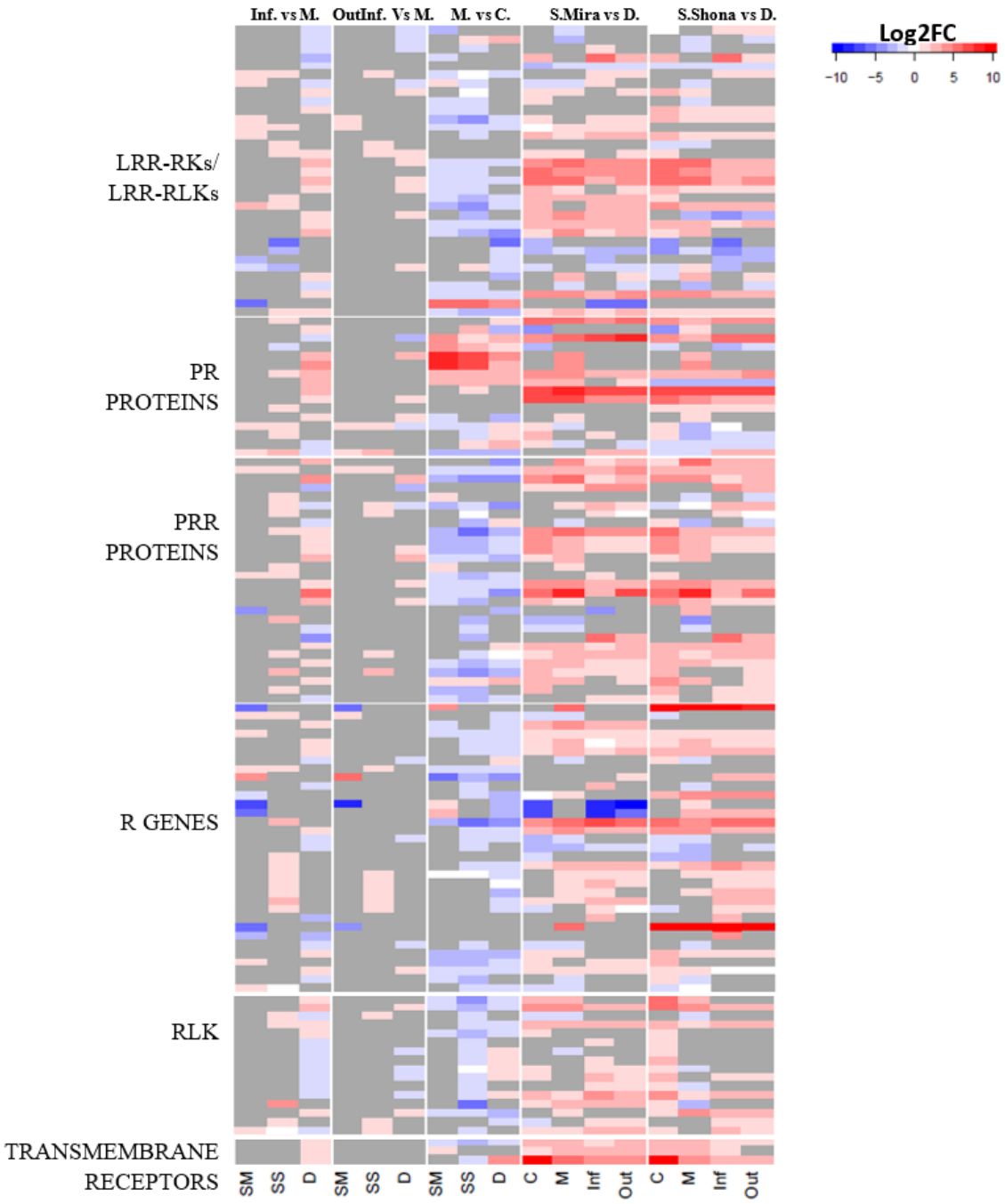


Figure 4.16 Heatmap of the DEGs in the infected area (Inf-48 vs mock-48) that encode transmembrane receptors (PRRs), intracellular receptors (R genes), and other genes that detect or combat pathogen infection.

Table 4.9 WAK proteins that were DE in any of the three varieties in the infected area. The table shows the log2-foldchange of the genes, where (-) means not significant expression. The table also shows the expression of these genes outside the infected area and if there is a significantly higher or lower (in log2FC) expression of any of these genes in each resistant variety compared with Duke.

Soltub v.6 Gene ID	Soltub v.6 Gene Function	*A. thaliana* gene ID	*A. thaliana* gene name	Infected area			Outside infected area			Sarpo Mira vs Duke				Sarpo Shona vs Duke			
				Sarpo Mira	Sarpo Shona	Duke	Sarpo Mira	Sarpo Shona	Duke	Control	Mock	Inf. area	Out. Inf.	Control	Mock	Inf. area	Out. Inf.
Soltu.DM.11G025150	wall associated kinase	AT1G21270	WAK2	-	1.13	-	-	0.96	-	1.84	2.09	2.84	2.81	2.24	0.83	2.33	2.14
Soltu.DM.10G019470	wall associated kinase-like	AT1G21230	WAK5	-	-	1.62	-	-	-	2.53	2.37	1.45	1.79	3.37	2.65	1.68	1.78
Soltu.DM.09G000290	wall-associated kinase	AT1G21270	WAK2	-	2.46	-	-	2.49	-	2.88	2.05	1.68	1.63	2.20	-	-	1.79
Soltu.DM.09G008330	wall-associated kinase	AT1G21270	WAK2	-	-	1.39	-	-	-	3.38	2.13	-	1.40	3.98	2.45	-	1.17
Soltu.DM.09G008370	wall-associated kinase	AT1G21270	WAK2	-	2.04	-	-	-	-	2.61	-	-	-	3.08	-	1.56	1.50
Soltu.DM.09G009220	wall-associated kinase	AT1G21230	WAK5	-	-	-1.16	-	-	-	-	-	1.71	1.55	1.72	-	1.49	1.05

Table 4.10 R genes DE in any of the three varieties inside the infected area (Inf-48 vs Mock-48). The table shows the log2-foldchange of the genes, where (-) means non-significance.

Soltub v.6 Gene ID	Soltub v.6 Gene Function	Infected area			Outside-Inf. area			Sarpo Mira vs Duke				Sarpo Shona vs Duke			
		Sarpo Mira	Sarpo Shona	Duke	Sarpo Mira	Sarpo Shona	Duke	Control	Mock	Inf. area	Out. Inf.	Control	Mock	Inf. area	Out. Inf.
Soltu.DM.01G003400	NB-ARC domain-containing disease resistance protein	-5.21	-	-	-5.14	-	-	-	5.31	-	-	9.70	9.18	9.08	8.98
Soltu.DM.02G003760		1.22	0.76	-	1.30	-	-	-1.60	-0.90	-	-	-0.76	-	-	-
Soltu.DM.04G001800		-	-	1.41	-	-	-	3.46	3.93	2.89	3.28	-	-	-	-
Soltu.DM.04G001850		1.14	-	-	-	-	-	1.47	1.67	1.87	2.03	1.55	1.77	1.63	1.64
Soltu.DM.04G003630		-	-	1.42	-	-	-	0.97	2.18	0.62	1.38	1.95	2.25	1.42	1.77
Soltu.DM.04G006280		-	-	1.14	-	-	-	1.58	2.84	2.02	2.17	2.47	2.81	2.22	2.43
Soltu.DM.05G000210		-	-	-1.24	-	-	-1.20	-	-1.22	-	-	0.89	-1.03	-	-
Soltu.DM.06G004990		0.84	1.15	-	-	0.96	-	-	-	-	-	-	-	-	-
Soltu.DM.06G011640		4.67	-	-	5.33	-	-	-	-	-	1.97	-	-	2.92	2.70
Soltu.DM.06G020880		-	-	-1.67	-	-	-	-	-	2.30	-	-	-1.21	-	-
Soltu.DM.08G000870		-1.15	-	-	-	-	-	-0.65	0.94	-	-	2.35	3.73	3.98	3.61
Soltu.DM.08G000910		-6.86	-	-	-7.76	-	-	-6.33	-	-8.91	-9.99	-	1.95	-	-
Soltu.DM.08G000930		-6.12	-	-	-	-	-	-7.13	-	-7.72	-5.23	-	3.16	2.61	2.46
Soltu.DM.08G015940		-	2.28	-	-	-	-	4.72	5.93	6.28	6.08	5.34	3.78	5.74	5.58
Soltu.DM.08G018740		-	-	1.44	-	-	-	2.85	4.47	2.81	2.75	3.59	4.25	2.70	3.17
Soltu.DM.08G021580		-	-	-1.49	-	-	-1.72	-2.78	-1.70	-	-	-1.95	-1.63	-	-
Soltu.DM.09G029540		-	-	-1.29	-	-	-	-3.36	-2.76	-1.16	-1.95	-0.91	-2.22	-	-1.91
Soltu.DM.10G022100		-	1.60	-	-	-	-	-1.77	-	-	-	-2.56	-2.43	-	-
Soltu.DM.11G007110		-	1.38	-	-	-	-	1.35	2.95	3.42	2.32	1.40	2.17	4.18	2.60
Soltu.DM.11G007180		-	1.09	-	-	0.90	-	-	0.90	1.59	1.42	-	0.75	1.92	1.68
Soltu.DM.11G007200		-	1.27	-	-	0.95	-	-	0.79	2.30	1.69	-	-	1.77	1.43
Soltu.DM.11G007330		-	1.73	-	-	1.46	-	-	1.24	0.99	-	-	1.36	2.46	2.14
Soltu.DM.11G007390		-	2.39	-	-	1.98	-	0.94	2.04	2.55	2.13	-	-	2.70	2.45
Soltu.DM.11G007410		-	1.62	-	-	1.42	-	-0.93	-	0.73	0.51	-1.01	-	1.84	1.77
Soltu.DM.11G025480		-	-	-3.10	-	-	-	-	-	2.47	-	-	-	3.00	-
Soltu.DM.04G001710	Putative late blight resistance protein homolog R1A-3	-5.69	-	-	-4.66	-	-	-	5.80	-	-	9.61	10.08	10.31	10.23
Soltu.DM.11G007030	Putative late blight resistance protein homolog R1C-3	-2.94	-	-3.09	-	-	-	-	-	-	-	-	-	3.55	-
Soltu.DM.02G011360	disease resistance family	-	-	-1.51	-	-	-1.42	-1.66	-1.81	-	-	-	-1.75	-	-

Soltu.DM.07G001090	protein / LRR family protein	-	-	1.77	-	-	-	1.92	1.61	-	-	2.10	2.08	1.03	1.04
Soltu.DM.07G004320		1.49	-	-	-	-	-	1.95	-	0.92	0.84	2.24	-	-	-
Soltu.DM.04G003570	Disease resistance protein (CC-NBS-LRR class) family	-	-	1.08	-	-	0.89	1.69	2.05	1.25	1.10	1.24	1.29	0.69	0.53
Soltu.DM.08G003430	Disease resistance protein (NBS-LRR class) family	-	-	-1.11	-	-	-	-	-1.68	-	-	0.94	-1.22	-	-
Soltu.DM.02G004040	disease resistance protein (TIR-NBS-LRR class), putative	1.01	0.65	-	-	-	-	-1.78	-0.99	-	-	-1.00	-0.51	-	-

Since R genes trigger a hypersensitive response and generate resistance against pathogens, and the WAK receptors recognize damage in the cell wall and have been previously associated with a resistant phenotype, it was decided to identify if these genes tended to have a consistently higher or lower expression in the resistant varieties compared with Duke.

As observed with the genes related to the cell wall (**Figure 4.15**), the majority of these genes with consistently higher or lower expression in the resistant varieties than in Duke did not have a significant difference inside (Inf48 vs Mock48) or outside (OutInf48 vs Mock48) the infected area (**Figure 4.17**). In the resistant varieties, a greater proportion of R genes had a consistently higher rather than lower expression. Interestingly, *Soltu.DM.02G001820* was the most highly expressed R gene and was consistently overexpressed in both resistant varieties in all treatments. The second most highly expressed consistent R gene, *Soltu.DM.04G001710*, was only consistently overexpressed in Sarpo Shona and was annotated as a "putative late blight resistance protein homolog R1A-3". Neither of these two genes had a significant difference inside the infected area (**Figure 4.17**). Most of these R genes did not significantly change their expression in response to the infection itself. Both *Soltu.DM.02G001820* and *Soltu.DM.04G001710*, and 5 other R genes with higher expression in the resistant varieties, located within a region of chromosome 4 of reference genotype DM, where additional R genes were annotated (**Figure 4.18**).

More WAK genes had also a constitutively higher expression in the resistant varieties than in Duke, 7 in Sarpo Mira and 6 in Sarpo Shona. Only one WAK gene in Sarpo Mira (*Soltu.DM.09G000280*) and another in Sarpo Shona (*Soltu.DM.09G009180*) had a lower expression than the susceptible variety (**Table 4.11**). In addition, 6 beta-1,3-glucanases that were not differentially expressed inside or outside of the infected areas in the resistant varieties, showed higher expression in these varieties than in Duke in almost all treatments (**Table 4.12**), including *Soltu.DM.01G005190* which was in the top 20 consistent DEGs (**Table 4.7**).

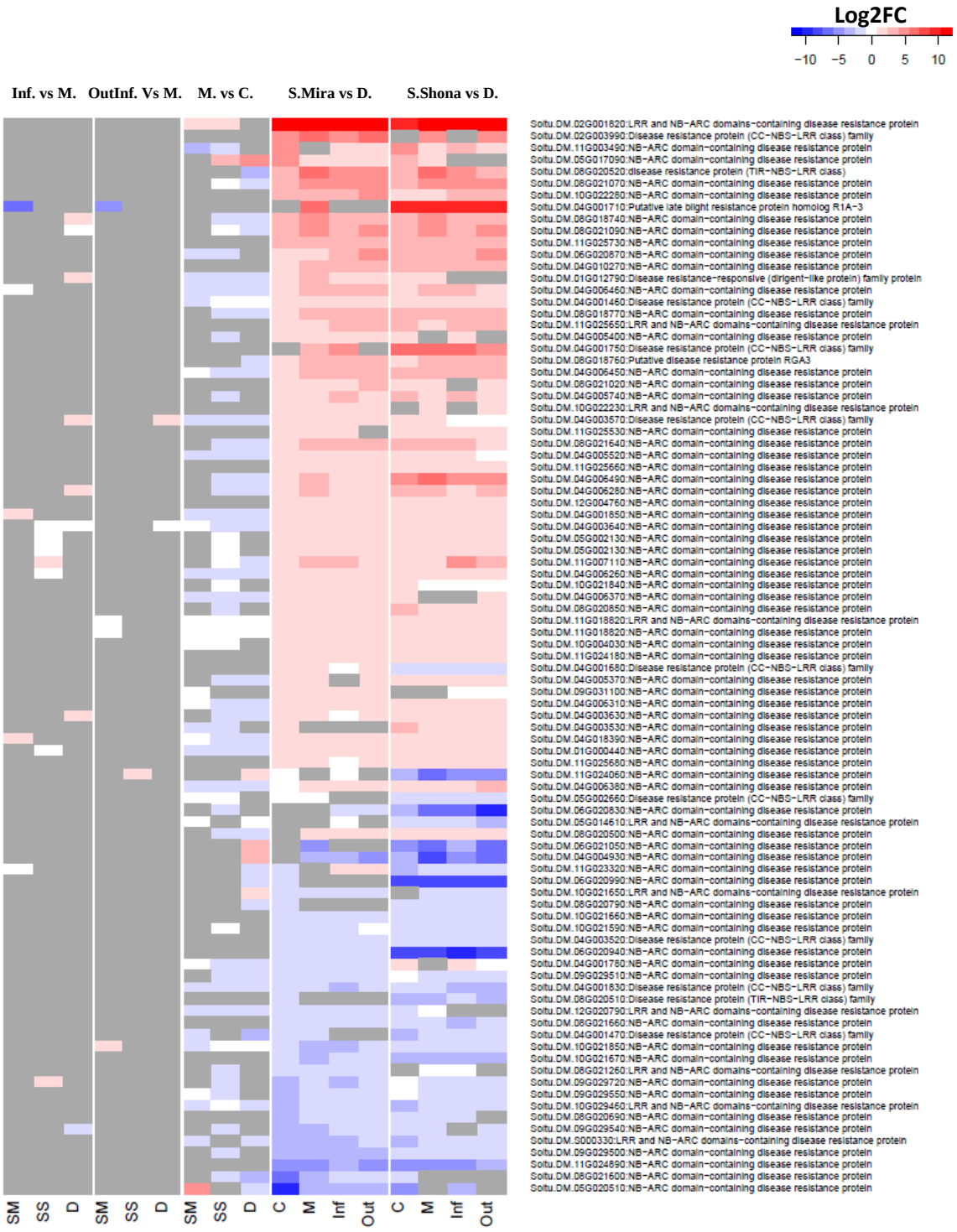


Figure 4.17 R genes with a consistently higher or lower expression in either of the two resistant varieties compared with Duke during all the treatments.

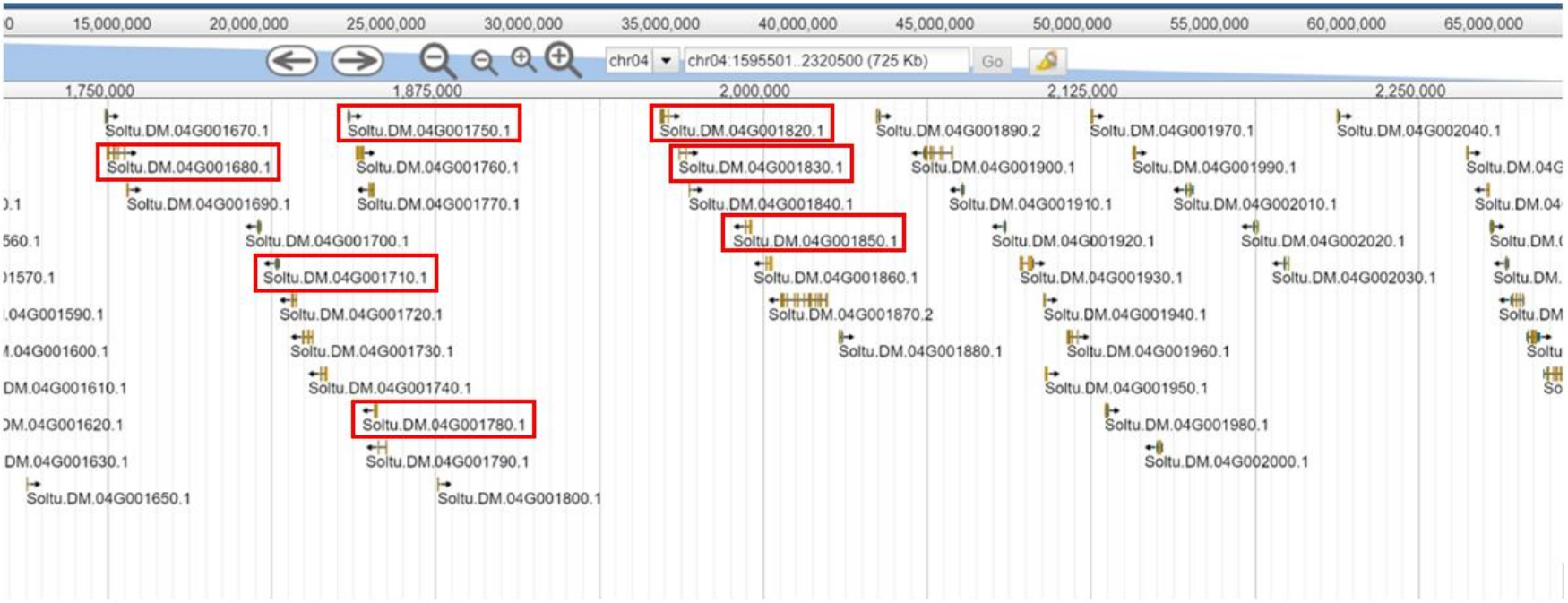


Figure 4.18 Region of potato chromosome IV containing a cluster of R genes. Some of these genes (boxed in red) had a constitutively higher expression in the resistant varieties than in the susceptible Duke of York, in control leaves, mock infected leaves and infected leaves.

Table 4.11 WAK genes with a consistently higher or lower expression in the resistant varieties than in Duke of York. Also shows the log2-foldchange of these genes from control to the inside and outside the infected area at 48 h.p.i. Red and blue indicate a higher or lower log2-foldchange, respectively. No values indicates that there was not a significant difference in the respective comparison.

Soltub v.6 Gene ID	Soltub v.6 Gene Function	Infected area vs Mock			Outside-Inf. area vs Mock			Sarpo Mira vs Duke				Sarpo Shona vs Duke			
		SM	SS	D	SM	SS	D	Control	Mock	Inf. Area	Out.Inf.	Control	Mock	Inf. Area	Out.Inf.
Soltu.DM.09G000300	wall-associated kinase	-	-	-	-	-	-	6.96	6.04	3.66	4.49	7.76	3.61	-	-
Soltu.DM.09G000290		-	2.46	-	-	2.49	-	2.88	2.05	1.68	1.63	2.20	-	-	1.79
Soltu.DM.10G019550		-	-	-	-	-	-	2.00	1.21	1.70	2.02	2.44	1.53	1.22	1.81
Soltu.DM.10G019480		-	-	-	-	-	-	1.99	1.67	1.54	1.77	2.63	1.73	1.56	1.53
Soltu.DM.11G025150		-	1.13	-	-	0.96	-	1.84	2.09	2.84	2.81	2.24	0.83	2.33	2.14
Soltu.DM.09G009230		-	-	-	-	-	-	1.77	1.79	2.34	2.22	3.02	2.40	3.61	3.04
Soltu.DM.09G009180		-	-	-	-	-	-	-	1.11	-	0.75	-1.48	-2.21	-2.08	-2.21
Soltu.DM.09G009250		-	-	-	-	-	-1.77	-	-	2.22	2.84	2.14	1.61	3.14	3.62
Soltu.DM.09G000280		-	-	-	-	-	-	-1.91	-3.75	-3.08	-4.99	-	-	-	-
Soltu.DM.10G019470	wall associated kinase-like	-	-	1.62	-	-	-	2.53	2.37	1.45	1.79	3.37	2.65	1.68	1.78

Table 4.12 Beta 1,3-glucanase genes with a consistently higher or lower expression in the resistant varieties than in Duke of York. Also shows the log2-foldchange of these genes from control to the inside and outside the infected area at 48 h.p.i. Red and blue indicate a higher or lower log2-foldchange, respectively. No values indicates that there was not a significant difference in the respective comparison.

Soltub v.6 Gene ID	Soltub v.6 Gene Function	Infected area vs Mock			OutInfected area vs Mock			Sarpo Mira vs Duke				Sarpo Shona vs Duke			
		Sarpo Mira	Sarpo Shona	Duke	Sarpo Mira	Sarpo Shona	Duke	Control	Mock	Inf. area	OutInf.	Control	Mock	Inf. area	OutInf.
Soltu.DM.01G005190	beta-1,3-glucanase	-	-	-	-	-	-	9.61	12.19	8.32	10.45	8.10	11.29	7.70	9.99
Soltu.DM.01G005260		-	-	2.01	-	-	1.57	6.48	6.51	4.14	4.37	5.11	4.86	3.36	3.32
Soltu.DM.01G005210		-	-	-	-	-	-	4.46	4.62	3.25	3.60	2.12	2.62	1.84	2.09
Soltu.DM.01G005200		-	-	-	-	-	-	3.86	4.39	3.58	3.63	0.89	2.33	1.81	1.74
Soltu.DM.10G027550		-	-	-	-	-	-	1.26	1.55	1.21	1.55	-	1.25	1.07	1.37
Soltu.DM.02G033060		-	1.4	-	-	-	-	-	-	-	-	1.23	1.43	1.77	1.89

4.3.9. Responses to the infection propagated outside the infected area

From the enrichment analysis (**Figure 4.12**), the DEGs found in the intersected area between the infected and outside infected area in each variety (**Table 4.6**), the top 20 most up- and down-regulated genes (**Table 4.7**), and the genes related to the cell wall modification grouped in the heatmap in **Figure 4.14**, modification of the different components of the cell wall caused by the infection were probably propagated to different zones outside the infected region(**Table 4.13**). Similar changes observed in both areas, and in both the resistant varieties, but not observed in the susceptible Duke, were the upregulation of genes encoding enzymes for lignin synthesis, downregulation of XTHs, and change in plant invertase/PMEI. Specific changes to each resistant variety were for Sarpo Mira a decrease of rhamnagalacturonate lyase family protein and an increase in proline-rich extensin like receptor kinase (PERK), and for Sarpo Shona decrease of a mannan synthase, in the expression of a pectin acetylesterase family protein and more genes involved in cutin transport (**Table 4.13**).

Genes within the GO:0006355: “regulation of transcription, DNA-templated”, which was an enriched term in only Sarpo Shona, were examined to identify transcription factors inside and outside the infected area. Repression and activation of basic-leucine zipper TFs, TFs with NAC domain, and WRKY TFs were observed. NAC domain containing proteins were upregulated in both areas in the three varieties, while upregulation inside and outside the infected area of basic zipper TFs was observed in only the resistant varieties. A variety specific response was observed with the upregulation of WRKYs in both areas at 48 h.p.i in only Duke of York (**Table 4.14**).

Regarding to the hormone response, enriched GO terms found in any of the three varieties at 48 h.p.i included those related to the response or signalling pathway of ABA (GO:0009737-“response to abscisic acid”, GO:0009738-“abscisic acid-activated signalling pathway”), jasmonic acid (GO:2000022-“regulation of jazmonic acid mediated signalling pathway”) and ethylene (GO:0009873-“ethylene-

activated signalling pathway"); almost all of them were enriched in Duke of York, while in Sarpo Shona only one was enriched (GO:0009873), and in Sarpo Mira none of them (**Figure 4.10-A**). Genes related to ABA included PP2C genes, downregulated in Sarpo Shona and Duke of York at 48 h.p.i, but only in Duke of York in both collected areas of the leaves. A light-harvesting chlorophyll B-binding protein (*Soltu.DM.12G029100*) was upregulated in only both resistant varieties, and several genes encoding white-brown complex homolog proteins were upregulated in Sarpo Shona. In addition, PYL genes were upregulated in almost exclusively the infected area in Sarpo Shona and Duke, but only one gene in Duke of York also was upregulated outside the infected area.

JA related genes were downregulated almost exclusively in the susceptible Duke of York, with some genes downregulated both within and outside the infected area, including genes encoding jasmonate-zim-domain proteins (JAZ). In the case of ethylene, different genes encoding ethylene responsive element (ERF) domain proteins and ERF binding factor were differentially expressed in Sarpo Shona and Duke of York; however, while Sarpo Shona upregulated the expression of these genes, Duke downregulated them (**Table 4.15**)

Table 4.13 Modification of the cell wall components at 48 h.p.i in each variety. This table shows the transcriptomic changes for each component of the cell wall observed inside and outside the infected area. The vertical small arrows indicate the upregulation (↑) or downregulation (↓) of genes modifying the respective component. Dotted arrows indicate potential spread of gene expression. Common changes found inside and outside the infected area observed in both resistant varieties are in yellow, while specific to one resistant variety are in orange.

CELL WALL COMPONENTS	SARPO MIRA		SARPO SHONA		DUKE	
	Infected area	Outside infected area	Infected area	Outside infected area	Infected area	Outside infected area
LIGNIN	↑↑↑lignin - - - - - - - - - - - - - - - ▸	↑↑lignin	↑↑↑lignin - - - - - - - - ▸	↑↑lignin	↑↑↑↓↓lignin - - - - - - - - - - ▸	↑↓lignin
CELLULOSE	↑Cellulose degradation		↓↓Cellulose degradation ↓Cellulose synthesis (*At* CESA6)	↓Cellulose degradation ↓Cellulose synthesis (AtCESA6)	↑↑↓Cellulose degradation - - - - - - - - - - ▸ ↑Cellulose synthesis (AtCOB)	↑Cellulose degradation
HEMICELLULOSE	↓XTH - - - - - - - - - - - - - - - ▸	↓XTH	↓↓XTH - - - - - - - - ▸ ↑CSL ↑xylosidase (*AtXYL2*)	↓XTH	↑↑↑↓↓XTH - - - - - - - - - - ▸ ↑CSL - - - - - - - - - - ▸ ↑xylan modification (BXL2) - - - - - - ▸	↑↑↑XTH ↑CSL ↑xylan modification (AtBXL2)
			↓Mannan synthase (*AtCSL9*) - - - - - - - ▸	↓Manan(CSL9)		
PECTIN	↑↑pectin lyase superfamily like - - - - ▸	↑pectin lyase superfamily like	↑↑pectin lyase superfamily like - - - - - - - ▸	↑pectin lyase superfamily like	↑↓pectin lyase superfamily like - - - - ▸	↑pectin lyase superfamily like
	↑↓↓plant invertase/PMEI - - - - ▸	↑↓plant invertase/PMEI(1)	↓↓plant invertase/PMEI - - - - - - ▸	↓plant invertase/PMEI(1)	↑↓↓plant invertase/PMEI	
			↓pectin acetylesterase family protein - - - ▸	↓pectin acetylesterase family protein	↑↓pectin acetylesterase family protein	
	↓Rhamnogalacturonate lyase family protein - - - - ▸	↓Rhamnogalacturonate lyase family protein	↑Rhamnogalacturonate lyase family protein			
CUTIN	↑Cutin transport		↑↑↑Cutin transport - - - - - - - - ▸	↑↑↑Cutin transport	↑↓cutin transport - - - - - - - - - - - - - ▸	↓cutin transport
GLYCOPROTEIN	↑expansin like B1		↑expansin like B1 ↑extensin ↓Fasciclin-like arabinogalactan		↑expansin A4 ↑extensin - - - - - - - - - - - - - - - ▸ ↑↓HGRP ↓Arabinogalactan	↑extensin ↑↓HGRP
	↑proline-rich extensin like receptor kinase (PERK) - - - ▸	↑proline-rich extensin like receptor kinase (PERK)			↓proline-rich extensin like receptor kinase (PERK)	

Table 4.14 Transcription factors found in the GO:0006355 - "regulation of transcription, DNA-templated". This table shows the log2FC of some of the transcription factors found in GO:0006355. These genes were DE within or outside the infected area in any of the 3 potato varieties. (-) is for genes with no significant change.

Soltub v.6 Gene ID	Soltub v.6 Gene Function	Sarpo Mira		Sarpo Shona		Duke	
		Inf48 vs Mock	OutInf48 vs Mock	Inf48 vs Mock	OutInf48 vs Mock	Inf48 vs Mock	OutInf48 vs Mock
Soltu.DM.04G035840	basic leucine-zipper	-	-	1.68	1.27	-	-
Soltu.DM.07G023890	Basic-leucine zipper (bZIP) transcription factor family protein	-	-	-5.1	-4.9	-2.92	-
Soltu.DM.08G011730		2.2	2.53	-	-	-	-
Soltu.DM.10G017560		-	-	-1.22	-	-0.86	-
Soltu.DM.06G017300	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	-	-	1.1	1.05	-	-
Soltu.DM.07G024010		-1.8	-	-	-	-	-
Soltu.DM.12G029330		1.08	1.2	-	-	-1.17	-
Soltu.DM.05G016710	NAC domain containing protein	-	-	1.94	-	-	-
Soltu.DM.06G028380		-	-	-1.28	-	-	-
Soltu.DM.07G014750		-	-	1.77	-	1.28	-
Soltu.DM.07G028450		1.44	1.29	-	-	-1.52	-
Soltu.DM.08G022950		-	-	-	-	3.32	2.57
Soltu.DM.07G024720		-	-	-	-	1.73	1.9
Soltu.DM.04G000840	NAC-like, activated by AP3/PI	-	0.77	1.32	1.38	-	-
Soltu.DM.05G005300		1.28	1.21	1.14	1.01	-	-
Soltu.DM.02G028310	WRKY DNA-binding protein	-	-	-	-	-1.18	-1
Soltu.DM.05G006300		1.86	-	2.59	-	-	-
Soltu.DM.05G023360		-	-	-	-	-	2.07
Soltu.DM.05G026450		-	-	1.34	1.32	-	-
Soltu.DM.06G011680		-	-	-	-	-	-5.5
Soltu.DM.06G026100		-	-	3.91	-	-	-
Soltu.DM.09G009490		-	-	-	-	-1.72	-
Soltu.DM.12G004050		-	-	-	-	-1.29	-0.88
Soltu.DM.03G022860	WRKY family transcription factor	-	-	-	-	-3.39	-2.53

Table 4.15 DEGs related to hormones expressed in any of the three potato varieties. This table shows the log2FC of part of the *DEGs* found in the GO:0009737-"response to abscisic acid", GO:0009738-*"*abscisic acid-activated signalling pathway*",* GO:2000022-*"*regulation of jasmonic acid mediated signalling pathway*", and* GO:0009873-*"*ethylene-activated signalling pathway*"*. These genes were DE within or outside the infected area in any of the 3 potato varieties. (-) is for genes with no significant change.

Soltub v.6 Gene ID	Soltub v.6 Gene Function	Sarpo Mira		Sarpo Shona		Duke	
		Inf48 vs Mock	OutInf48 vs Mock	Inf48 vs Mock	OutInf48 vs Mock	Inf48 vs Mock	OutInf48 vs Mock
Genes related to ABA							
Soltu.DM.02G030840	ABI five binding protein	-	-	-	-	-1.33	-1.4
Soltu.DM.04G000490		-	2.55	-	-	-	-
Soltu.DM.07G000120	Glycosyl hydrolase chitinase insertion domain	1.13	0.9	1.03	0.84	-	-0.8
Soltu.DM.07G000160		-	-	1.02	-	-	-
Soltu.DM.03G022710	Highly ABA-induced PP2C gene	-	-	-	-	-2.61	-1.88
Soltu.DM.03G012480	Protein phosphatase 2CA	-	-	-1.71	-	-1.85	-1.39
Soltu.DM.05G023010		-	-	-1.15	-	-1.38	-1.12
Soltu.DM.02G004650	Late embryogenesis abundant (LEA)	-	-	1.18	-	-	-
Soltu.DM.07G024910	Light-harvesting chlorophyll B-binding protein	1.35	2.22	-	-	-	-
Soltu.DM.12G029100		1.07	1.62	1.09	1.57	-	-
Soltu.DM.03G013340	PYR1-like	-	-	2.59	-	3.15	-
Soltu.DM.05G022460		-	-	2.26	-	1.81	-
Soltu.DM.06G010300		-	-	1.21	-	0.91	-
Soltu.DM.10G022490		-	-	-	-	1.30	1.01
Soltu.DM.01G044620	White-brown complex homolog protein	-	-	2.67	4.51	-	-
Soltu.DM.05G021330		1.13	-	2.08	1.53	-	-
Soltu.DM.05G021350		-	-	2.17	1.41	-	-
Soltu.DM.05G021360		-	-	2.08	1.59	-	-
Genes related to JA							
Soltu.DM.03G036980	Jasmonate-zim-domain protein	-	-	-	-	-1.16	-1.11
Soltu.DM.03G036980		-	-	-	-	-1.16	-1.11
Soltu.DM.08G007100		-	-	-	-	-2.43	-2.44
Soltu.DM.08G007150		-	-	-	-	-1.47	-
Soltu.DM.12G026270		-	-	-	-	-1.82	-1.65
Soltu.DM.12G004050	WRKY DNA-binding protein	-	-	-	-	-1.29	-0.88
Soltu.DM.08G004690	WRKY family transcription factor	-	-	-	-	-2.43	-1.89
Soltu.DM.08G028850		-	-	-	-	-1.66	-
Genes related to ethylene							
Soltu.DM.02G016660	Ethylene responsive element binding	-	-	1.42	-	-	-
Soltu.DM.02G017280	ERF domain protein	-	-	-	-	-1.97	-1.2
Soltu.DM.07G020090		0.88	-	-	0.97	-1.28	-0.85
Soltu.DM.12G022800		-	-	-	-	-1.28	-
Soltu.DM.09G021200	Ethylene response	-	-	2.68	3.39	-	-
Soltu.DM.08G000970	Ethylene responsive element binding factor	-	-	3.08	2.72	-	-
Soltu.DM.08G024150		-	-	2.78	-	-	-
Soltu.DM.08G024160		-	-	2.88	2.37	-	-
Soltu.DM.04G008930		-	-	-	-	-	-1.12

4.4. Discussion

Transcriptomic changes were observed in potato leaves infected with *P. infestans* to identify changes in the cell wall components and identify whether some of these changes correlate with a resistant phenotype in two resistant varieties, Sarpo Mira and Sarpo Shona, compared with one susceptible variety, Duke of York.

In the two resistant varieties, a reaction against the infection was observed at 24 h.p.i in the form of a high level of callose deposition and in Sarpo Shona with necrotic spots on the leaves. In the susceptible variety Duke of York, low callose deposition was observed at 24 h.p.i and necrotic spots at 48 h.p.i. The accumulation of callose in the cell wall at the site of infection was reported many years ago. It was proposed that callose deposition provides a physical barrier against the infection to decrease the speed of entry of the pathogen, rather than to stop the infection (Voigt and Somerville, 2009). This was mainly because callose deposition has been correlated with a decrease in pathogen accumulation, as observed in *Arabidopsis* against *C. higginsianun* (Shimada *et al*., 2006). However, complete penetration resistance was also observed in *Arabidopsis* against powdery mildew conferred by an early and high amount of callose deposition (Ellinger *et al*., 2013). In contrast, a lack of callose accumulation in *Arabidopsis* lines, due to non-expression of the glucan synthase-like 5 (GSL5) gene, a callose synthase involved in callose production, did not significantly reduce the level of entry of the pathogen, suggesting callose deposition has no major effect on resistance (Jacobs *et al*., 2003).

On the other hand, callose deposition at the site of haustoria has also been proposed to restrict nutrient uptake by the pathogen from the host cell (Voigt and Somerville, 2009). Although the relationship between callose accumulation and resistance phenotypes is inconclusive, a greater level of callose accumulation was observed in the resistant varieties than in the susceptible in an early reaction at 24 h.pi., which was particularly pronounced in Sarpo Mira. Surprisingly, upregulation of genes related to callose deposition was observed in Sarpo Mira in every evaluated condition, even in

the control samples, which were not inoculated and were sampled the same day of the infection. This callose deposition reaction not produced by infection could be caused by mechanical damage to the plant during leaf detachment, since callose deposition is also a reaction against abiotic stress and wounding (Voigt and Somerville, 2009) and can occur as soon as minutes after damage (Galway and McCully, 1987). However, callose is deposited at the site of the damage, and according to the observed DEGs, upregulation of callose synthases responsible for callose synthesis (Chen and Kim, 2009; Ellinger and Voigt, 2014), was not observed in any treatment at 48 h.p.i. Furthermore, Sarpo Mira showed leaves with dark spots since they were growing in the pot in the greenhouse, which were not observed in plants collected from the field (**Table 3.4 in Chapter 3**). Therefore, the reaction of callose deposition at least in this variety may be highly likely to have occurred as a response to wounding damage caused by insects in the greenhouse, though this was not seen in the other two varieties.

Overall, more differential expression of genes was observed in the susceptible Duke of York than in either resistant variety, Sarpo Mira or Sarpo Shona, at 48 h.p.i., and cell wall-related genes were involved in these changes. These results are consistent with the previous work of Ali *et al.* (2014), who evaluated two incompatible (Sarpo Mira and SW93-1015) and one compatible (Desiree) interaction at 6, 24, and 72 h.p.i., and observed a different response between the two resistant varieties. At the early time point (6 h.p.i), the two resistant varieties, Sarpo Mira and SW93-1015, had a lower number of DEGs than the susceptible Desiree. However, at the two later time points, more DEGs were observed in the susceptible variety Desiree and the resistant variety SW93-1015 than in Sarpo Mira, being more notable at 72 h.p.i, showing a different reaction of the two resistant varieties to the infection (Ali *et al*., 2014). The authors suggested that while Sarpo Mira possesses its resistance through the action of R genes, SW93-915 was able to resist infection through a constitutive accumulation of H_2O_2 and pathogen-related proteins from before the infection, without compromising its growth (Ali *et al*., 2012). According to these results, it is probable that our variety

Sarpo Mira could still have less transcriptomic change at earlier hours, such as 24 h.p.i., than Duke, but to determine if this response occurs in both resistant varieties, Sarpo Mira and Sarpo Shona, it will be necessary to sequence the transcriptome at that time point. Ali *et al*., also reported that a higher number of proteins in the apoplast increased their abundance in the incompatible than in the compatible interaction at 24h than at the other 2 time points, further supporting the notion that the cell wall plays an important role in the resistance response.

Yogendra and Kushalappa (2016) reported higher expression of genes related to the cell wall at 48 h.p.i. in two potato resistant varieties compared to a susceptible one. These genes encode enzymes that modify the cell wall, such as pectinesterases, xyloglucan endotransglucosylase-hydrolases (XTHs), expansin, among others; and genes involved in the cell wall reinforcement, such as extensin. However, not all these genes were significantly higher in both resistant varieties. Indeed, only one receptor protein kinase was overexpressed in both of them, while the rest were specific to one variety. In our resistant varieties, changes in the expression of genes related to the cell wall were observed and some of these changes were associated with the level of resistance to *P. infestans*, i.e., were common to both resistant varieties. These included widespread changes in genes relating to lignin, pectin, and hemicellulose components of the cell wall.

4.4.1. Specific changes in expression of cell wall related genes

Expression changes in genes related to lignin synthesis were a common response in all three varieties, but these changes were greater in the resistant varieties. Indeed, one of the 20 most upregulated DEGs in response to infection in Sarpo Mira and Sarpo Shona was a laccase gene (*Soltu.DM.04G028320*) involved in lignin polymerization, while in the susceptible variety the expression of this gene somewhat decreased with the infection. Moreover, the susceptible variety downregulated other genes relating to lignin biosynthesis including genes encoding phenylalanine ammonia lyases (PALs), the first enzymes in the phenylpropanoid pathway that leads to monolignol

biosynthesis. There is evidence that lignin accumulation in the casparian-strip can prevent the invasion of the pathogenic bacterium *P. syringae pv. Tomato* in *Arabidopsis* (Lee *et al*., 2019). Lignin accumulation was also observed in cotton and *Camelia sativa* after pathogen attack and was associated with a resistant phenotype against fungal pathogens (Xu *et al*., 2011; Eynck *et al*., 2012). In *C. sativa*, more accumulation of lignin was observed in resistant plants even before the infection (Eynck *et al*., 2012). The same may be occurring in our resistant potato varieties since they had a higher expression of some lignin-related genes than Duke even before the infection. It was also reported that the production of lignin in the cell wall after pathogen attack was mediated by the R genes RPM1 and RPS2 during the ETI response (Lee *et al*., 2019). It would be interesting to evaluate the cell wall lignin abundance in our resistant varieties by further experimental analyses, to associate the accumulation of lignin before and after infection with the expression of the R genes observed in Sarpo Shona and Sarpo Mira.

The expression of genes relating to hemicellulose components was markedly different in the susceptible variety Duke of York compared with the two resistant varieties, with many more genes differentially expressed in response to the infection in Duke. In particular, many more XTH genes were upregulated in response to the infection in the susceptible Duke at 48 h.p.i compared with the resistant varieties that only downregulated XTH genes. In contrast, Yogendra *et al*., found a lower expression of XTHs in the susceptible variety, though for only two XTH genes. In Duke of York, only one XTH had a lower expression and 8 had a significantly higher expression compared to the resistant varieties. XTHs are enzymes capable of both disassembling and re-ligating the bond between cellulose and xyloglucan, remodelling the state of loosening of the cell wall (Lampugnani, *et al*., 2018). Previous work has shown that XTHs can play an important role in the defence of *Glycine max* against the parasitic nematode *Heterodera glycines*. Increasing the expression of *XTH43* led to the accumulation of xyloglucan chains with a shorter length and it was proposed that this could limit the plant cell wall expansion and thereby the pathogen infection (Niraula *et al*., 2021). Further

experimental work is needed to determine if the observed differences in the expression of XTHs could affect the rigidity or elasticity of the cell wall in the resistant compared with the susceptible potato varieties after infection.

Besides the differences in the expression of XTHs between susceptible and resistant varieties, we also observed other changes that imply more widespread xylan remodelling in Duke. For example, Duke upregulated 2 cellulose synthase-like (CSL) genes, which are involved in hemicellulose backbone synthesis, and 1 glycosyl hydrolase gene involved in xylan remodelling (in *A. thaliana*: AtBXL2). Other changes in the hemicellulose components have also been associated with resistance to pathogens, though the effects may vary according to the pathosystem. A high amount of attached xylose and a high level of acetylation in the hemicellulose has been associated with resistance to infection (Bacete *et al*., 2018), although this correlation has not been observed in all systems. For example, more acetylation conferred resistance in *Arabidopsis* against *Botrytis cinerea* (Manabe *et al*., 2011), but not to *Pseudomonas syringae* and *Xanthomoas oryzae* (Pogorelko *et al*., 2013). Obvious gene expression changes linked to such responses could not be detected in our varieties, although upregulation of *Soltu.DM.03G13940*, homologous to an alpha-xylosidase in *A. thaliana* (*AtXYL2*) that remove xylosyl residues from the side chains of xyloglucan (Sampedro *et al*., 2001), was observed only in the resistant variety Sarpo Shona. Therefore, this resistant variety would contain less xylose groups attached to the hemicellulose, which would be different from what was reported by Bacete *et al*., 2018.

Up- and down-regulation of many genes relating to the pectin component of the cell wall was observed in all three varieties. Higher expression of some pectin lyase-superfamily proteins was observed in both resistant varieties in response to infection, compared with Duke, with two pectin lyase-like proteins being in the top 20 most upregulated genes, but not being upregulated in Duke. According to the Spud database and the BlastP result stored in this database (http://spuddb.uga.edu/), the protein sequences of the pectin lyase-like superfamily genes have a

high similarity with PGs and pectate lyases (PLs) in different *Solanum* species. PLs and PGs produce OGA fragments, which especially with a reduced level of methyl esterification, are recognized as a DAMP that alerts the host cell against the infection and generates an immune response (Osorio *et al*., 2008). OGAs can be generated by the PGs and PLs released from the pathogen or produced by the host plant itself (Lorrai and Ferrari, 2021; Shin *et al*., 2021). Therefore, our resistant varieties could potentially be producing more OGA fragments during infection through the upregulation of specific members of this pectin-lyase superfamily enzyme group. Furthermore, a common response observed in all 3 varieties was the (mostly) downregulation of PMEIs. Therefore, if more OGAs were released from the resistant varieties, they would have a reduced level of methylesterification to trigger an immune response in potato leaves.

Although the accumulation of structural glycoproteins has been correlated with the resistance, specifically HGRP proteins (Deepak *et al*., 2010), a clear pattern of expression in related genes could not be associated with a resistance phenotype in common between the two resistant varieties. However, the susceptible variety downregulated more cell wall protein genes than the two resistant varieties. The 3 varieties changed the expression of expansin genes and a variety specific response was observed in Sarpo Mira with an overexpression of a proline-rich extensinlike receptor kinase (PERK) and in Sarpo Shona with downregulation of FASCICLIN-like arabinogalactan. However, a single expansin-like B1 gene (*Soltu.DM.08G001190*) was upregulated in only both resistant varieties and was in the top 20 most upregulated genes. Upregulation of expansin was previously observed in potato under *P. infestans* attack (Yogendra and Kushalappa*,* 2016). Expansins have been involved in the control of cell wall loosening and constriction of guard cells regulating the stomata closure/opening (Marowa *et al*., 2016) and their overexpression was associated with a decreasing stomatal density and a negative influence on the development of guard cells (Sampedro and Cosgrove, 2005; Marowa *et al*., 2016). *P. infestans* uses the stomata as an exit point of its newly formed hyphae to liberate sporangia (Farewell *et al*., 1969). To avoid pathogen dissemination,

stomata closure and guard cell death were observed in potatoes 4 hours after *P. infestans* infection (Yang *et al*., 2021). This behaviour in potato was observed as a common response among different varieties with different levels of resistance to this pathogen (Yang *et al*., 2021), however, the degree of this response in the different potato varieties has not been quantified. Since the stomata function as a door for P*. infestans* to disperse their sporangia, the increased expression of expansin observed here could reduce the stomatal density and be beneficial to the resistant varieties.

PERK is a class of EXT located in the plasma membrane and with an increased expression under fungal infection and wounding in *Brassica napus* (Silva and Goring, 2002; Liu *et al*., 2016). Its expression was associated with callose, cellulose, and pectin accumulation, since its altered expression produced aberrant amounts of these cell wall components (Haffani *et al*., 2006; Borasi *et al*., 2021). Among FASCICLIN-like arabinogalactans, a type of AGPs, 9 out of 11 evaluated genes were downregulated under virus (TuMV) and *P.syringae* infection in *Nicotiana benthamina*, but not under other two virus infections (Wu *et al*., 2020). Although the upregulation of PERK in Sarpo Mira is consistent with the resistant response observed in *B. napus*, more information is needed for the reaction of Fasciclin-like arabinogalactans under oomycete or fungus infection.

Several genes involved in cutin transport were expressed at a higher level in the resistant varieties even before the infection (in control leaves). In Sarpo Shona only, these same 5 genes were also upregulated in response to the infection. Cutin mobilization in Sarpo Shona was related to the upregulation of genes encoding white-brown complex (WBC) homolog proteins, which belong to an ATP-binding cassette (ABC) transporter-family G (ABCG) localized in the plasma membrane (Verrier *et al*., 2008). ABCG is involved in exporting cutin precursors into the apoplast, where cutin synthesis is completed and cutin is deposited (Elejalde-Palmett, 2021). In *Arabidopsis*, mutans with low cutin content by not expressing two glycerol-3-phosphate acyltransferase genes (*gpat4*/*gapt8*) showed susceptibility to necrotrophic A*lternaria brassicicola* (Li *et al*., 2007). In *Arabidopsis*, mutants not

expressing the RESURECTION1 gene showed a high concentration of cutin monomers and waxes and were susceptible to a biotrophic but resistant to a necrotrophic pathogen (Mang *et al*., 2009). Therefore, the level of cutin will affect plant defence depending on the type of pathogen. In our varieties, a higher expression of cutin transporters in healthy leaves may mean that the resistant varieties have more cutin in their cell walls, which may make these varieties already well prepared for pathogen attack.

Overall, we have observed more similarity in the response of the two resistant varieties compared with the susceptible Duke of York. This may be expected, not only because these varieties are resistant, but also share a higher genetic similarity. Nevertheless, as observed elsewhere (Ali *et al*. 2014), variety-specific resistance responses may also be important. Specific responses observed in only Sarpo Shona included less degradation of cellulose and greater cutin mobilization in the cell wall in response to infection. The relationship between cellulose biosynthesis and resistant phenotypes in the literature is inconclusive, as an alteration in its biosynthesis led to resistance in *Arabidopsis*, but to susceptibility in barley (Hernandez-Blanco et al., 2007; Douchkov *et al*., 2016; Bacete *et al*., 2018).

4.4.2. Resistant varieties are ready-prepared for pathogen attack

We have observed that while there are many changes that occur in response to infection in all three varieties, there are also many pre-existing differences in healthy control leaves that may prepare the resistant varieties to face the pathogen and/or enable a more rapid response. In barley, drought triggered more change expression in the evaluated susceptible variety than in the resistant one (Janiak *et al*., 2018). However, the transcriptomic data also showed that the resistant varieties may not need substantial change because some mechanisms were already activated under normal conditions enabling that plant to react quickly to the stress without a drastic change in gene expression (Janiak *et al*., 2018). Therefore, this strategy observed in barley may also occurred in our

resistant potato plants, since several genes encoding proteins involved in counteract pathogen infection were higher before the infection.

There were many more DEGs relating to the response to pathogens in the susceptible variety Duke compared with the resistant varieties, a response common across transmembrane receptors (PRR proteins), intracellular receptors (R genes), PR proteins and others involved in defence against pathogens. Rather than upregulating these genes in response to the pathogen, many of these genes were already expressed at a higher level in the resistant varieties in healthy control leaves. The consistently higher expression of many genes encoding WAK receptors in Sarpo Mira and Sarpo Shona, even before the infection, could allow them to have a better alert system to detect pectin degradation during the early stages of infection. WAKs are receptors linked to the pectin component and recognize polygalacturonic and OGA fragments in a Ca^{+}-dependent manner (Hématy *et al*., 2009). Overexpression of WAKs in *Arabidopsis* has been observed to confer resistance against *Botrytis cinera* (Brutus *et al*., 2010). They have also been identified within major QTL regions associated with resistance to pathogens (Diener and Ausubel, 2005; Zuo *et al.*, 2014; Hurni *et al.*, 2015;). Taking into consideration that pectin is one of the first cell wall components to be degraded during infection (Lionetti, Cevone, and Bellicampi, 2012; Lorrai and Ferrari, 2021), more pectin fragments and WAK receptors could generate a quicker response in the resistant varieties. According to the results of Ali *et al*., there may be less transcriptomic changes in some resistant varieties, especially Sarpo Mira, in the early hours of the infection (Ali *et al*., 2014), which could be related to the higher baseline expression of defence related genes (WAKs) that we have observed here. Therefore, it will be important to examine the transcriptomic changes of the three varieties at this earlier time point of infection, to fully understand the time course of the response to *P. infestans*.

Many R genes also showed a similar response, with many being expressed at a higher level in the two resistant varieties in all treatments compared with the susceptible. Activation of R proteins generates

a hypersensitive response provoking cell death, and their constitutive activation can compromise growth or the survival of the plant (van Wersch *et al*., 2020). Therefore, plants have mechanisms to control their gene expression, at the transcriptional and post-transcriptional levels (Lai and Eulgem, 2018), and their protein activation (van Wersch *et al*., 2020). Activation of R proteins occurs after effector recognition by its LRR domain, which can produce a conformational change or oligomerization of R proteins (Bentham *et al*., 2020). In our resistant varieties, the higher expression of R genes would likely lead to these varieties having more intracellular receptors to detect the effectors of *P. infestans*. These receptors may only be activated after the pathogen is detected, so their greater expression may not be affecting the development of the plant, but may allow a faster hypersensitive response once the infection occurs. Nevertheless, not all R genes were consistently upregulated in the resistant varieties, with a significant minority being consistently downregulated.

Among genes encoding PR proteins, there was consistently higher expression of beta-1,3-glucanase and basic chitinase genes in the resistant varieties. Several beta-1,3-glucanases had more than a 7-fold log2 fold change across all conditions in both resistance varieties. High expression of these genes could make an important contribution to the resistance response to *P. infestans* since the cell wall of oomycetes is composed of beta 1,3 glucan and cellulose (Bartnicki-Garcia, 1968). In potato, a beta 1,3 glucanase conferred a field resistant phenotype to potato and increased its expression under *P. infestans* attack, but with a much higher expression in the resistant than the susceptible potato variety (Tonòn, Guevara, and Daleo, 2002). None of our three varieties significantly upregulated these genes in response to the pathogen, but the higher baseline expression in the resistant varieties may be important to allow a rapid response to the pathogen. In the case of basic chitinase, the gene encoding for this PR protein, *Soltu.DM.10G018010*, had a consistent massive over-expression in the resistant varieties than in the susceptible Duke in control leaves and in the infected leaves both inside and outside of the infected areas, with Log2FC of more than 15 in every evaluated treatment. Chitinases are enzymes that participate in the first line of the plant defence response during PAMP-

triggered immunity (PTI) by degrading chitin, a major component of the fungal cell wall. Even though *P. infestans* has a cell wall composed mainly of cellulose rather than chitin, by having more chitinases in the cell wall, the resistant varieties may mount a quicker response to various pathogens.

4.4.3. No enrichment of genes involved in SA response

In response to pathogen attack, plants synthesize SA to activate plant defences. During infection, SA concentration fluctuates with pick hours depending on the plant-pathogen interaction. In watermelons infected with *Fusarium*, SA increased their concentration after 12 h.p.i. with a pick at 3 and 7 days post-infection (d.p.i) (Zhu *et al*., 2022). In *Populus tomentosa* after *Botryosphaeria dothidea* inoculation, SA increased after 6 h.p.i. with a pick at 3 d.p.i. (Li *et al*., 2018). This fluctuation was also observed in transcription factors that regulate SA synthesis. In potato infected with *P. infestans*, the expression of *StICSI1* (ISOCHORISMATE SYNTHASE 1), a key enzyme in the synthesis of SA, and StbZIP61 (basic region/leucine zipper motif), a transcription factor that regulates StICSI1 (Zhou *et al*, 2018) were upregulated at 6 h.p.i. with a pick at 12 and 18 h.p.i., respectively, from which their expression begins to decrease. In this thesis, since no biological process related to the SA biosynthesis pathway was observed to be enriched, SA was probably produced at an earlier time point than 48 h.p.i., as in the plants evaluated by Zhou *et al*.

It is also important to mention that SA accumulation can be inhibited by JA signalling (Yang *et al*., 2019). In our data, JAZ (JASMONATE ZIM DOMAIN PROTEIN), which suppresses the JA response (Yang *et al*., 2019), had no significant change in any of the resistant varieties but was downregulated in only the susceptible variety at 48 h.p.i. The no differential expression or downregulation of JAZ may indicate that repression of JA response would not be occurring. This could indicate that SA signalling could be suppressed by JA at the evaluated time point in our potato varieties.

4.4.4. Systemic responses to infection

It is well known that infected leaves can trigger an immune response in uninfected distant leaves of the plant. This mechanism, known as "Systemic Acquired Resistance" (SAR) (Ross, 1961), can be observed in uninfected regions neighbouring the infected areas and in distant uninfected leaves that give rise to a systemic reaction in the whole plant, referred to by Cordelier *et al*. as SAR_T and SAR_S, respectively (Cordelier *et al*., 2003). SA is a key hormone to generate SAR since without its accumulation in distal uninfected tissues, no SAR was observed (Klessig, Choi, and Dempsey, 2018). Accumulation of PR proteins in infected and distal uninfected regions is considered a marker of SAR (Klessig, Choi, and Dempsey, 2018). Different transport signals have been proposed to travel from the infected toward the uninfected region. They include SA, its inactive form methyl salicylate (MeSA), Aza, G3P, DA, Lys, and Pip (Klessig, Choi, and Dempsey, 2018). In potato, the conversion of MeSA to an active SA by the methyl-esterase 1 (StMES1) was necessary to activate SAR in regions far from the infected region, where an accumulation of PR1 and beta glucosidase (PR2) was observed (Manosalva, *et al*., 2010). In this chapter, no enrichment of GO terms related to SA was found in the evaluated potato varieties, and only a few differentially expressed PR genes, such as basic chitinase and beta 1,4 glucanase, were observed outside the infected area.

Importantly, to verify SAR it would be necessary to infect the regions outside of the infected areas to evaluate if a resistance response occurred. Nevertheless, some of the transcriptomic changes observed in the infected areas of the leaf were also observed in the non-infected areas of the same leaves. This included changes in the expression of genes related to the modification of the cell wall components. Specific to both resistant varieties was the overexpression of genes for lignin biosynthesis and downregulation of plant invertase/PMEI (therefore more unmethylesterfied pectin) and XTHs. A specific response in Sarpo Mira was the downregulation of a gene encoding a rhamnagalacturonate lyase family protein and upregulation of a proline-rich extensin like receptor

kinase (PERK), while specific to Sarpo Shona was a reduced expression of genes for a pectin acetylesterase family protein and a mannan synthase.

It was previously observed at the transcriptomic level in *Arabidopsis* that after 2 days post-infection with *Pseudomonas syringae* pv. maculicola, SAR was detected in healthy upper leaves, far from the infected leaf, by changes in gene expression that involved genes inside the functional category "cell wall", "extracellular", among others (Gruner, *et al*., 2013). Downregulated genes related to the cell wall modification, indicating a decrease in cell wall loosening and extension, included an expansin-like A1 (EXPLA1), Fasciclin-like arabinogalactan-proteins (FLA9, FLA8, FLA13), polygalacturonase (AT3G06770), arabinogalactan proteins (AGP21, AGP9, AGP26), extensin-like protein (ELP), and xyloglucan endotransglucosylase/hydrolases (XTH31, XTH7) (Gruner *et al*., 2013). In tomato, SA pre-treatment generated upregulation of genes for lignin and cellulose synthesis and downregulation of PME genes to help plants withstand Cadmium stress (Jia *et al*., 2021). In *A. thaliana*, a SAR response was observed by an increase in lignin, cellulose, and pectin content in leaves. Also, an increase in cell wall thickness, stomatal closure, and resistance to *H. arabidopsidis* was reported (Qi *et al*., 2022). A common response observed in both Sarpo Mira and Sarpo Shona with these previous results was the downregulation of XTHs and changes in lignin content. Therefore, it is very likely that these changes in the cell wall are part of a systemic response in potato, observed in distal uninfected tissue to prevent future *P. infestans* infection.

Various transcription factors also changed with expression significantly in both infected and uninfected areas. This included TFs containing NAC domain, WRKY, bZIP, and MYB, among others. The overexpression of NAC and MYB TFs, specifically NAC43 and MYB8, has been associated with an increase of cell wall thickness in potato under *P. infestans* attack (Yogendra *et al*., 2017). Therefore, some of the NAC and MYB may regulate changes in the cell wall composition. However, further

microscopy analyses would be needed to determine any changes in cell wall thickness in our varieties.

4.4.5. Conclusions and future work

We have shown that there are commonalities and differences in the transcriptomic response between potato varieties that differ in their level of resistance to *P. infestans*, many of which involve genes related to the plant cell wall. All three varieties show widespread upregulation of genes involved in the synthesis of lignin, though this response is much stronger in the two resistant varieties. There are many commonalities in the response of the two resistant varieties that we do not observe in the susceptible variety, most notably the higher expression of many defence-related genes, including R genes, and WAK receptors, even more pathogen attack. This may enable the resistant varieties to mount a more rapid and hence more effective response when the pathogen first appears at the leaf surface. However, variety-specific responses may also be important in the resistant response.

All these results regarding transcriptomic change of genes related to the composition of the cell wall can be corroborated by detecting the concentration of the different components in the cell wall. This could be done using the COMPP technique, demonstrated in chapter 4. In the case of glycoproteins and the different receptors mentioned here, a proteomic approach could be followed. Since the transcripts are exposed to post-transcriptional regulation and *P. infestans* secretes different enzymes that degrade host components, a proteomic analysis could detect whether the expressed genes actually lead to the accumulation of their respective encoded proteins. It is also important to evaluate the response of potato plants at different time points of the infection to determine which genes are specific to an early and late response, and if some early response genes could be key to resisting the infection. In addition, more evidence needs to be provided for the correlation between the change of specific cell wall components with a resistant response. The use of mutant plants that

lack key genes related to lignin or xyloglucan synthesis would help to see if these components are indeed linked to the resistant phenotype towards *P. infestans* in potato. It will also be important to examine if the observed results are specific to the strain used here or can also be observed under infection with different strains of *P. infestans*.

CHAPTER 5: Similarities and differences in leaf abiotic and biotic stress responses

5.1. Introduction

Many types of stresses negatively affect potato production, with drought and *P. infestans* as the two most damaging (Nasir and Toth, 2022; Savary *et al*., 2019). Different types of biotic and abiotic stress can affect plants simultaneously, which implies that adaptation or tolerance/resistance mechanisms are required to deal with both. Crosstalk between biotic and abiotic stress involves the activation of different mechanisms. This includes the activation or repression of transcription factors, ROS production, changes in intracellular Ca^{2+} levels, as well as hormone regulation (Kissoudis *et al*., 2014, Nejat and Mantri, 2017). Among the different phytohormones, ABA has been widely associated with the response to abiotic stress, but also with the response to pathogen attack. Stomatal closure caused by ABA signalling can play a protective role for plants under attack from pathogens whose entry point is through the stomata (Lim *et al*., 2015). Therefore, at the beginning of an infection, the ABA signalling pathway triggered by the recognition of pathogen structures, like flagellin 22 (flg22), can limit pathogen invasion (Melotto *et al*., 2006). Although *P. infestans* employs a structure called an appressorium to enter the host cell, this pathogen still needs the stomatal aperture to release sporangia and liberate zoospores (Farrel, Preece, and Wren, 1969), therefore modulation of stomatal aperture/closure is also relevant in potato plants infected with *P. infestans*.

High levels of ABA have been associated with a susceptible response once the pathogen has already invaded the host (Cao, Yoshioka, and Desveaux, 2011). However, a recent study has shown that the susceptibility or resistance produced by ABA depends on the pathogen lifestyle, ABA concentration in the plant, and plant age (Stevens, Johnston, and Lune, 2023). The susceptibility produced by ABA is associated with its ability to suppress SA signalling. Since SA is involved in cell wall reinforcement, production of antimicrobial compounds such as chitinases, glucanases, and phytoalexin, and in the

systemic acquired resistance (SAR), the accumulation of ABA after pathogen infection would not be beneficial for plants (Cao, Yoshioka, and Desveaux, 2011, van Butselaar and Van den Ackerveken, 2020). Therefore, hormone regulation is an important mechanism involved in the response and tolerance to individual abiotic and biotic stresses and the antagonistic or complementary cross-talk between them (Ku *et al*., 2018).

Changes in cell wall components and expression of transcription factors, such as WRKY, MYB, NAC, ERF, and HSF are also part of the crosstalk between these two types of stress (Kissoudis *et al*., 2014). As an example, the transcription factor ATAF1 (*Arabidopsis thaliana* activator factor 1), part of the NAC transcription factor family, was involved in the response against abiotic and biotic stress (Wu *et al*., 2009; Nejat and Mantri, 2017). In addition, plants possess a family of receptor-like kinases (RLKs) located in the cell membrane that activate the first line of defence against pathogens, called PAMP/DAMP-triggered immunity (PTI). RLKs can also mediate the response to different environmental stresses, such as drought, salt, and cold (Ye *et al*., 2017). Activation of RLKs can also regulate stomatal closure and activate the ABA signalling pathway, and their overexpression can confer tolerance to drought (Ye *et al*., 2017). Therefore, elements from the pathogen immunity are also an integral part of the abiotic stress response. Reflecting on this, Nejat and Mantri propose a reframing of PTI as STI or "Stress Triggered Immunity", because of the commonalities in the response to pathogens and various types of abiotic stress (Nejat and Mantri, 2017).

Since drought and *P. infestans* are the two principal problems affecting potato production worldwide, this chapter aims to identify overlapping responses between these stresses that are associated with a tolerant/resistant phenotype. To address this aim, the transcriptomic response of leaf tissue to drought stress and rewatering from Chapter 3 will be compared with the transcriptomic response of leaf tissue to late blight from Chapter 4 in the tolerant/resistant and susceptible varieties. This analysis aims to identify key genes associated with tolerance to drought and resistance

to blight, thus identifying candidate genes to be tested in different *S. tuberosum* cultivars under different types of stress in order to develop cultivars with improved yield and quality.

5.2. Material and methods

5.2.1. Biological material, experimental design and analysis

Data from 2 independent experiments were analysed using existing methods developed for the first experiment described in Chapter 3 (**Figure 5.1**) and the second experiment in Chapter 5 (**Figure 5.2**).

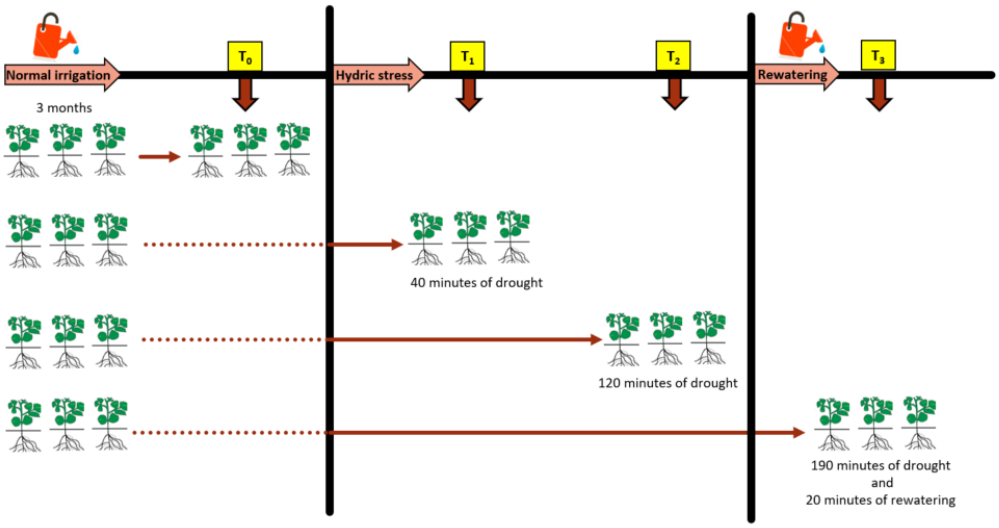


Figure 5.1 Experimental design to simulate drought stress. After 3 months of irrigation, potato plants were exposed to hydric stress. Samples were collected at the time when the tolerant plants decreased photosynthetic rate by 25% and 60%, and recovery 60% of the initial rate after rewatering. These responses were observed at 40 min (early response-T_1) and 120 min (late response-T_2) after removing the water supply, and after 20 minutes of re-watering (recovery phase-T_3). At these time points, leaf and root samples were collected from the susceptible and the tolerant varieties. Control plants, without hydric stress, were also collected (Control-T_0). There were 3 biological replicates (independent plants) at each time point.

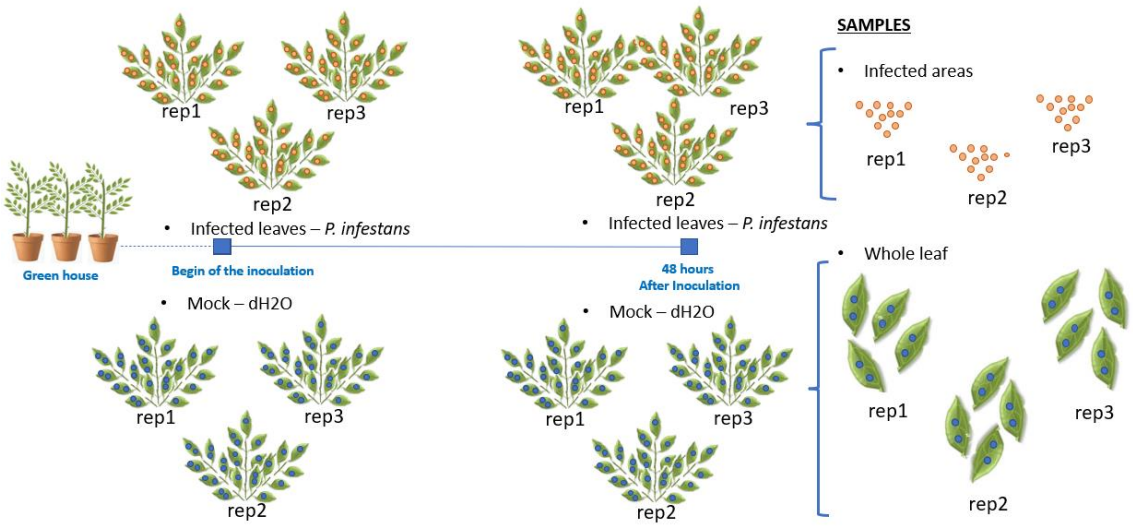


Figure 5.2 Experimental design for infection with *P. infestans*. Representation of the treatments applied to one potato variety during the detached infection assay. Collection of samples was carried out 48 h.p.i. Only the transcriptomic profile of the infected area was analysed on the infected leaves, while the whole leaf was analysed for the mock samples. The same treatment was applied to the three potato varieties.

5.2.2. Identification of transcription factor binding sites and cis-regulatory elements.

In the annotation of the potato genome v6, each potato gene ID is associated with an *A. thaliana* gene ID. The *A. thaliana* ID was used to identify genes that are activated by a specific transcription factor (TF) in the AthMap database (Bulow, Brill, and Hehl, 2010). This database identifies potential transcription binding sites (TFBSs) in *A. thaliana* genes, which were predicted by using positional weight matrices or by a pattern-based screening (Bulow, Brill, and Hehl, 2010). In this database, the tool 'Gene Identification' was used to find TFBSs in a region 500 bases upstream and 50 bases downstream of *A. thaliana* genes and obtain potential target genes regulated by a specific TF. Then, the resulting *A. thaliana* target gene IDs were used to search the corresponded potato genes in the annotation file of the potato genome v6, allowing the association of a potato transcription factor with its possible gene targets in potato (**Figure 5.3**).

To corroborate that these potato genes are putatively activated by a specific transcription factor, the sequence of 1,000 bp upstream of each gene was extracted from Spud Database

(http://spuddb.uga.edu/) to identify the *cis*-regulatory elements. This analysis was carried out with PLACE, a database of plant *cis*-acting regulatory DNA elements (Higo *et al*., 1999) (**Figure 5.3**).

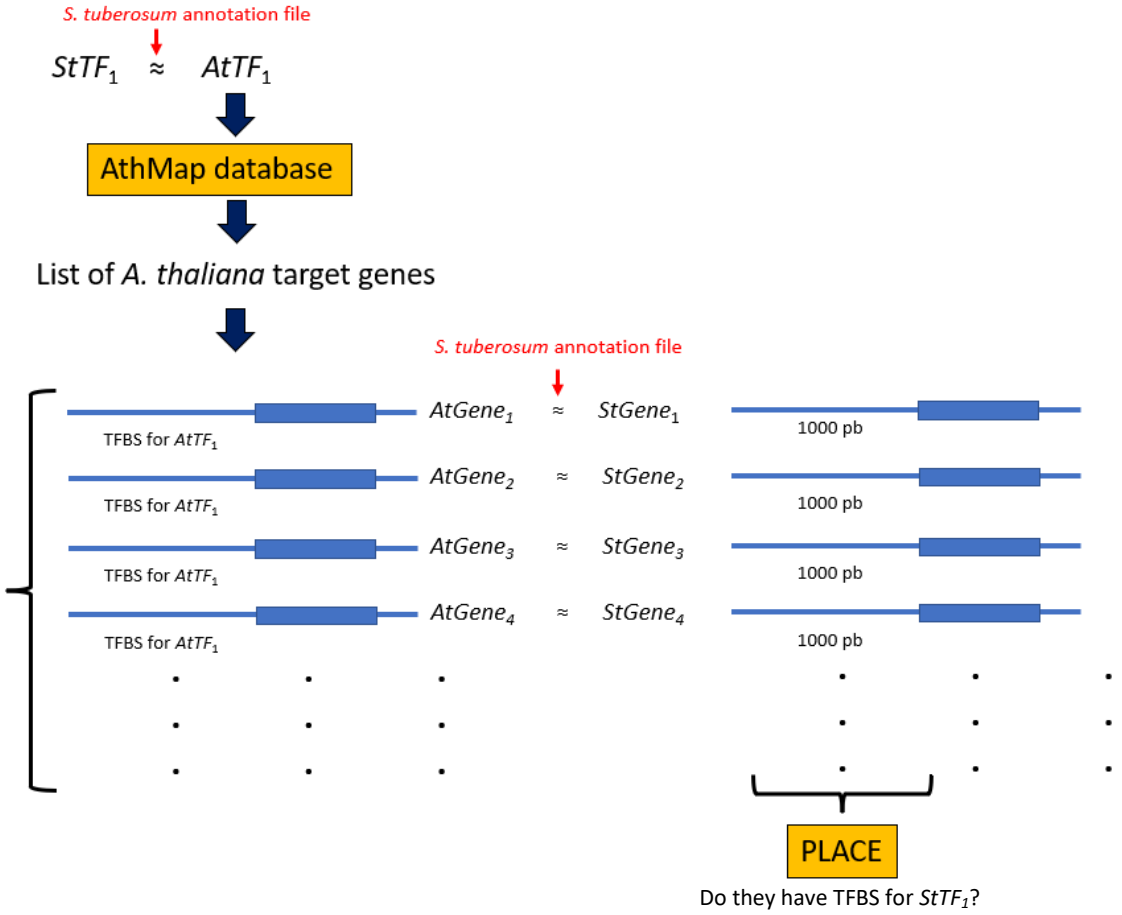


Figure 5.3 Identifying targets genes of a specific transcription factor in potato. The potato genome v6 annotation file was useful for this analysis since it contained the corresponded *A. thaliana* gene ID for each *S. tuberosum* gene. The AthMap database identified *A. thaliana* genes with transcription factors binding sites (TFBS) for a specific transcription factor of *A. thaliana* (AtTF). With the PLACE program, the identification of TFBS in a group of potato genes was analysed by confirming the presence of a TFBS in the sequence 1,000 bp upstream of the candidate gene sequence.

5.3. Results

The transcriptomic response of potato leaves exposed to drought or infected with *P. infestans*, from two independent assays using different varieties for each type of stress, was compared to identify common DEGs within the enriched biological processes involved in the response to both types of stress. There were no common DEGs observed among the top 20 most up or downregulated genes in response to each type of stress.

5.3.1. Enriched GO terms under drought stress or *P. infestans* infection

Biotic and abiotic stresses can activate common biological processes that include transcription factor activity, hormone signalling, pathogen defence responses, and cell wall modification (Nejat and Mantri, 2017). Therefore, enriched GO terms related to these processes were selected in each experiment to compare the responses of tolerant and susceptible varieties to both types of stress (**Table 5.1**).

There were no enriched GO terms in common between the response of the tolerant variety to drought and the response of the two resistant varieties to late blight. In general, many more biological processes were enriched in response to drought than under *P. infestans* infection. This was observed in the biological processes related to transcription factors, pathogen response, and hormone response, while for processes related to cell wall modification a similar number of terms were enriched under *P. infestans* infection and in response to drought (**Table 5.1**). All the enriched GO terms relating to response to pathogen or hormone activity were enriched for upregulated genes in the response to drought, while under *P. infestans* infection, the majority of these terms were enriched for downregulated genes. In general, more terms were enriched in the tolerant than in the susceptible variety exposed to drought, while more terms were enriched in the susceptible than in the resistant varieties infected with *P. infestans* (**Table 5.1**), though this was not true for cell wall related processes.

Table 5.1 Enriched GO terms in potato plants under drought stress or *P. infestans* infection. "Up" and "Down" indicate if the enrichment was for a group of up or downregulated DEGs.

Enrichment GO terms	Drought						*P. infestans*		
	Tolerant			Susceptible			SS	SM	D
	T1	T2	T3	T1	T2	T3	Inf 48 vs Mock 48		
Transcription factors									
GO:0006355: regulation of transcription, DNA-templated	Up	Up	Up	-	-	-	-	Up	-
GO:0043433: negative regulation of sequence-specific DNA binding transcription factor activity	Down	-	-	Down	-	-	-	-	-
GO:0045892: negative regulation of transcription, DNA-templated	Up	-	-	-	-	-	-	-	-
GO:2000143: negative regulation of DNA-templated transcription, initiation	Up	-	-	-	-	-	-	-	-
Response to pathogen									
GO:0002239: response to oomycetes	-	Up	-	-	-	-	-	-	-
GO:0009611: response to wounding	-	Up	Up	-	-	-	-	-	Down
GO:0010200: response to chitin	Up	Up	Up	-	-	Up	-	-	Down
GO:0042742: defence response to bacterium	-	Up	Up	-	-	Up	-	-	-
GO:0050832: defence response to fungus	-	Up	Up			Up	-	-	-
GO:0071323: cellular response to chitin	-	Up	Up	-	-	-	-	-	-
GO:0031347: regulation of defence response	-	-	-	-	-	-	-	-	Down
GO:0002215: defence response to nematode							-	-	Up
GO:0006952: defence response	-	-	-	-	-	-	-	Up	-
Hormone related									
GO:0009737: response to abscisic acid	Up	Up	Up	-	-	-	-	-	Down
GO:0009738: abscisic acid-activated signalling pathway	Up	Up	Up	-	Up	Up	-	-	Down
GO:0080142: regulation of salicylic acid biosynthetic process	-	Up	Up	-	-	-	-	-	-
GO:2000022: regulation of jasmonic acid mediated signalling pathway	-	Up	Up	-	-	-	-	-	Down
GO:0009873: ethylene-activated signalling pathway	-	-	-	-	-	-	-	Up	-
Cell wall related									
GO:0046274: lignin catabolic process	-	-	-	-	-	Up	-	-	Down
GO:0009807: lignan biosynthetic process	-	-	-	-	Down	-	-	-	-
GO:0010143: cutin biosynthetic process	Down	-	-	-	-	-	-	-	-
GO:0009827: plant-type cell wall modification	Up	-	-	-	-	Up	-	-	-
GO:0009828: plant-type cell wall loosening	-	-	-	-	-	Down	-	-	-
GO:0010411: xyloglucan metabolic process	-	-	-	-	-	-	-	-	Up
GO:0009809: lignin biosynthetic process	-	-	-	-	-	-	Up	Up	Up
GO:0042546: cell wall biogenesis	-	-	-	-	-	-	-	-	Up
GO:0080051: cutin transport	-	-	-	-	-	-	-	Up	-

5.3.2. DEGs inside the GO terms enriched under the response to biotic and abiotic stress.

All the DEGs inside the enriched GO terms shown in **Table 5.1** were identified for all 5 potato varieties exposed to drought or infected with *P. infestans*. Within the analysed GO terms, the majority of the DEGs were specific to the drought response (**Figure 5.4, Figure 5.5, Figure 5.6, Figure 5.7)**. There were more genes differentially expressed only in the two potato varieties, Negrita and Wila Huaka Lajra, after drought stress (but not in any of the three potato varieties infected with *P. infestans*) compared with the number of genes expressed only in the three varieties exposed to *P. infestans* (but not under any drought treatment). In addition, more genes were significantly differentially expressed in only the tolerant variety Negrita and specific to drought or rewatering treatment than only in the two tolerant varieties Sarpo Mira and Sarpo Shona and specific to the *P. infestans* infection. A selection of these genes is listed in **Table 5.2**.

Table 5.2 DEGs differentially expressed in only the tolerant varieties in response to a specific stress (abiotic or biotic). The selected DEGs are within the enriched GO terms that relate to the activity of transcription factors (GO:0006355, GO:0043433, GO:0045892, GO:2000143). This only a short list of the total transcription factors that follow this pattern and the complete table is provided in **Appendix-Table 5.1.**

Potato gene ID v6	Gene function	Drought						P. infestans		
		Tolerant			Susceptible			SS	SM	D
		T1	T2	T3	T1	T2	T3	Inf 48 vs Mock 48		
Soltu.DM.06G010320	DRE-binding protein 2A	2.07	-	-	-	-	-	-	-	-
Soltu.DM.05G022450	DRE-binding protein 2A	2.02	-	-	-	-	-	-	-	-
Soltu.DM.12G005300	bifunctional nuclease in basal defense response	2.28	2.50	-	-	-	-	-	-	-
Soltu.DM.03G037200	GRAS family transcription factor	1.85	1.94	-	-	-	-	-	-	-
Soltu.DM.10G024900	growth-regulating factor	-1.99	-1.63	-1.97	-	-	-	-	-	-
Soltu.DM.03G021700	heat shock protein	-	4.67	-	-	-	-	-	-	-
Soltu.DM.10G002230	NAC domain containing protein	-	4.61	-	-	-	-	-	-	-
Soltu.DM.12G026290	Integrase-type DNA-binding superfamily protein	-	-	5.10	-	-	-	-	-	-
Soltu.DM.06G012920	Integrase-type DNA-binding superfamily protein	-	-	6.16	-	-	-	-	-	-
Soltu.DM.04G008290	Integrase-type DNA-binding superfamily protein	-	-	-	-	-	-	1.75	2.14	-
Soltu.DM.02G027750	BTB and TAZ domain protein	-	-	-	-	-	-	1.75	1.72	-
Soltu.DM.05G005300	NAC-like, activated by AP3/PI	-	-	-	-	-	-	1.28	1.14	-
Soltu.DM.10G011330	phytochrome A	-	-	-	-	-	-	-1.39	-1.09	-

Common DEGs among only the 3 tolerant/resistant varieties that were responding to drought stress (T_1 and T_2) or to *P. infestans* infection were identified. These 8 genes included one transcription factor (*Soltu.DM.11G004620*-AtNAC50), genes that respond to pathogens (*Soltu.DM.11G002890*-Protein kinase superfamily protein, *Soltu.DM.05G006300*-AtWRK70, *Soltu.DM.02G033940*-AtLHT1, *Soltu.DM.02G020560*-AtCERK1), and a gene that responds to ABA (*Soltu.DM.11G002700*-AtHVA22E) (**Table 5.3**). Two genes, *Soltu.DM.03G030960*-AtWRKY40 and *Soltu.DM.12G006670*-AtCRK25, were also significantly upregulated in the tolerant/resistant varieties during both stresses but were downregulated in the susceptible under *P. infestans* infection (**Table 5.3**). These 8 genes were selected as candidate genes important in the tolerance/resistant response against both drought stress and *P. infestans* infection in potato.

The cell wall related genes that were DE under drought or *P. infestans* infection do not show some specific common genes responding in only tolerant/resistant varieties under both types of stress. There were some genes responding to a specific stress, or with opposite expression pattern, like genes encoding plant invertase/pectin methylesterase inhibitor superfamily proteins and cytochrome P450, family 98, subfamily A, polypeptides **(Figure 5.7**).

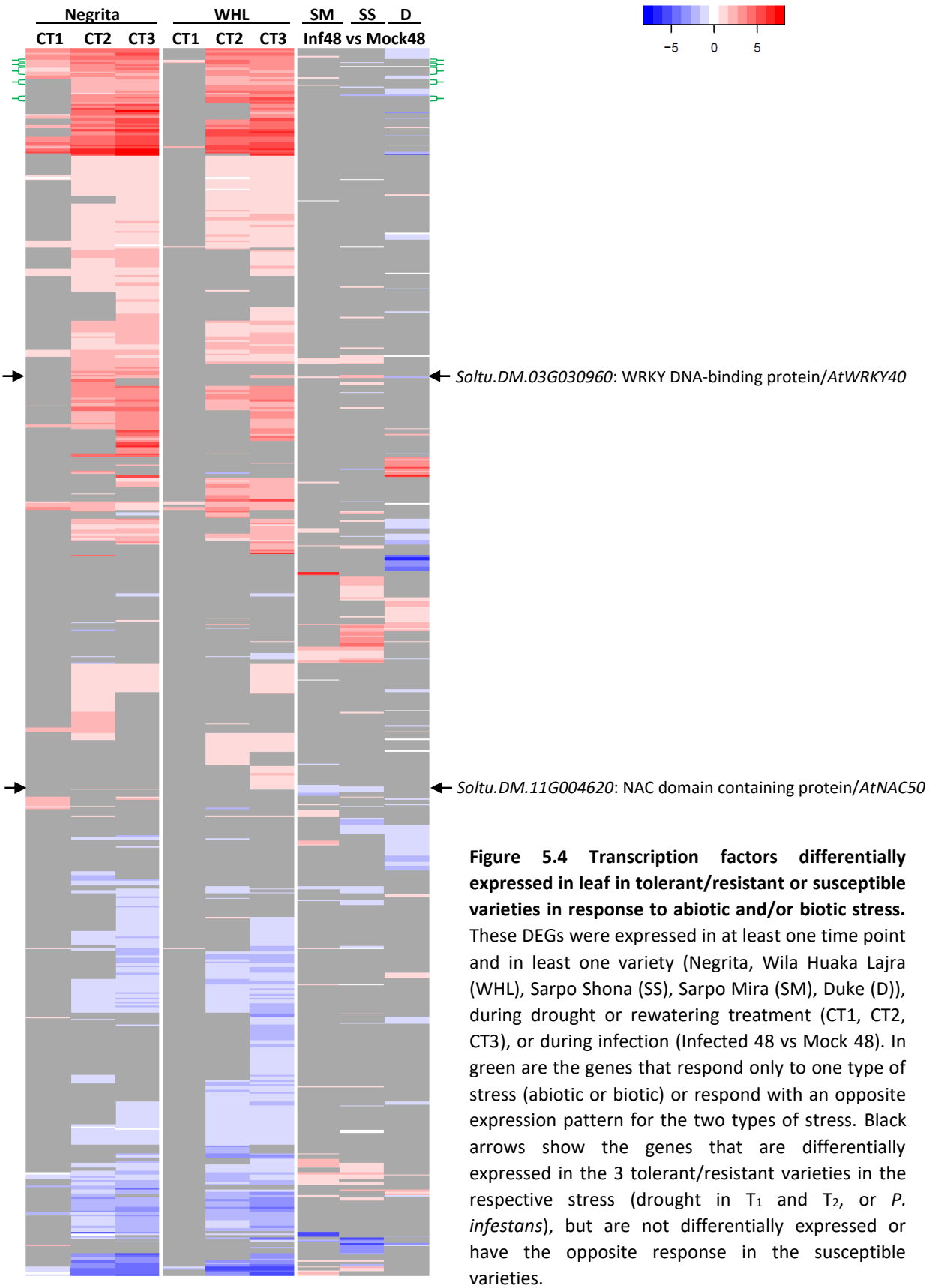
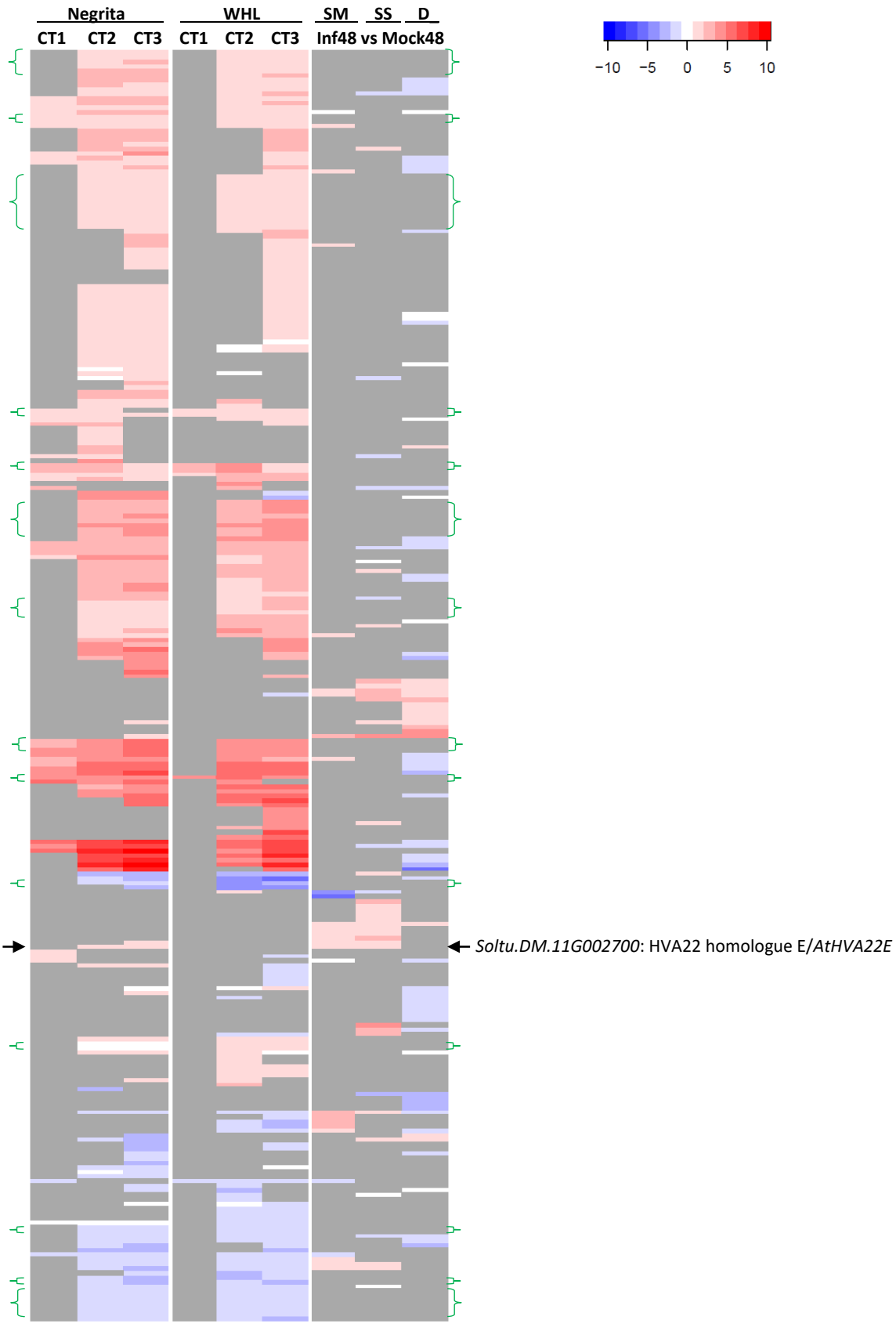


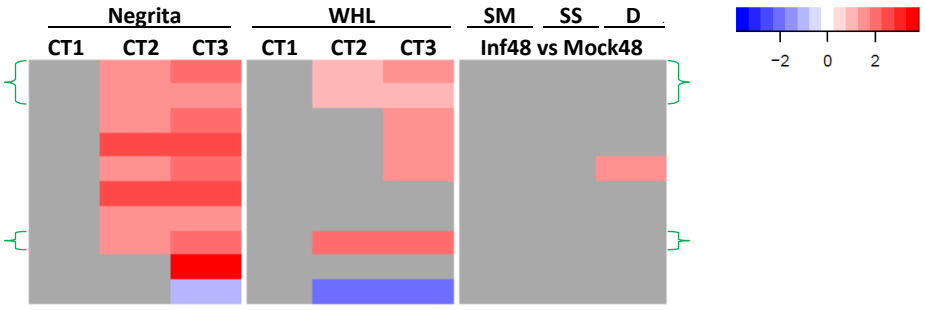
Figure 5.4 Transcription factors differentially expressed in leaf in tolerant/resistant or susceptible varieties in response to abiotic and/or biotic stress. These DEGs were expressed in at least one time point and in least one variety (Negrita, Wila Huaka Lajra (WHL), Sarpo Shona (SS), Sarpo Mira (SM), Duke (D)), during drought or rewatering treatment (CT1, CT2, CT3), or during infection (Infected 48 vs Mock 48). In green are the genes that respond only to one type of stress (abiotic or biotic) or respond with an opposite expression pattern for the two types of stress. Black arrows show the genes that are differentially expressed in the 3 tolerant/resistant varieties in the respective stress (drought in T_1 and T_2, or *P. infestans*), but are not differentially expressed or have the opposite response in the susceptible varieties.

Figure 5.5 Pathogen response related DEGs expressed in leaf in tolerant/resistant or susceptible varieties in response to abiotic and/or biotic stress. These DEGs were expressed in at least one time point and in least one variety (Negrita, Wila Huaka Lajra (WHL), Sarpo Shona (SS), Sarpo Mira (SM), Duke (D)), during drought or rewatering treatment (CT1, CT2, CT3), or during infection (Infected 48 vs Mock 48). In green are the genes that response in only abiotic or in only biotic stress or respond with an opposite expression pattern for the two types of stress. Black arrows show the genes that are differentially expressed in the 3 tolerant/resistant varieties in the respective stress (drought in T1 and T2, or *P. infestans*), but are not differentially expressed or have the opposite response in the susceptible varieties.

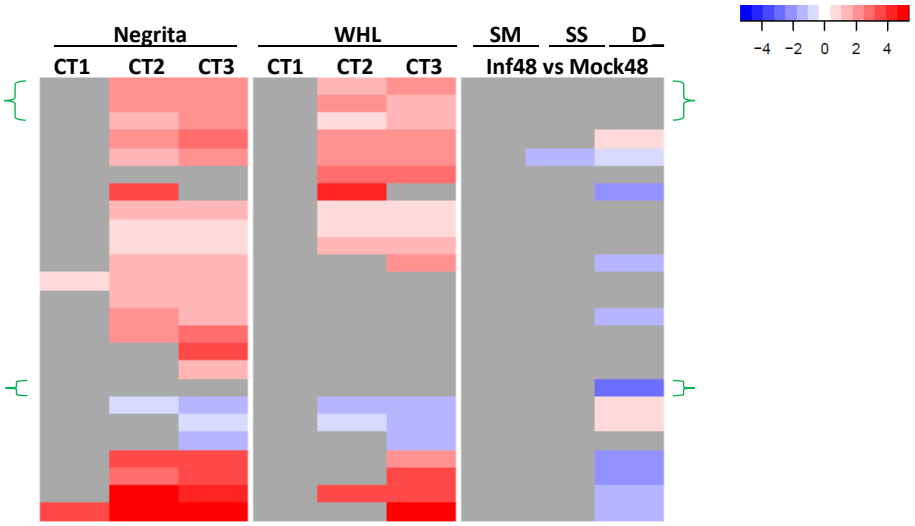
(A) ABA (GO:0009737-response to ABA/GO:0009738-ABA-activated signalling pathway)



(B) SA (GO:0080142: regulation of salicylic acid biosynthetic process)



(C) JA (GO:2000022-regulation of jasmonic acid mediated signalling pathway)



(D) Ethylene (GO:0009873-ethylene-activated signalling pathway)

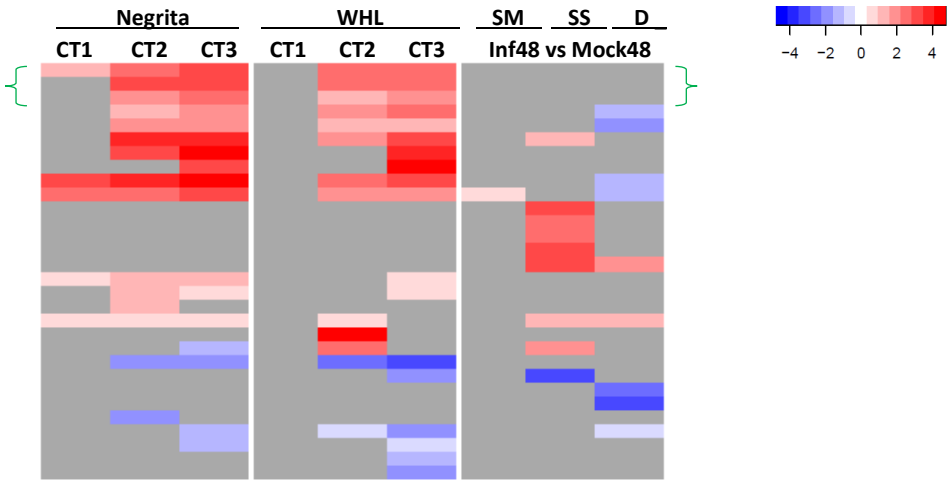


Figure 5.6 Hormone related genes differentially expressed in leaf in tolerant/resistant or susceptible varieties in response to abiotic and/or biotic stress. These DEGs were expressed in at least one time point and in least one variety (Negrita, Wila Huaka Lajra (WHL), Sarpo Shona (SS), Sarpo Mira (SM), Duke (D)), during drought or rewatering treatment (CT1, CT2, CT3), or during infection (Infected 48 vs Mock 48). In green are the genes that response in only abiotic or in only biotic stress or respond with an opposite expression pattern for the two types of stress. Black arrows show the genes that are differentially expressed in the 3 tolerant/resistant varieties in the

respective stress (drought in T_1 and T_2, or *P. infestans*), but are not differentially expressed or have the opposite response in the susceptible varieties.

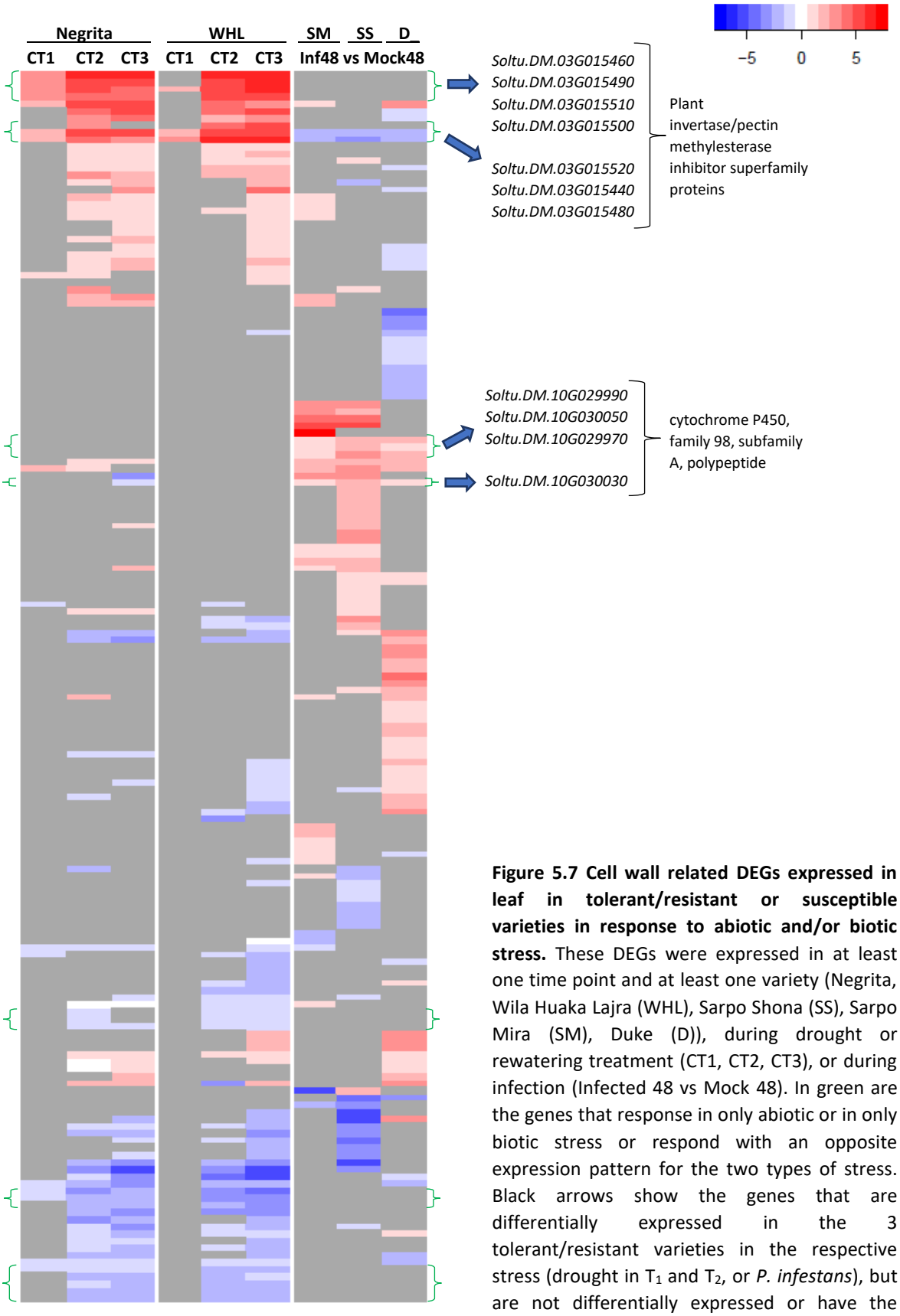


Figure 5.7 Cell wall related DEGs expressed in leaf in tolerant/resistant or susceptible varieties in response to abiotic and/or biotic stress. These DEGs were expressed in at least one time point and at least one variety (Negrita, Wila Huaka Lajra (WHL), Sarpo Shona (SS), Sarpo Mira (SM), Duke (D)), during drought or rewatering treatment (CT1, CT2, CT3), or during infection (Infected 48 vs Mock 48). In green are the genes that response in only abiotic or in only biotic stress or respond with an opposite expression pattern for the two types of stress. Black arrows show the genes that are differentially expressed in the 3 tolerant/resistant varieties in the respective stress (drought in T_1 and T_2, or *P. infestans*), but are not differentially expressed or have the

Table 5.3 Genes that respond differently in the tolerant/resistant and the susceptible varieties under both types of stress. These genes were selected from the DEGs that were inside the selected enriched GO terms shown in **Table 5.1** . The table shows the log2FC of each gene in each time point of each type of stress, in the early (T_1), late (T_2) and recovery (T_3) response to drought, and after 48 hours of infection with *P. infestans*. (-) means the gene did not have a significant log2FC in the respective time point or comparison.

Potato gene ID v6	Gene function	Athaliana gene ID	Athaliana gene name	Drought						*P. infestans*		
				Tolerant			Susceptible			SS	SM	D
				T1	T2	T3	T1	T2	T3	Inf 48 vs Mock 48		
Transcription factor												
Soltu.DM.03G030960	WRKY DNA-binding protein	AT1G80840	WRKY40	-	2.24	2.49	-	-	2.17	1.34	1.88	-2.05
Soltu.DM.11G004620	NAC domain containing protein	AT3G10480	ANAC050	-	0.91	0.71	-	-	0.43	-1.02	-0.82	NA
Respond to Pathogen												
Soltu.DM.11G002890	Protein kinase superfamily protein	AT5G65530	AT5G65530	-	2.19	2.46	-	-	1.28	0.5	0.53	NA
Soltu.DM.05G006300	WRKY DNA-binding protein	AT5G15130	WRKY70	-	-1.74	-	-	-	-	1.86	2.59	NA
Soltu.DM.02G033940	lysine histidine transporter	AT5G40780	LHT1	-	1.06	1.20	-	-	-	0.87	0.91	NA
Soltu.DM.02G020560	chitin elicitor receptor kinase	AT3G21630	CERK1	1.0	1.08	0.73	-	-	-0.77	0.8	0.72	NA
Soltu.DM.12G006670	cysteine-rich RLK (RECEPTOR-like protein kinase)	AT4G05200	CRK25	-	2.09	-	-	-	-	0.72	0.67	-1.08
Hormone related – ABA												
Soltu.DM.11G002700	HVA22 homologue E	AT5G50720	HVA22E	-	1.32	1.43	-	-	-	1.32	0.84	NA

5.3.3. Putative target genes regulated by specific transcription factors.

From the 3 transcription factors responding differently in the tolerant/resistant and susceptible varieties under both stresses (*Soltu.DM.03G030960 – AtWRKY40*, *Soltu.DM.11G004620 – ANAC050*, and *Soltu.DM.05G006300 – AtWRKY70,* in **Table 5.3**), it was possible to identify putative gene targets for Soltu.DM.03G030960 – AtWRKY40 using the information in the AthMap database.

This *AtWRKY40* gene had 991 putative *A. thaliana* gene targets. The IDs of these genes were searched in the annotation file of the potato genome v6. From these 991 genes, 400 were linked to 822 potato gene IDs. Among these 822 potato genes, 419 were differentially expressed in at least one treatment of the drought assay (T_1, T_2, or T_3) or during *P. infestans* infection. The differential expression of these 419 genes was represented in a heatmap to observe their response under both types of stress (**Figure 5.8**).

From the 419 genes, 67 responded only to drought (T_1 or T_2) in only the tolerant variety Negrita, but not in the susceptible Wila Huaka Lajra, and most of them were upregulated genes, while 9 genes responded only to the *P. infestans* infection in only both resistant varieties Sarpo Mira and Sarpo Shona, but not in Duke of York, most of which were downregulated genes (**Figure 5.9**). Additionally, within the 419 genes, 4 genes could be identified that were DE under both types of stress (T_1 and T_2 in drought treatment and 48 hours post infection with *P. infestans*) in all the tolerant/resistant varieties, but not in the susceptible ones (**Figure 5.9**, **Table 5.4**).

Analysis of the 1,000 bp upstream region of the 4 DEGs that were putative targets of the WRKY40 TF and differentially expressed in the tolerant/resistant varieties under both types of stress (**Table 5.4**), confirmed the location of the W-box (C/TTGACT/C) domain, which is the binding site for the WRKY gene (**Appendix-Table 5.2**).

Figure 5.8 Putative genes regulated by *Soltu.DM.03G030960* - *AtWRKY40* under drought or re-watering treatment or *P. infestans* infection.

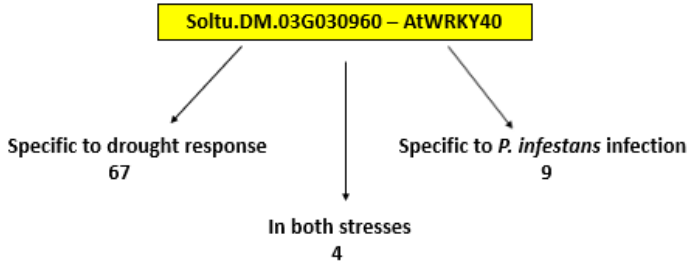


Figure 5.9 Putative genes regulated by *Soltu.DM.03G030960* (*AtWRKY40*) in tolerant/resistant varieties. The figure shows the number of DEGs in only the tolerant variety under only drought stress (67 genes), only in the response of the resistant varieties to *P. infestans* infection (9 genes) or in the response of all tolerant/resistant varieties under both types of stress (4 genes).

Table 5.4 Genes putatively regulated by the *Soltu.DM.03G030960* (*AtWRKY40*) gene in only the tolerant/resistant varieties in response to drought and *P. infestans* infection. The table shows the log2FC of each gene in each time point of each type of stress: T_1 or T_2 early and late response to drought, and after 48 hours of infection with *P. infestans*.

Gene ID	Gene_Name	Athaliana gene ID	Athaliana gene name	Drought				*P. infestans*		
				Tolerant		Susceptible		SS	SM	D
				T1	T2	T1	T2	Inf 48 vs Mock 48		
Soltu.DM.04G010950	heat shock transcription factor B3	AT2G41690	AtHSFB3	-	2.78	-	-	2.09	2.62	-
Soltu.DM.12G006670	cysteine-rich RLK (RECEPTOR-like protein kinase)	AT4G05200	AtCRK25	-	2.09	-	-	0.72	0.67	-1.08
Soltu.DM.02G020560	chitin elicitor receptor kinase	AT3G21630	AtCERK1	1.00	1.08	-	-	0.80	0.72	-
Soltu.DM.10G003320	conserved hypothetical protein	AT2G33180	AT2G33180	-	-0.69	-	-	0.86	0.80	-

5.4. Discussion

Potato yield is affected by various kinds of biotic and abiotic stress, but the most devastating factors are drought and *P. infestans* infection. With the aim of identifying common candidate tolerance genes activated or repressed by these two stresses, the reaction against drought and blight of potato varieties with different degrees of resistance was compared. This comparison was focused on the biological processes that are known to be important in plant responses to biotic and abiotic stresses, including processes related to transcription factors, responses to pathogen attack, hormone regulation and cell wall modification (Nejat and Mantri 2017).

Overall, transcriptional reprogramming in response to drought stress was much more widespread compared with the response to blight. As expected, most responses were specific to one type of stress, though some common responses could be identified. In total, 8 genes were associated with the response of tolerant/resistant varieties against drought and *P. infestans* within the selected groups of GO terms enriched in one or more stress conditions. They were genes encoding transcription factors, annotated in *A. thaliana* as WRKY40 (*Soltu.DM.03G030960*), WRKY70 (*Soltu.DM.05G006300*), and ANAC050 (*Soltu.DM.11G004620*); genes within the biological processes related to the pathogen attack, such as protein kinase superfamily protein (*Soltu.DM.11G002890*), lysine histidine transporter (*Soltu.DM.02G033940*-LHT1), chitin elicitor receptor kinase (*Soltu.DM.02G020560*-CERK1), cysteine-rich RLK (*Soltu.DM.12G006670*-CRK25); and a gene within the biological process related to the response to ABA, the HVA22 homolog E (*Soltu.DM.11G002700*-HVA22E).

The WRKYs are a large family of transcription factors involved in different plant biological processes. In the potato genome, 79 WRKY genes were identified and through *in-silico* prediction and expression analysis, a number of them were linked to the response to different abiotic stresses, such as heat and drought (Zhang *et al*., 2017). WRKY TFs recognize the W-box *cis*-regulatory element

([(T)TGAC(C/T)]) in the promoter of their target genes and are involved in the response to biotic as well as abiotic stresses. In *Arabidopsis*, different members of the WRKY TF family change their expression in response to salicylic acid treatment or infection with *Pseudomonas syringe* (Dong, Chen, and Chen, 2003), and W-box elements were identified in the promoters of a group of pathogen-related (PR) genes (Eulgem *et al*., 1999, Maleck, 2020).

Specifically, the expression WRK40 has been observed to increase in response to drought and ABA treatment in *Arabidopsis* (Chen *et al*., 2010). When overexpressed, WRKY40 can interact with WRKY60 to inhibit the response of ABA, suggesting that both WRKYs could also be involved in negative feedback regulation of the ABA signalling pathway (Chen *et al*., 2010). It has been reported that in the ABA signalling pathway, WRKY40 can control the expression of ABI5, a bZIP TF that positively regulates ABA signals. When ABA is not present, WRKY40 interacts with the histone demethylase JMJ17, which interacts with the ABI5 chromatin and removes the activating methylation mark, H3K4me3; consequently, ABI5 is not expressed. In the absence of ABA, WRKY40 can also repress the binding activity of HY5, which is a bZIP TF that stimulates ABI5 expression. In the presence of ABA, the interaction of WRKY40 with JMJ17 and HY5 is abolished and the expression of ABI5 is activated (Wang *et al*., 2021c). This behaviour is well correlated with the fact that under high levels of ABA, WRKY40 is translocated from the nucleus to the cytosol to interact with the protein ABAR located in the chloroplast envelope (Shang *et al*., 2010), removing the repression of *ABI5* expression.

In pepper, WRKY40 inhibits the expression of genes involved in the tolerance to bacteria *Xanthomonas campestris* pv. Vesicatoria, such as genes related to the JA and SA signalling pathway. Therefore, the degradation of WRKY40 was associated with resistance to the pathogen. Interestingly, the effector XopS of *X. campestris* can inhibit the proteosome degradation of WRKY40 to induce susceptibility in the host. The invasion of the pathogen was favoured by the inhibition of stomatal

closure, which occurs in the presence of WRKY40 (Raffeiner *et al*., 2022). Therefore, overexpression of WRKY40 has been positively associated with stomatal opening and with negative regulation of the ABA signalling pathway and is involved in the crosstalk between abiotic and biotic stress responses.

In the potato varieties, upregulation of WRKY40 was observed under both types of stress (drought and *P. infestans* infection), only in the tolerant varieties. WRKY40 increased its expression in response to drought, as observed also in *Arabidopsis* (Chen *et al*., 2010), in the late response, where a higher number of genes that respond to ABA were also upregulated (**Figure 5.6-A**) in the tolerant compared with the susceptible variety. Also in the tolerant variety only (as described in Chapter 3), there was an early upregulation of many other genes involved in negative regulation of the ABA response, including ABI five binding proteins that facilitate the ubiquitin-mediated degradation of ABI5 and many protein phosphatase PP2C genes, indicating earlier, stronger negative regulation of the ABA signalling pathway in response to drought stress, which could be beneficial in terms of minimising detrimental effects of too much ABA (Gietler *et al*., 2020). In addition, the tolerant variety was able to maintain higher rates of photosynthesis throughout the drought stress treatments, which could indicate that the stomata were more open compared with the susceptible variety. In this way, the upregulation of WRKY40 may play an important role in the tolerance phenotype through negative regulation of ABA signalling and avoiding a drastic stomatal closure to resist prolonged drought events. In response to *P. infestans*, the high expression of WRKY40 observed in the resistant varieties in response to infection could enhance stomatal opening, which may not be a beneficial response for the plant (Raffeiner *et al*., 2022). Meanwhile, the susceptible variety Duke of York downregulated WRKY40, so the role of WRKY40 in the resistance response to *P. infestans* and its relationship with stomatal opening/closure requires further investigation.

Among the target genes detected for WRKY40 in the potato genome v6 were the cysteine-rich RLK (*Soltu.DM.12G006670*-AtCRK25) and the chitin elicitor receptor kinase (*Soltu.DM.02G020560*-

AtCERK1), and both were upregulated in the late response of the tolerant variety to drought and in the response to *P. infestans* only in the resistant varieties. CRK is part of the large family of RLKs that possess an extracellular domain to detect external stimuli or ligands, a transmembrane domain, and an intracellular kinase domain, which is activated after stimulus recognition. CRK expression has been observed to increase under SA, pathogen, and drought exposure (Ohtake, Takahashi, and Komeda, 2000, Acharya *et al*., 2007, Marshall *et al*., 2012). This overexpression observed in different CRKs has produced tolerance to osmotic stress (Tanaka *et al*., 2012) or pathogen infection (Acharya *et al*., 2007). CRKs regulate stomatal aperture, stomatal density, and development in response to abiotic stress and pathogen infection (Bourdais *et al*., 2015; Arellano-Villagómez *et al*., 2021). Different CRKs play different roles; while the expression of CRK2, CRK5, and CRK31 reduce water loss, the expression of CRK45 increases it. CRKs probably regulate stomatal aperture after ROS perception, since mutant *Arabidopsis* lines not expressing CRK5, CRK7, CRK20, and CRK28 genes produced ROS after flg22 elicitation, but not stomatal closure (Bourdais *et al*., 2015). Although CRK25 has not been functionally characterised, it may regulate stomatal closure like other characterised CRKs, since its expression was associated with WRKY40. While the expression of *Soltu.DM.12G006670* - AtCRK25 was upregulated in the tolerant/resistant varieties in response to both types of stress, it was downregulated in the susceptible Duke of York variety in response to blight, likely due to the downregulation of WRKY40.

CERK1 is a membrane receptor, a PRR with an extracellular lysin motif (LysM-PRR) involved in the recognition of chitin structure, which as a consequence activates downstream signals involved in an immune response, such as ROS production and MAPK activation (Miya *et al*., 2007; Yang, Wang, and Liu, 2022). In *Arabidopsis*, the main receptor for chitin is LYK5, which interacts and forms a complex with CERK1 to trigger an immune response (Cao *et al*., 2014) when chitin is perceived. Although CERK1 can also bind chitin, this interaction is not as strong as with LYK5 (Cao *et al*., 2014). In rice, CEBiP is the chitin receptor that interacts with CERK1 (Shimizu *et al*., 2010; Hayafune *et al*., 2014).

CERK1 was also involved in the crosstalk between the resistance/tolerance response against pathogens and salt stress and may explain why plants pre-treated within chitin have improved salt tolerance in *Arabidopsis* (Espinoza, Liang, and Stacey, 2017). The overexpression of CERK1 in *Arabidopsis* under salt stress produced similar transcriptomic changes to those observed by chitin exposure and CERK1 represents the link between salt stress and innate immunity to fungi triggered by chitin (Espinoza, Liang, and Stacey, 2017). Interestingly, the same was not true for the response to osmotic rather than salt stress, which the authors explained by the physical link between CERK1 and ANNEXIN1 (ANN1), which is a key channel involved in salt stress (Espinoza, Liang, and Stacey, 2017). In contrast, in our potato varieties, CERK1 is indeed part of the cross-talk between drought stress and response to pathogens as it is upregulated in the tolerant/resistant varieties in response to both stresses.

Although CERK1 was mainly related to the recognition of chitin, other ligands can also be directly recognized by CERK1 and activate an immune response. These ligands include the β-glucans, which are abundant in the cell wall of fungi and oomycetes and makes CERK1 a likely receptor for *Phytophthora* structures (Naveed *et al*., 2020; Yang, Wang, and Liu, 2022). Regarding drought, a transcriptomic analysis of a potato variety Kenxin1 showed that a CERK1 gene (*PGSC0003DMG400016433*) decreased its expression in response to the rehydration treatment, after a drought period of 14 days (Liu, Meng, and Chen, 2018). Although CERK1 is not well characterized in potato, it was involved in stomatal closure in response to chitin exposure (Ye *et al*., 2020) and could play an important role in resistance/tolerance to both types of stress.

WRKY70 plays a role in the connection between the SA and JA signalling pathways during pathogen attack. While SA is associated with the resistance to biotrophic and hemibiotrophic pathogens, the JA and ET hormones are associated with the resistance to necrotrophic pathogens (Glazebrook, 2005). SA triggers a systemic acquired resistance, which provokes a hypersensitive response that kills the

host cell. This is beneficial against biotrophic pathogens because they need living cells to grow, but it is detrimental against necrotrophic pathogens, because they feed from dead cells (Glazebrook, 2005, Mengiste 2012). It was reported that WRKY70 stimulates the expression of genes in the SA pathway and represses genes that respond to JA, while it is also activated by SA and inactivated by JA (Li *et al*., 2004). Consistent with this, the overexpression of WRKY70 promoted resistance to biotrophic pathogens (Li *et al*., 2006). Since *P. infestans* is a hemibiotrophic pathogen, the observed upregulation of WRKY70 in resistant varieties is likely a beneficial response against the infection in which SA is involved. Interestingly, however, we do not observe many differentially expressed genes related to the SA response in any of the three potato varieties (**Figure 5.6 -B**), which may be related to the fact that although WRKY70 positively regulates SA signals, WRKY70 together with WRKY40 negatively regulates SA biosynthesis (Wang, Amornsiripanitch, and Dong, 2006).

WRKY70 and WRKY54 are also involved in the regulation of stomatal aperture. Under drought, wild type *Arabidopsis* overexpressed WRK70 and WRK54. However, double mutants not expressing both genes increased their tolerance to drought, despite reduced proline levels and reduced expression of genes that respond to drought, including ABA-responsive genes, NCED3, PP2C, and LEA14 heat shock proteins. In these mutants, the improved tolerance was associated with a better capacity to maintain water status due to increased stomatal closure compared to wild type (Li *et al*., 2013). Therefore, WRKY70 and WRKY54 would act as negative regulators of stomatal closure by negatively controlling ABA signalling and SA biosynthesis (Li *et al*., 2013). Contrary to this result, Xiang *et al*., 2021, observed that *Arabidopsis* lines overexpressing WRKY70 from *Myrothamnus flabellifolia* (a desiccation-tolerant woody plant that can recover after drastic events of drought) had increased drought tolerance. The transformed *Arabidopsis* plants had longer roots, greener leaves, more stomatal closure, less lipid peroxidation, more antioxidant activity, more proline content, and higher expression of NCED3, RD29, P5CS (Pyrroline-5-carboxylic acid synthetase) genes than wild type. It was suggested that the contrasting responses to drought between plants overexpressing *AtWRKY70*

and *MfWRKY70* could be caused by the difference in the amino acid sequences of their WRKY domains (Xiang *et al*., 2021). In our potato varieties, the observed increase in expression of WRKY40 coupled with reduced expression of WRKY70 was associated with drought-tolerance. Since the tolerant variety was able to maintain a higher photosynthetic rate, this would suggest reduced stomatal closure compared with the susceptible variety, though additional data on stomatal aperture would be needed to directly address this question.

LHT1 transports amino acids lysine and histidine from the soil into the root, and into leaf cells (Hirner *et al*., 2006). Its expression was observed in lateral roots and leaves, more in older than young leaves (Hirner *et al*., 2006). For biotrophic and hemibiotrophic pathogens, these amino acids are a convenient source of nutrition. In *Arabidopsis*, mutant lines not expressing LHT1 showed increased resistance to biotrophic and hemibiotrophic pathogens. Mutants showed more callose deposition, more hypersensitive reaction, and expression of genes involved in the SA pathway (Liu *et al*., 2010). In addition, the accumulation of free radicals, such as H_2O_2 and NO, was observed in mutant lines. In *Arabidopsis*, it was suggested that the upregulation of LTH1 could be triggered by the pathogen to avoid the death of the host cells (Tünnermann *et al*., 2022). However, its accumulation could also be part of the plant's strategy to mobilize amino acids outside the infected area to restrict nutrient availability for the pathogen (Masclaux-Daubresse *et al*., 2010, Tünnermann *et al*., 2022). The latter explanation could be most likely in our case because the observed upregulation of LHT1 in tolerant potato varieties was not observed in the susceptible variety. Expression of LHT genes was also observed to be modulated under abiotic stress, consistent with our observations. An LHT gene from Panax ginseng, homologous to LHT1 in *Arabidopsis*, was overexpressed under salt stress, as well as under ABA, SA, and MeJA treatment (Zhang, *et al*., 2013). Wheat also showed changes in the expression of an LHT gene under salt stress, not LHT1 but LHT7 (Wan *et al*., 2017).

NAC domain-containing proteins are a large family of transcription factors involved in plant growth and development, and in the response to different environmental stresses or pathogen attack (Yuan *et al*., 2019). Different NACs trigger different levels of resistance against pathogens. For example, while overexpression of *ANAC042* decreased resistance to *P. syringae* pv. Tomato, the overexpression of ANAC062 increased the resistance against the same pathogen (Yuan *et al*., 2019). NAC TFs are also involved in stomatal immunity by controlling the aperture or closure of stomata, where ABA and JA are probably involved (Yuan *et al*., 2019). In potato, two NAC TFs, StNTP1 and StNTP2, were targets of a *P. infestans* effector. This effector prevented the translocation of the two NACs into the nucleus and increased plant susceptibility to infection (McLellan *et al*., 2013). In the tolerant Sarpo potato varieties, downregulation of *ANAC050* was observed, but not in the susceptible variety, though its exact role is unclear. The opposite was observed in the response to drought, with the tolerant variety upregulating the expression of ANAC050. NAC proteins also regulate ABA synthesis by regulating the expression of the NCED enzymes (Yuan *et al*., 2019) and are involved in drought and salinity tolerance, through both ABA dependent and independent pathways (Nuruzzaman, Sharoni, and Kikuchi, 2013, Shen *et al*., 2017, Jiang *et al*., 2019). In rice, the increase in ABA levels was associated with the expression of OsNAC2 TF, which interacts with the promoter of *NCED3* gene and activates its expression (Jiang *et al*., 2019). In potato, ABA or drought treatment significantly increased StNAC053 expression, and in transgenic *Arabidopsis* lines, the overexpression of this *StNAC053* gene provided a better drought tolerance than wild type lines (Wang *et al*., 2021).

ABA has an important role in the response against both evaluated stresses by regulating stomatal closure, which allows the plants to reduce water loss under drought and also to stop the dispersion of sporangia after *P. infestans* infection (Farell, Preece, and Wren., 1969). Although the data indicate that the ABA signalling pathway was activated in response to drought, this activation was not observed in response to *P. infestans* attack. Indeed, the susceptible variety Duke of York showed enrichment of downregulated genes that respond to ABA, suggesting a possible inactivation of ABA

signalling and as a consequence a possible stomatal opening at 48 h.p.i. It was previously observed that stomata opening occurs in potato leaves incubated in dark after 8 hpi with *P. infestans* (Yang *et al*., 2021). This response is modulated by *P. infestans* through triacyglycerol breakdown and starch degradation to re-open guard cells in favour of the infection. Yang *et al* did not evaluate the ABA signalling pathway, but the analyses showed that stomata stopped being sensible to ABA under maximum stomatal aperture at 48 h.p.i. (Yang *et al*., 2021). ABA also favours the resistance by promoting callose deposition (Asselbergh *et al*., 2007, Luna *et al*., 2011, Shwarzenbacher, 2020). In our potato varieties, the ABA was probably activated at an early time point where guard cells still respond to this effect and callose deposition is carried out, which was observed in our 3 varieties, but in less amount in the susceptible variety. However, ABA was also associated to be negative against infection since ABA inactivates SA, JA, and ET response, and therefore suppressing the resistance against biotrophic and necrotrophic pathogens (Anderson, 2004, Audenaert, 2002). Recently, it was predicted that the effect in the resistance of the exogenous application of ABA depends on the age of the plant, the lifestyle of the pathogen, and the ABA concentration (Stevens *et al*., 2023). Specifically, with *P. infestans*, the application of more than 500 uM of ABA produced susceptibility in tomato plants of different ages for which it was mentioned that ABA would be inducing this susceptibility by repressing SA signalling (Stevens et al., 2023).

ABA signalling is regulated by WRKY TFs. Specifically, the interaction among WRKY18, WRKY40 and WRKY60 has shown to be important for ABA signalling, being WRKY60 target of WRKY18 and WRKY40, and while WRKY18 and WRKY60 positively regulate ABA signalling, WRKY40 had an opposite effect. It has been shown that this interaction plays an important role in abiotic stress as well as plant defence (Chen *et al.*, 2010). Interestingly, in this thesis, among the transcription factors that were identified as a part of the response to both stresses were members of the family WRKY, specifically WRKY40 and WRKY70. These transcription factors could contribute to the resistance

observed in the evaluated potato varieties by connecting ABA signalling and the modulation of stomata closure, as well as, intersecting the signalling of ABA with other hormones, such as SA.

5.4.1. Conclusions and Future Work

Regulation of stomatal opening/closure appears to be central, not only to enable the plant to withstand drought, but also in battling *P. infestans* infection, where stomatal closure and guard cell death help the plant to resist infection (Yang *et al*., 2021). A rapid stomatal closure and death have been observed 4 to 8 hours after infection with *P. infestans* and was a common response among different potato varieties with different degrees of resistance/susceptibility (Yang *et al*., 2021). In response*, P. infestans* can induce stomatal opening by modulating starch and lipid degradation, generating free sugar that allows an increase in cellular turgor, to continue the infection and life cycle (Yang *et al*., 2021). Therefore, a more detailed investigation of stomatal immunity to both types of stress is warranted in relation to the expression of the key transcription factors identified (WRKY40, WRKY70, ANAC50) and their target genes, particularly the transmembrane receptors involved in the recognition of pathogen cell wall component or other ligands (CRK25, CERK1). Possible next steps involve overexpressing or repressing the expression of the identified genes to observe the effect on varietal responses to *P. infestans* and drought, ideally in the same set of varieties. Drought stress and late blight infection will not usually be observed together in a potato field, due to the humidity requirement of *P. infestans* for sporangia production and dispersal. Therefore, although the dual exposure of plants to different forms of stress is usually more realistic, in this case, a separate evaluation of the two types of stress is appropriate.

CHAPTER 6: General discussion

6.1. Introduction

Potato plants are considered important for food security due to their high nutritional value. However, they are highly susceptible to *P. infestans* and drought, which are the two major factors decreasing their yield. Therefore, I conducted a glycome and transcriptomic analyses of potato varieties with different levels of tolerance to drought and resistance to *P. infestans* infection to identify genetic and cellular components that influence the tolerance of potato to these stressors. Although the cell wall is a dynamic structure that is regulated during cell growth and development, and under different types of stress, there are no studies that specifically describe the dynamics of the potato cell wall and commonalities under drought and *P. infestans*. In this scenario, I focused on describing how the cell wall and cell wall-related genes are regulated under these two stressors and identifying which changes are specifically observed in tolerant/resistant genotypes. In addition, I also analysed how genes that change their expression under the evaluated stressors and encode transcription factors, receptors, and genes involved in hormone signalling could participate in the tolerant/resistant response of the evaluated potato varieties. Furthermore, the autotetraploid condition of cultivated potato presents a challenge for genetic improvement of this crop. Nowadays, breeding programs widely apply GWAS to identify genetic markers associated with a desirable phenotype in natural populations. Because GWAS is based on the concept of linkage disequilibrium, I developed a theoretical model to describe linkage disequilibrium and its decay in autotetraploid species based on the factors influencing their meiosis.

6.2. Cell wall composition and its modification in drought and blight tolerant phenotypes.

Employing a glycome analysis using Comprehensive Microarray Polymer Profiling (CoMPP) as described in Chapter 3, I identified that tolerant potato varieties grown in the field possessed higher levels of specific components of the cell wall in the absence of any visible infection. These components, which were less abundant in the susceptible varieties, can predispose the plants to have a more effective barrier against *P. infestans*. I also observed by a transcriptomic analysis, that genes involved in the synthesis of these cell wall components can be modified under infection (Chapter 5) and drought conditions (Chapter 3), with some of these changes specific to tolerant varieties. Although the potato varieties employed in the drought and *P. infestans* infection assay were different, I found that similar cell wall components and genes were responding to both types of stress. These included changes in pectin, hemicellulose, glycoprotein, and lignin components (**Table 6.1**).

Pectin was one of the structures whose composition was significantly different in the resistant and susceptible varieties to *P. infestans* before the infection. The glycome analysis indicated that leaves from resistant varieties had homogalacturonans with a higher degree of methylesterification and RG-I with more arabinan residues. A higher level of methylesterification would protect the host cell against degradation by pathogen secreted polygalacturonases (PGs) and pectato lyases (PLs), which has been shown in many different plant pathosystems (Lionetti *et al*. 2012; Lionetti, Cevone, and Bellicampi, 2017). This can be observed in the higher levels of methylesterified pectin that positively correlated with resistance of potato tubers to *P. carotovorum*, of bean to *Colletotrichum lindemuthianum*, and of tomato to *Ralstonia solanacearum* (McMillan *et al*., 1993; Boudart *et al*., 1998, Wydra *et al*., 2006). In potato, jasmonic acid modulates the degree of pectin methylesterification to protect pectin from degradation by the PLs produced by the soft rot pathogen

Dickeya dadantii (Taurino *et al*., 2014), though here we have shown that pre-existing differences in abundance may also be important.

Table 6.1 Composition of the cell wall in potato varieties exposed to drought and *P. infestans* infection. In black are the components observed directly in the CoMPP analysis and in light blue are the components extrapolated from the CoMPP and the transcriptomic analyses. Up and down arrows indicate higher or lower amount in the tolerant (TOL) or susceptible (SUS) variety. (-) indicates a change not found in the respective tissue in any variety.

Cell Wall Component	Pre-existing structures Leaf	Response to the infection Leaf	Response to drought Leaf	Response to drought Root
PECTIN				
Pectin methylesterification	↑TOL	↓TOL&SUS	↑TOL	↓TOL
Arabinan (in RG-I)	↑TOL			
Pectin acetylation	-	↑TOL		
WAK proteins	-	↑TOL		
HEMICELLULOSE				
Xyloglucan	↑TOL	↑ SUS (XTH)	↑↓TOL&SUS	↓TOL&SUS(XTH)
Xylan	↑TOL	↑SUS (*AtBXL2*)		
Xylose	-	↑TOL (*AtXYL2*)		
Mannan	↑TOL	↓TOL *(AtCSLA9*)		
GLYCOPROTEIN				
Expansin like B 1 (EXPLB1)- *Soltu.DM.08G001190*	-	↑TOL		↑TOL
Extensin	↑TOL	↑ TOL ↑SUS	-	-
LIGNIN				
Lignin synthesis	-	↑TOL	-	↑TOL

On the other hand, arabinan is an important component of the cell wall of guard cells (Jones *et al*., 2003). In *Commelina cummunis*, arabinan helps maintain the flexibility of the cell wall and avoids the formation of a rigid cell wall structure created by the homogalacturonan (Jones *et al*., 2003). Regulation of the stomatal aperture is important during the infection with *P. infestans* since through this structure the pathogen can disperse its sporangia outside the infected host cell to continue propagating the infection. Yang *et al*. (2021) observed that at the beginning of the infection, potato leaves close and destroy their own stomata, but after 8 hours of the infection, *P. infestans* can manipulate this aperture in the host by producing metabolic changes in potato, where there is the

participation of ROS, triacylglycerol, and starch. However, until now, no studies have correlated cell wall composition, regulation of stomatal opening, and *P. infestans* tolerance.

Since arabinan and homogalacturonan are important components that influence the flexibility of the guard cell, they might be important factors that control the closing of guard cells after *P. infestans* attack. On the other hand, the high amount of arabinan observed in tolerant varieties to *P. infestans* could be beneficial not only in the response to this pathogen but also to drought. Indeed, African plants that can resurrect after periods of extreme drought possess a high amount of arabinan in the pectin component of their cell walls, conferring flexibility during drought and rehydration (Moore *et al*., 2008). At the extreme, plants that lack arabinan are unable to open or close their stomata because a rigid structure is formed by bridges of Ca^{2+} between homogalacturonan pectin (Jones *et al*., 2003). Besides the influence on cell wall flexibility, higher levels of RG-I in tolerant varieties, as a consequence of high levels of arabinan, may provide a dual benefit by also enhancing the accumulation of pectin-derived damps that can trigger an immune response in plants (Jiménez-Maldonado *et al*., 2018).

In pectin, the degree of cell wall methylesterification probably changed after drought and *P. infestans* attack, since the transcriptomic analyses indicated a change in the expression of INV/PMEI superfamily proteins, which include pectin methylesterase inhibitors (PMEIs). Resistant leaves infected with *P. infestans* and roots from tolerant plants exposed to drought downregulated more INV/PMEIs than the susceptible varieties, which would generate less methylated (or more unmethylated) pectin in the tolerant varieties. Unmethylated pectins are more susceptible to PGs and PLs and will generate more OGA fragments, which are recognized by WAK receptors. The transcriptomic analysis also suggested that resistant varieties to *P. infestans* possess more WAKs receptors, which would make the recognition of OGAs more rapid or efficient and enable a faster immune response to be triggered. In the case of drought, the unmethylated pectin fragments could

bond with each other by crosslinking with Ca^{2+} to generate an "egg-box" structure. This structure confers rigidity to the cell wall and in *Allium fistulosum* the formation of egg-box reduced the permeability of the cell wall and conferred tolerance to dehydration (Forand *et al*., 2022).

In contrast to the downregulation observed in roots, tolerant potato leaves exposed to drought upregulated PMEI, which would allow them to maintain higher levels of pectin methylesterification. Under drought, one of the most important mechanisms generated by the plant is the regulation of stomatal aperture/closure. The degree of pectin methylesterification in guard cell walls was observed to play a key role in the regulation of stomatal closure (An *et al*., 2008, Amsbury *et al*., 2016, Wu *et al*., 2017). During extreme heat stress, *pme34* mutant lines showed more greatly altered activity of PMEs and PGS, and a heat sensitive phenotype due to the important effect of methylesterification levels on guard cell wall flexibility (Huang *et al*., 2017; Wu *et al*., 2017). In addition, activation of PME34 was observed to be dependent on ABA (Huang *et al*., 2017; Wu *et al*., 2017). Also in *Arabidopsis*, the demethylesterification of pectin, produced by PME6, was important to regulate stomatal closure, since the high level of methylesterification by the non-expression of PME6 produced insensitive guard cells to CO_2 and ABA (Amsbury *et al*., 2016). Furthermore, *Arabidopsis* overexpressing a PMEI from pepper, *CaPMEI1,* which would maintain more methylesteried pectin, increased tolerance to drought in *Arabidopsis* by showing less water loss than the wild type leaves (An *et al*., 2008). The results of An *et al*, suggest that higher levels of methylesterification in pectin would be favourable to stomatal closure in drought tolerant varieties. All these results show that PME and PMEI are important enzymes influencing the flexibility and functionality of guard cells during abiotic stress. Therefore, I conclude that both arabinan and the degree of pectin methylesterification are important factors that may confer tolerance/resistance to potato varieties against both stressors and need to be experimentally evaluated more deeply in the future (**Figure 6.1**).

There were also some specific characteristics observed in response to *P. infestans*. These included the downregulation of pectin acetylesterases that would produce more pectin with acetyl groups and was associated with resistant response to this pathogen. This could be explained by the fact that acetylated pectin confers protection against endopolygalacturonases (Bonnin, *et al*., 2003), thus tolerant plants would be protected from a complete degradation by keeping part of the pectin acetylated. Therefore, downregulation of PAE and more WAK receptors would also be two key features of resistant potato varieties to *P. infestans* (**Figure 6.1**).

In the hemicellulose component, a high amount of xyloglucan, xylan, and mannan was also part of the pre-existing composition of resistant varieties to *P. infestans.* A higher abundance of xylans could create a more effective structural barrier against *P. infestans*, while a higher abundance of mannans and xyloglucans may generate more fragments that produce a stronger DAMP response (Claverie *et al*, 2018, Zhang *et al*., 2019). The transcriptomic analysis showed that the abundance and/or structure of these three components might be modified after *P. infestans* infection. Changes in xyloglucan could be produced by the change in the expression of xyloglucan endotransglucosylase/hydrolases (XTHs). These enzymes are capable of both disassembling and re-ligating the bond between cellulose and xyloglucan, and as a consequence, remodel the state of loosening of the cell wall (Lampugnani, *et al*., 2018). Here in potato, the susceptible variety to *P. infestans*, Duke of York, showed more upregulation of XTHs compared with tolerant varieties, although some downregulation of XTHs was also observed in all three varieties. Leaf and root of the two potato varieties exposed to drought changed the expression of several genes encoding XTHs. In root, both susceptible and tolerant varieties downregulated XTHs, with a stronger response in the tolerant variety. This suggests that overall, downregulation of XTH activity may be favourable in the response of potato to both drought and *P. infestans*. In wheat, downregulation of XTH was observed under drought in the apical zone of the roots, but to a greater extent in susceptible cultivars (Iurlaro *et al*., 2016). Downregulation of XTHs was also observed in leaves of *Arabidopsis* plants exposed to

drought (Clauw *et al*., 2015). In contrast, upregulation of XTHs was observed in rice roots under drought stress (Yang *et al*., 2006) and the constitutive expression of XTH3 in leaves of hot pepper produces tolerance to drought and salt (Cho *et al*., 2006). Because the expression of XTHs produces different effects, depending on the type of XTH and the species, further analyses are needed in potato to unravel the benefits of XTH downregulation observed here.

Another specific response associated with the resistance to *P. infestans* was the upregulation of a xylosidase (*Soltu.DM.01G013940 – AtXYL2*) that remove xylosyl residues, and downregulation of a mannan synthase (*Soltu.DM.10G024740 - AtCSL9*) in resistant plants. In plants, mannan functions as a reservoir of carbohydrates in seed, which later works as a source of nutrients during plant development and under drought, where it can also help to maintain water potential (Moreira and Filho, 2008, Ahl *et al*., 2019). The presence of mannan in the thick cell wall of epidermal cells suggested a possible function as a barrier against pathogens (Handford *et al*., 2003). In this thesis, more mannan was observed in resistant varieties to *P. infestans* before infection, which could be providing a stronger barrier in the cell wall to prevent pathogen infection or mannan oligosaccharide fragments (MOS) could be as a source of DAMPs (Zhang *et al*., 2019). However, a decrease in the expression of a mannan synthase observed only in Sarpo Shona is difficult to reconcile with this hypothesis. Change in the metabolism of mannose was found as a marker of the transition between biotrophic and necrotrophic phases of *P. infestans* infection (Botero *et al*., 2018). If *P. infestans* could use mannan from the plant host as a source of energy for this transition, then downregulation of mannan synthesis could produce less input of mannan for the pathogen.

In relation to glycoproteins, extensin and expansin composition varied depending on the degree of resistant phenotype. Resistant varieties to *P. infestans* had more extensins than the susceptible varieties without being exposed to the pathogen. Meanwhile, the expression of extensin genes increased in response to infection in Sarpo Shona and Duke, which could lead to an increase in

extensin abundance in both tolerant and susceptible varieties. Extensins are part of the HGRP family of proteins that can fortify the cell wall by cross-linking in response to pathogen attack and thus restrict pathogen entrance at the point of the infection (Deepak *et al*., 2010). Their role in plant defence against pathogens has been widely reported (Esquerré-Tugayé and Mazau 1974; Esquerré-Tugayé and Lamport 1979; Esquerré-Tugayé *et al*., 1979; and Wei and Shirsat, 2006). Regarding expansin, the gene *Soltu.DM.08G001190*, a homologue of *AtEXPLB1*, was highly upregulated in roots exposed to drought and leaves infected with *P. infestans* in only the resistant varieties. EXLB1 was previously reported to be upregulated in potato plants during drought (Moon *et al*., 2018; Gong *et al*., 2015). More expansin would produce more cell wall relaxation and loosening (Cosgrove, 2015), and its expression allows the growth of lateral roots and tolerance to drought (Marowa *et al*., 2016). The upregulation of expansin genes was also previously observed in potato under *P. infestans* infection (Yogendra *et al*., 2016). Expansin was also involved in the control of cell wall loosening and constriction of guard cells and as a consequence in the regulation of stomatal closure/opening (Marowa *et al*., 2016). The overexpression of expansin was associated with a decrease in stomatal density, with a destabilization of cellulose and a decrease in cell wall thickness of the guard cell, which influence negatively the development of guard cells (Sampedro and Cosgrove, 2005, Marowa *et al*., 2016). Under *P. infestans* infection, if the increased expression of expansin also leads to a reduction of stomatal density, there would be fewer exit points for the pathogen to disperse its sporangia.

Lignin was also a component that according to the transcriptomic analyses, likely had increased abundance in the cell wall of tolerant varieties in response to both drought stress in root and *P. infestans* in leaf. Moreover, the tolerant varieties had higher expression of some genes involved in lignin synthesis even before the infection, suggesting a higher baseline level of lignin in the cell wall, as in *C. sativa* (Eynck *et al*., 2012), though lignin was not investigated in the CoMPP analysis. Interestingly, under drought and *P. infestans* infection, the expression of a laccase gene involved in

lignin polymerisation, *Soltu.DM.04G028320*, was highly upregulated in the resistant varieties Sarpo Mira, Sarpo Shona, and Negrita. Lignin accumulation was observed to be favourable under drought and pathogen infection due to its role in fortifying the cell wall, reducing water leakage (Karlova *et al*., 2021), and pathogen entrance (Lee *et al*., 2019). In potato, Sprenger *et al*., found a constitutive higher expression of genes for lignin biosynthesis in drought tolerant compared with susceptible plants growing in a greenhouse (Sprenger *et al*., 2016). Moon *et al*., reported upregulation of a laccase gene (Laccase 14) in tolerant potato plants at 6, 12, 24, and 48 hours after drought treatment (Moon et al., 2018). Tubers of the variety Orion, which was resistant to *P. infestans*, accumulated lignin faster after infection compared with the susceptible variety Pentald Beauty (Herderson and Friend, 1979). A proteomic analysis of Sarpo Mira leaves showed an enrichment of the phenylpropanoid biosynthetic pathway (Xiao *et al*., 2019). Consequently, pre-existing high lignin levels and its dynamic accumulation both play a role in the response to the two stressors in potato. Lignin accumulation in potato may occur as a consequence of the activation of R genes during the ETI response, as observed in *Arabidopsis* (Lee *et al*., 2019). Moreover, under *P. infestans* infection, some transcriptomic changes were observed in common between infected areas and the neighbouring uninfected areas from the same leaf in only the resistant varieties. These changes suggested an accumulation of lignin and changes in methylesterification. Although systemic acquired resistance (SAR) could not be corroborated outside the infected area, changes in the expression of genes involved in these two cell wall components indicate the action of salicylic acid (SA). In tomato, SA pre-treatment influenced cell wall composition by upregulating genes for lignin and cellulose synthesis and downregulating PME genes to enable plants to withstand cadmium stress, with a corresponding increase in cell wall thickness (Jia *et al*., 2021). In *A. thaliana*, the elicitor LY56-24-2 induced SAR by upregulating genes of the SA pathway and increased the lignin, cellulose, and pectin content in leaves, increasing the cell wall thickness and stomatal closure, and boosting resistance to *H. arabidopsidis*, or to *P. cubensis* in cucumber (Qi *et al*., 2022).

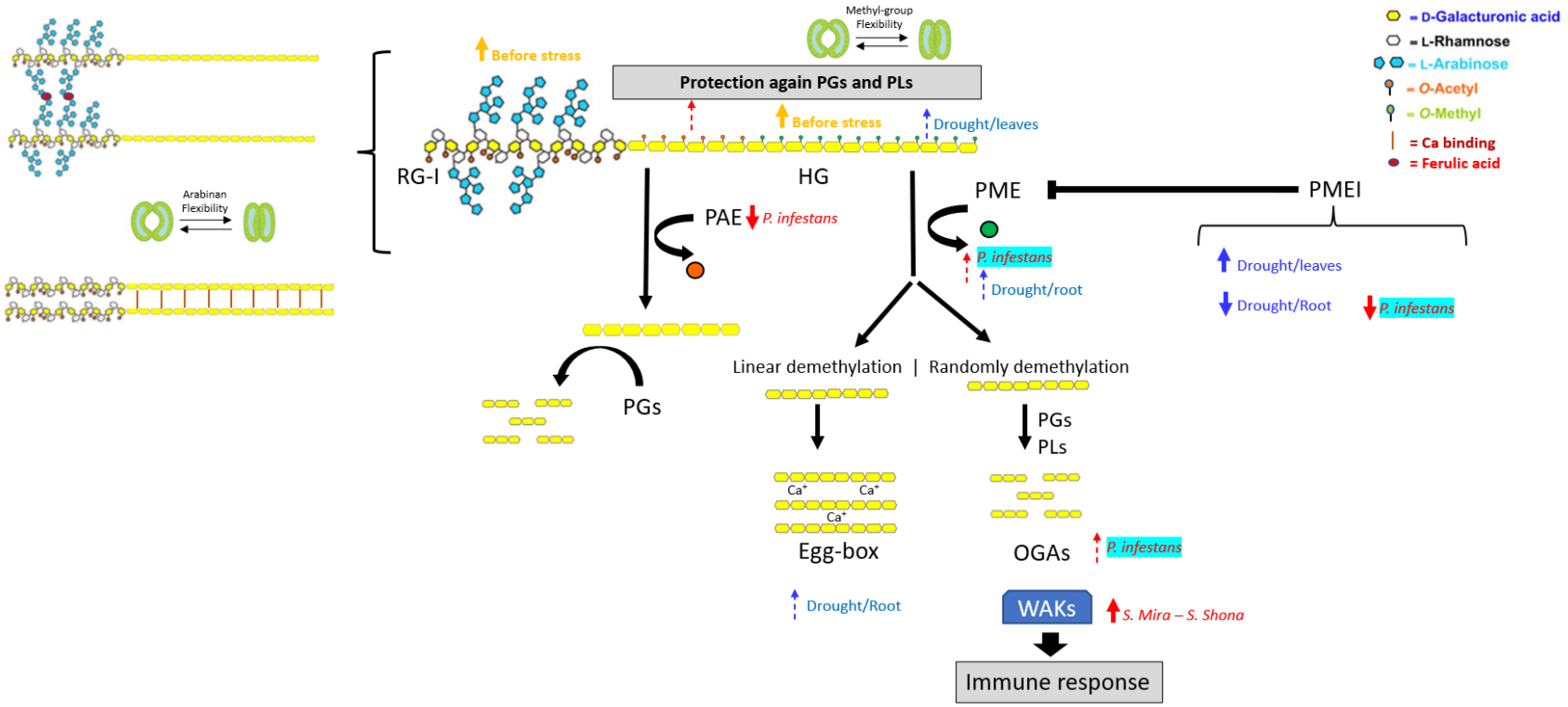


Figure 6.1 Pectin composition before stress and its modification after drought and *P. infestans* infection. Changes (indicated by red and blue arrows) observed in the tolerant potato varieties were different from those in the susceptible varieties, except for the downregulation of PMEI, which was observed in both tolerant and susceptible varieties to *P. infestans*. Dotted arrows indicate an inferred consequence of a non-dotted arrow. Reduced expression of PMEI under *P. infestans* infection and drought (in root) would lead to more demethylesterified pectin (DM-pectin). DM-pectin could undergo two consequences; one is to form of a rigid egg-box structure crosslinked with calcium (beneficial against drought), another is to produce OGAs (beneficial against *P. infestans* infection). Under infection, OGAs can be recognized by WAKs receptors. WAKs genes had higher expression in both tolerant varieties. The recognition of WAKs favours the triggering of an immune response. On the other hand, downregulation of PMEI observed under drought in tolerant leaves would lead to more methylesterified pectin (M-pectin). The CoMPP analysis revealed that tolerant leaves of potato varieties to *P. infestans* had more M-pectin. The degree of methylesterification influences the stomatal closure/aperture. Pectin with more methyl and acetyl groups (by downregulating PAE genes, which was observed in only the tolerant varieties to *P. infestans*) is better protected from PG enzyme attack. More arabinan was also observed in tolerant varieties to *P. infestans* before the infection. Arabinan content in the cell wall of guard cells confers flexibility. Without arabinan, homogalacturonan pectin forms bridges of calcium between each other, giving rigidity to the guard cell walls. Adapted from Jones *et al*., 2003; Harlot *et al*., 2010; and Wormit and Usadel, 2018).

6.3. Transcription factors involved in the tolerance to drought and *P. infestans* in potato.

Transcription factors modulate the expression of genes during different physiological events and under different types of stress. Different TFs have been implicated in the response of biotic and abiotic stress in different species, working as a switch to modulate plant physiology in order to tolerate the stress. Here, the transcriptomic analysis of potato varieties stressed with drought and *P. infestans* also showed an enrichment of TF genes in response to stress, although not in all the varieties.

The results from the simulation of drought (Chapter 3) showed enrichment of TF genes since the early stress response, more strongly in the leaf tissue of tolerant than susceptible plants, while in root the response was similar in both varieties. Some of these TFs were not only responding to drought, but were also influenced by rewatering and were identified as genes with a rapid response to water. Interestingly, a group of these genes had only a quick response in the tolerant variety, but not in the susceptible. These TFs differentially expressed in leaves included a heat shock transcription factor and a basic helix-loop-helix (bHLH) DNA-binding superfamily protein, while in root there were different TFs with NAC domain, MYB-like, and with a basic region/leucine zipper motif. It was previously reported that the family of these transcription factors respond to drought in potato (Vasquez-Robinet *et al*., 2008; Sprenger *et al*., 2016, Moon *et al*. 2018, Chen *et al*., 2020), but specific genes in these families were not associated with a rapid response to drought and re-watering specifically in the tolerant genotypes. In potato, while Vasquez-Robinet *et al*. and Sprenger *et al*. analysed the transcriptomic response under late or extreme drought, Moon *et al*. and Chen *et al*. analysed only tolerant potato varieties.

Regarding potato plants infected with *P. infestans*, only Sarpo Shona was enriched with genes encoding TFs (GO:0006355). However, within this GO term, there were some TF that were

overexpressed in both resistant varieties, such as WRKY and TF with a NAC domain, which were not differentially expressed in Duke. Participation of these TFs in stress signalling has been extensively reported, and some were functionally characterized, mainly in *Arabidopsis*.

In Chapter 6, the joint analysis of all GO terms related to the TFs and enriched in drought or during *P. infestans* infection allowed the identification of some TFs whose expression was regulated under both types of stress and specifically in the tolerant/resistant varieties. These common genes included 2 WRKY TFs and one NAC domain containing protein (*Soltu.DM.03G030960*, *Soltu.DM.05G006300*, and *Soltu.DM.11G004620*), homologous to *AthWRKY40, AtWRKY70, and ANAC50* in *Arabidopsis*, respectively, and whose functions have been linked to the regulation of stomatal closure/opening (Li *et al*., 2013; Yuan *et al*., 2019; Xiang *et al*., 2021; Raffeiner *et al*., 2022). While in drought this regulation is important to minimize water loss, under *P. infestans* infection this regulation is important because from this structure the pathogen disseminates sporangia (Farrel, Preece, and Wren, 1969). In potato tolerant leaves, while the expression of WRKY40 was upregulated under both stresses, the expression of WRKY70 and ANAC50 showed an opposite direction under drought versus *P. infestans* infection (**Figure 6.2**).

In *Arabidopsis*, the individual expression of WRKY40 had a positive effect on stomatal opening (Raffeiner *et al*., 2022), but the effect of WRKY70 varied depending on its individual analysis or together with other WRKY TFs (Li *et al*., 2013; Xiang *et al*., 2021). Therefore, the particular effect of both WRKY40 and WRKY70 in potato need to be experimentally validated under both stressors coupled with stomatal assays to determine their effects on stomatal closure. Specifically, to identify if a particular expression pattern of both genes could favour the resistance/tolerance to the two types of stress. This same is true for ANAC50, although NAC TFs were associated with stomatal closure (Yuan *et al*., 2019), the specific effect of NAC50 remains to be elucidated.

6.4. Regulation of guard cells in response to drought and *P. infestans* infection.

The main phytohormone that regulates the stomatal closure/aperture is ABA. While in drought, a rapid response to ABA is important at the beginning of the stress to avoid or regulate water loss, in *P. infestans* this regulation would be important later, after the pathogen completes its life cycle and needs to disseminate its sporangia. This occurs after 3 to 4 days from the beginning of the infection depending on the host, pathogen strain, and environmental conditions (Leesutthiphonchai *et al*., 2018).

The modulation of ABA in our tolerant/resistant varieties under both types of stress could be linked to the differential expression of WRKY40, WRKY70, and the NAC TFs. It was previously reported that WRKY40 and NAC TFs modulate stomatal closure/opening through the regulation of ABA (Chen *et al*., 2010; Wang *et al*., 2021; Yuan *et al*., 2019). For WRKY70, its regulation was linked with JA and SA (Li *et al*., 2006, Li, Brader, and Palva, 2004), therefore this gene could be influenced indirectly by ABA, which interacts with these two hormones, inhibiting JA and SA signalling (Anderson *et al*., 2004, de Torres Zabala *et al*., 2009) (**Figure 6.2**).

Specifically, under drought, one of the most remarkable differences between tolerant and susceptible varieties was the early expression of genes in leaf and root that respond to and regulate ABA, as well as genes involved in its synthesis like *NCED*, particularly in leaf (*Soltu.DM.08G015120* – *AtNCED1/AthNCED3*), which was consistent with more rapid regulation of stomatal closure in the tolerant variety. In contrast, at 48 h.p.i with *P. infestans*, only the susceptible Duke of York showed enrichment of downregulated genes related to the response to ABA and the ABA-activated signalling pathway, in GO:00097937 and GO:00097938, respectively. Interestingly, both terms were enriched for upregulated genes in the tolerant variety Negrita in the early response to drought, and since the late response in the susceptible variety. Therefore, independently of their degree of tolerance, the response of the potato varieties to ABA was opposite under drought versus infection.

Some of these genes within the enriched GO terms observed in Duke of York were also DE in Sarpo Shona. Both varieties downregulated PP2C genes, but more so in Duke; this would reduce the negative feedback regulation of ABA signalling, to keep the stomata closed and thus not allow the dissemination of sporangia. While under drought, upregulation of PP2C genes allowed more controlled stomatal closure in the tolerant than in the susceptible variety. In fact, in potato, the upregulation of PP2C was previously reported under drought (Gong *et al*., 2015; Chen *et al*., 2020) and a genetic marker associated with drought tolerance was close to a PP2C gene (Schumacher *et al*., 2021). It is interesting to note that low levels of ABA have been associated with a resistant response because of its negative influence on SA signalling (Cao, Yoshioka, and Desveaux, 2011), and that the tolerant variety Sarpo Mira did not show DE of many genes related to ABA compared with Sarpo Shona and Duke of York, showing variety-specific responses are important.

As previously commented, the composition of the cell wall will influence stomatal closure/aperture. Pectin with arabinan side chains confer flexibility to the guard cell (Jones *et al*., 2003) and methylesterified pectins affect guard cell flexibility and hence stomatal movement (An *et al*., 2008; Amsbury *et al*., 2016; Wu *et al*., 2107). In our resistant variety to *P. infestans*, the observed higher abundance of arabinan in the cell wall would allow more flexibility to regulate the stomatal aperture/closure. Therefore, the aperture/closure of stomata is closely linked to the response to ABA, which can be regulated by WRKY and NAC TFs, PP2C, and NCED genes, and affected by the cell wall composition (**Figure 6.2**).

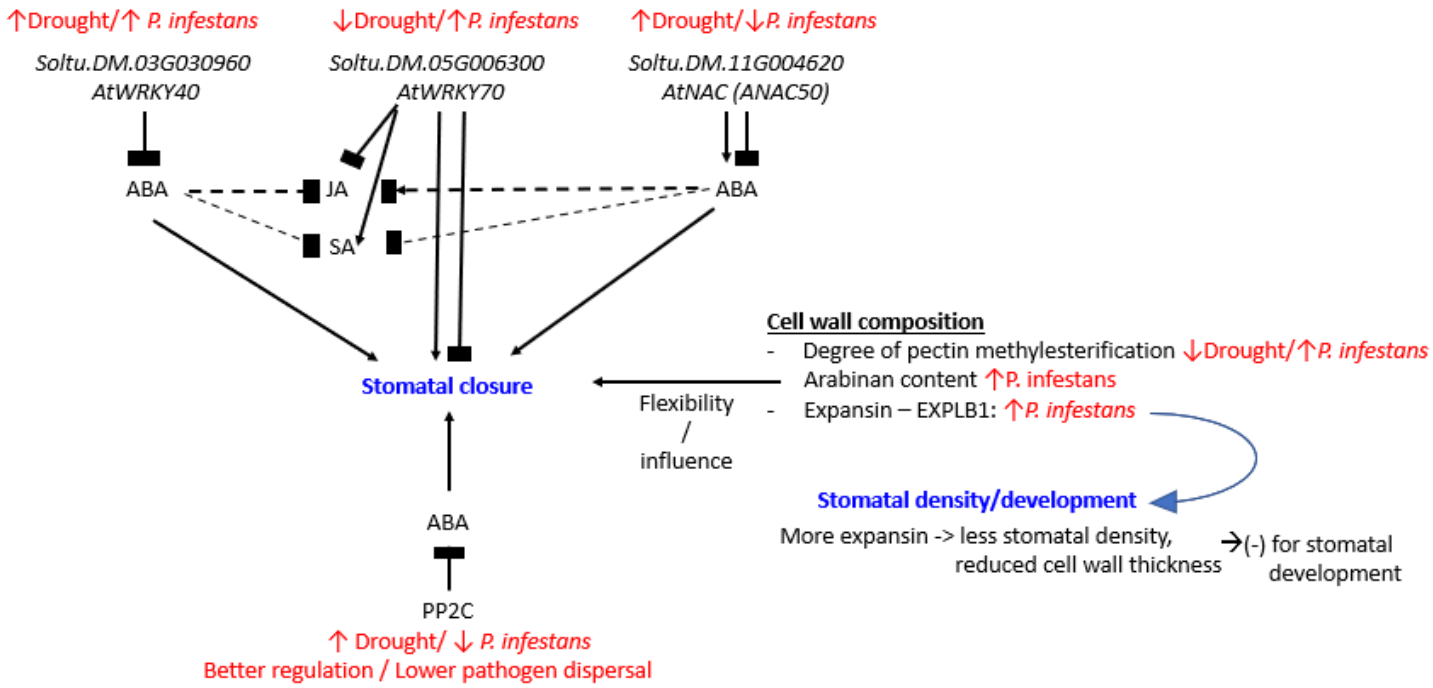


Figure 6.2 Factors influencing the regulation of stomatal closure/aperture during drought and *P. infestans* infection. In red are the transcriptomic changes occurring in the leaves of tolerant varieties under drought or *P. infestans* infection. The black arrows indicate a positive (normal arrowhead) or negative (blocked arrows) influence.

6.5. Expression of pathogen related genes under biotic and abiotic stress

Different genes involved in the response to pathogens were not only DE under *P. infestans* but also under drought stress. Roots of the tolerant varieties upregulated 3 basic chitinases (*Soltu.DM.07G005400*, *Soltu.DM.07G005390*, *Soltu.DM.02G022960*) specific to this tissue and to the tolerant variety during the two time points of the stress, and responding rapidly to re-watering. Interestingly, under *P. infestans* infection, *Soltu.DM.07G005390* was upregulated in Sarpo Shona and had a consistently higher expression during all treatments in both resistant varieties than in Duke. Another basic chitinase, *Soltu.DM.10G018010*, was the most highly overexpressed gene (log2FC > 15) in the two resistant varieties compared with the susceptible across all experimental conditions, before and after disease stress. It was observed that *P. infestans* causes the formation of cytoplasm aggregation at the site of the infection (Takemoto *et al*., 1997). This cytoplasm aggregation functions as a barrier against infection and its role was associated with papilla formation (Bushnell and Berquist, 1975). The formation of this aggregation in potato cells was observed to be dependent on the actin rearrangement, where chitinase would participate due to its capacity to bind actin filaments (Takemoto *et al*., 1997). Therefore, the increase of chitinase observed here during infection could be associated with this function.

Similarly, the beta-1,3-glucanase (*Soltu.DM.02G033060*) responded rapidly to drought and re-watering in only the tolerant root and had a constitutively higher expression in only Sarpo Shona in treatment conditions. Another beta-1,3-glucanase, *Soltu.DM.01G005190*, was in the top 20 most highly overexpressed genes in both resistant varieties in all treatments compared with the susceptible variety. β-1,3 glucanases hydrolyse glycosidic bonds in the glucans of the fungal cell wall, to protect against fungal pathogens (Oide *et al*., 2013). Although chitinase and beta-1,3-glucanase also participate in plant development (Passarinho and Vries, 2002, Balasubramanian *et al*., 2012), these two genes would be part of the crosstalk and may protect potato against both *P. infestans* and

drought. Similarly, in pepper, a basic chitinase 2 (*CaChi2*) was responding and involved in the crosstalk between drought, salinity, ABA, and fungal infection (Hong *et al*., 2002).

Another response specific to tolerant varieties included upregulation of terpene synthase genes in response to both types of stress. Drought stressed roots upregulated 3 terpene synthases in the early response to stress, while *P. infestans* infected leaves upregulated 2 terpene synthases, one of which was in common between the two types of stress, *Soltu.DM.01G040930*. Terpene synthases are a large family of genes involved in plant development and in response to stress (Tholl, 2006). A type of terpenoid is the phytoalexin, an antimicrobial compound that is rapidly accumulated in potato after *P. infestans* infection and has been associated with tolerance against this pathogen (Yoshioka *et al*., 2019). Upregulation of terpenoid synthase was also observed under drought in other plant species, however, its function has not been resolved (Savoi *et al*., 2016).

6.6. Phytohormones and its defensive role in potato

Phytohormones play an important role in regulating plant growth and development and modulate different signalling pathways in response to stress. Under pathogen attack, SA, JA, and ethylene (ET) are considered the backbone of plant immunity, being modulated by ABA (Li *et al*., 2019). It is generally considered that SA is associated to the resistance to biotrophic and hemibiotrophic pathogen. As *P. infestans* is a hemibiotrophic pathogen, the accumulation of SA in response to the infection has been associated to be positive against the infection in potato (Halim *et al*., 2007). The activation of SA signalling triggers oxidative burst, MAPK activation, and activation of defence genes such as PR genes, and SAR. In our potato varieties infected with *P. infestans* not enrichment of biological processes involved in SA response or biosynthesis was observed. However, the enrichment of genes involved in the jasmonic acid signalling pathway was observed in Duke of York. This includes downregulated genes encoding jasmonate-zim-domain proteins, which function as a repressor of JA. Therefore, positive regulation of JA could inhibit SA response at the evaluated time point. The

negative regulation of SA by JA is mediated by NAC TFs. ANAC019, ANAC55, and ANAC72 repress isochorismate synthase 1 (ICS1), a key enzyme on SA biosynthesis, and as a consequence reduce SA level (Li *et al*., 2019; Yang *et al*., 2019).

In the tolerant variety Sarpo Mira enrichment of genes involved in ET-activated signalling pathway was detected. While upregulation of genes encoding ethylene-responsive element (ERF) binding factor was observed in both resistant varieties, more in Sarpo Mira, downregulation of these genes was observed in the susceptible Duke of York. In *N. benthamiana*, the activation of ET mediated signalling pathway was essential to generate resistance to *P. infestans* because ET promoted the production of phytoalexin to prevent the infection (Shibata, Kawakira, and Takemoto, 2010), which may be occurred in our resistant varieties.

There is a crosstalk between SA, ET, and JA in which molecules that regulate SA synthesis or response are involved. ICS1 could be possibly involved in this crosstalk because its repressor, EIN3 and ANAC19, positively regulated ET and JA signalling. The crosstalk can also be observed by NPR1 (non-expresser of pathogenesis-related genes 1), a key regulator of SA signalling. NPR1 mediates the activation of defence genes triggered by SA. Under low SA, NPR1 binds each other and remains in the cytosol, while under a high amount of SA, monomers of NPR1 are generated and translocated into the nucleus to activate the expression of defence genes by interacting with TGACG-binding transcription factors (Li *et al*., 2019). However, it is also suggested that NPR1 induces the expression of WRKY genes, specifically WRKY70. WRKY70 is also induced by SA, but can repress ET/JA responsive genes (Li *et al*., 2019).

It was recently predicted that the function of ABA in pathogen infection depends on the pathogen lifestyle and plant age (Stevens *et al*., 2023). In the specific case when plants are infected with hemibrotrophic pathogens, the increment of ABA generates susceptibility to the plant because this hormone inactivates SA signalling. On the other hand, ABA promotes callose deposition by repressing

the transcription of pathogenesis-related protein 2 (PR2). PR2 encodes a beta-1,3 glucanase, which could break callose to produce elicitors and induce defence response (Oide *et al*., 2013). Stomata closure produced by ABA can also limit pathogen infection (Bharath *et al*., 2021), which in *P. infestans* could prevent the release of newly formed sporangia. Therefore, this phytohormone is important to restrict pathogen invasion and dissemination. However, it will be important to evaluate the time points of these responses since the production of elicitors and the activation of SA is an important mechanism to tolerate the infection. The resistant varieties Sarpo Mira and Sarpo Shona had more callose deposition when they were infected with *P. infestans* TdT10 strain, but a low amount of callose was observed in the infection with blue 13 strain, although there was a positive correlation between an intermediate resistance and callose accumulation. In addition, the transcriptomic data evaluated in this thesis show that the transcription factors WRKY and NAC family members would be involved in the regulation of ABA and would be connected to ABA with the JA and SA pathway to regulate stomatal closure. Therefore, it is suggested that ABA would be playing an important role in the resistance observed in the evaluated potato varieties.

Although phytohormones levels were not quantified in this thesis, they should be analysed in the future and at different time points. This will allow us to connect the activation/repression of genes involved in the metabolic pathways of these hormones with their actual accumulation and to identify which specific mechanisms are activated during an early and late response.

6.7. Conclusion and future research

Specific cell wall components have been identified and related to the resistant response to *P. infestans* before and during the infection and key similarities identified with the response to drought stress. Analysis of organic and conventional farming systems indicated that the cell wall composition is influenced by the environment and by specific environment x genotype interactions. Nevertheless, common cell wall characteristics could be associated with a tolerant/resistant phenotype. The results

suggested that a cell wall conferring tolerance against *P. infestans* should have a high baseline amount of methylesterified pectin, arabinan, xyloglucan, xylan, mannan, and extensin. Changes in expression in response to the pathogen and associated with resistance involved genes related to methylated pectin, mannan, expansin, extensin, and lignin components. In the case of drought, key changes associated with a resistant phenotype also involved genes related to methylated pectin, expansin, and lignin components, which thus represent key similarities in the response to the two types of stress.

All the genes correlated with the tolerance phenotypes need to be experimentally validated, using mutant over-expression or knockout lines. COMPP analysis needs to be performed to validate the relationship between the transcriptomic changes and changes in cell wall composition in response to *P. infestans* using the samples already collected. This could be complemented with the use of antibodies in live cells to directly observe these changes during stress. For *P. infestans* infection, an analysis under different time points is necessary to observe and differentiate the early and late responses, considering that this pathogen has biotrophic and necrotrophic developmental stages. It will also be important to incorporate yield data into this work given the importance of developing potato varieties that tolerate biotic and abiotic stresses while maintaining high yield or yield stability.

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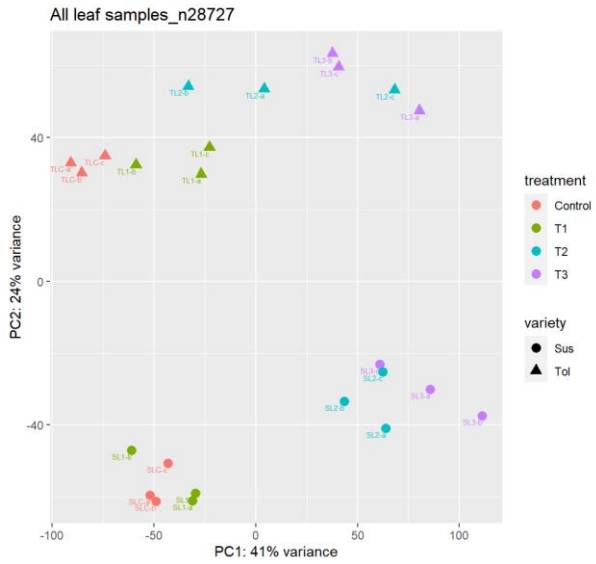
Appendix - Chapter 2

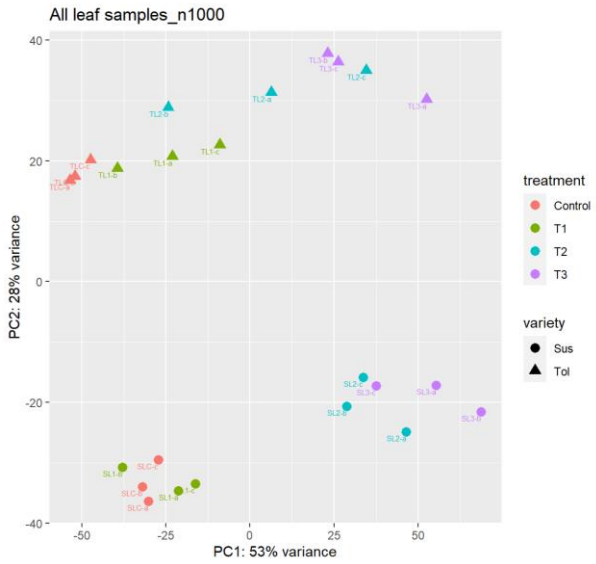
Appendix-Table 2.1 Number and percentage of reads before and after trimming, and mapping reads in each sample.

Library	Sample	Initial	Trimmed	%	Mapped	%
Replicate 1						
AA	TLC-1	26,304,956	26,156,706	99.44	22,777,230	86.59
AB	TRC-1	30,840,996	30,689,477	99.51	27,183,400	88.14
AC	SLC-1	27,976,839	27,813,683	99.42	23,799,346	85.07
AD	SRC-1	27,993,309	27,876,741	99.58	24,431,616	87.28
AE	TL3-1	25,424,875	25,118,280	98.79	22,160,674	87.16
AF	TR3-1	14,717,082	14,595,966	99.18	13,000,115	88.33
AG	SL3-1	20,067,948	19,863,932	98.98	17,280,268	86.11
AH	SR3-1	16,975,442	16,837,551	99.19	14,940,540	88.01
AI	TL1-1	17,079,619	16,928,170	99.11	14,987,216	87.75
AJ	TR1-1	19,780,525	19,622,591	99.20	17,364,649	87.79
AK	SL1-1	28,223,430	28,023,124	99.29	23,172,726	82.10
AL	SR1-1	27,415,791	27,233,457	99.33	23,998,857	87.54
AM	TL2-1	22,260,826	22,103,981	99.30	19,354,610	86.94
AN	TR2-1	23,292,392	23,065,199	99.02	20,639,123	88.61
AO	SL2-1	23,408,804	23,091,712	98.65	19,844,045	84.77
AP	SR2-1	24,620,110	24,397,771	99.10	21,557,819	87.56
Replicate 2						
BA	TLC-2	46,550,628	46,228,457	99.31	40,877,664	87.81
BB	TRC-2	30,706,596	30,215,910	98.40	27,145,973	88.40
BC	SLC-2	26,077,819	25,330,779	97.14	22,169,365	85.01
BD	SRC-2	35,020,751	34,514,605	98.55	30,536,729	87.20
BE	TL3-2	36,111,457	35,848,611	99.27	32,055,002	88.77
BF	TR3-2	39,146,541	38,586,768	98.57	34,664,659	88.55
BG	SL3-2	37,473,883	37,111,310	99.03	32,793,862	87.51
BH	SR3-2	46,512,109	45,954,048	98.80	40,720,535	87.55
BI	TL1-2	50,760,622	49,934,704	98.37	44,185,061	87.05
BJ	TR1-2	34,893,186	34,409,538	98.61	30,272,810	86.76
BK	SL1-2	22,931,546	22,784,776	99.36	19,934,398	86.93
BL	SR1-2	26,421,383	26,178,495	99.08	22,932,702	86.80
BM	TL2-2	43,378,704	43,026,919	99.19	38,436,768	88.61
BN	TR2-2	65,443,855	64,864,862	99.12	57,840,610	88.38
BO	SL2-2	37,499,070	36,960,678	98.56	32,322,585	86.20
BP	SR2-2	33,478,561	33,054,710	98.73	29,114,541	86.96
Replicate 3						
CA	TLC-3	23,835,480	23,648,008	99.21	20,944,308	87.87
CB	TRC-3	32,458,154	32,189,703	99.17	28,666,079	88.32
CC	SLC-3	44,146,851	42,459,608	96.18	36,741,837	83.23
CD	SRC-3	43,620,934	43,151,864	98.92	38,432,898	88.11
CE	TL3-3	51,048,669	50,507,050	98.94	45,143,511	88.43
CF	TR3-3	49,055,736	48,663,688	99.20	43,932,011	89.56
CG	SL3-3	40,740,771	40,235,487	98.76	33,879,880	83.16
CH	SR3-3	59,552,325	58,455,003	98.16	51,906,375	87.16
CI	TL1-3	46,415,103	44,879,577	96.69	39,301,492	84.67
CJ	TR1-3	33,341,378	32,625,336	97.85	29,075,254	87.20
CK	SL1-3	55,247,111	54,841,731	99.27	48,340,865	87.50
CL	SR1-3	54,778,319	54,342,632	99.20	47,851,250	87.35
CM	TL2-3	31,377,679	30,704,322	97.85	27,518,051	87.70
CN	TR2-3	40,295,595	39,361,665	97.68	35,046,577	86.97
CO	SL2-3	56,400,098	55,659,431	98.69	48,984,525	86.85
CP	SR2-3	37,945,230	37,681,160	99.30	33,724,034	88.88

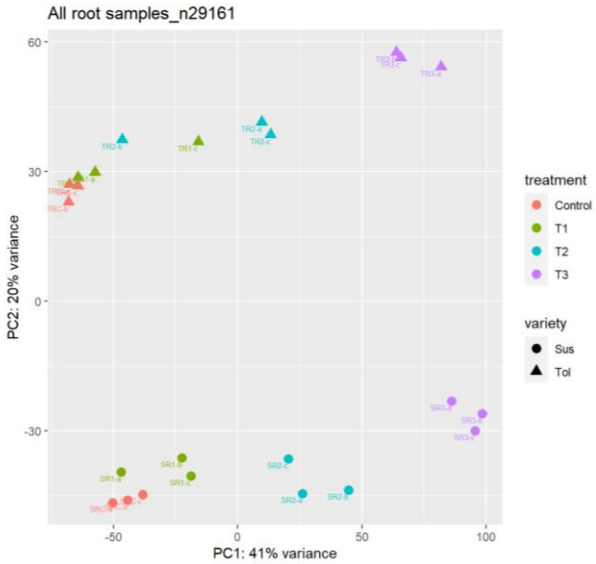
(A)



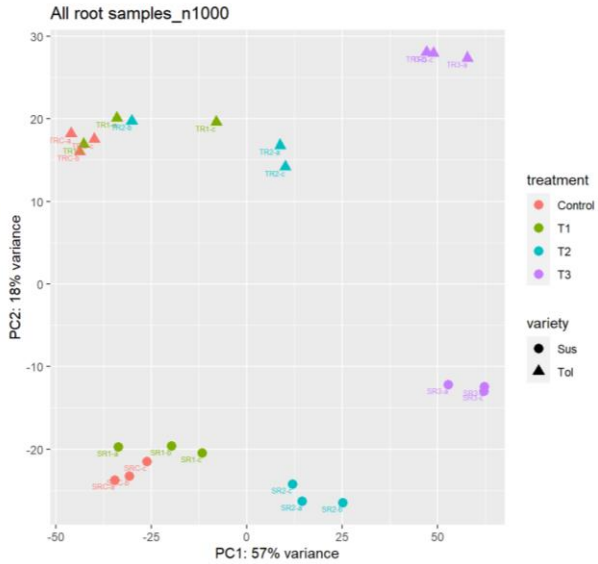
(B)



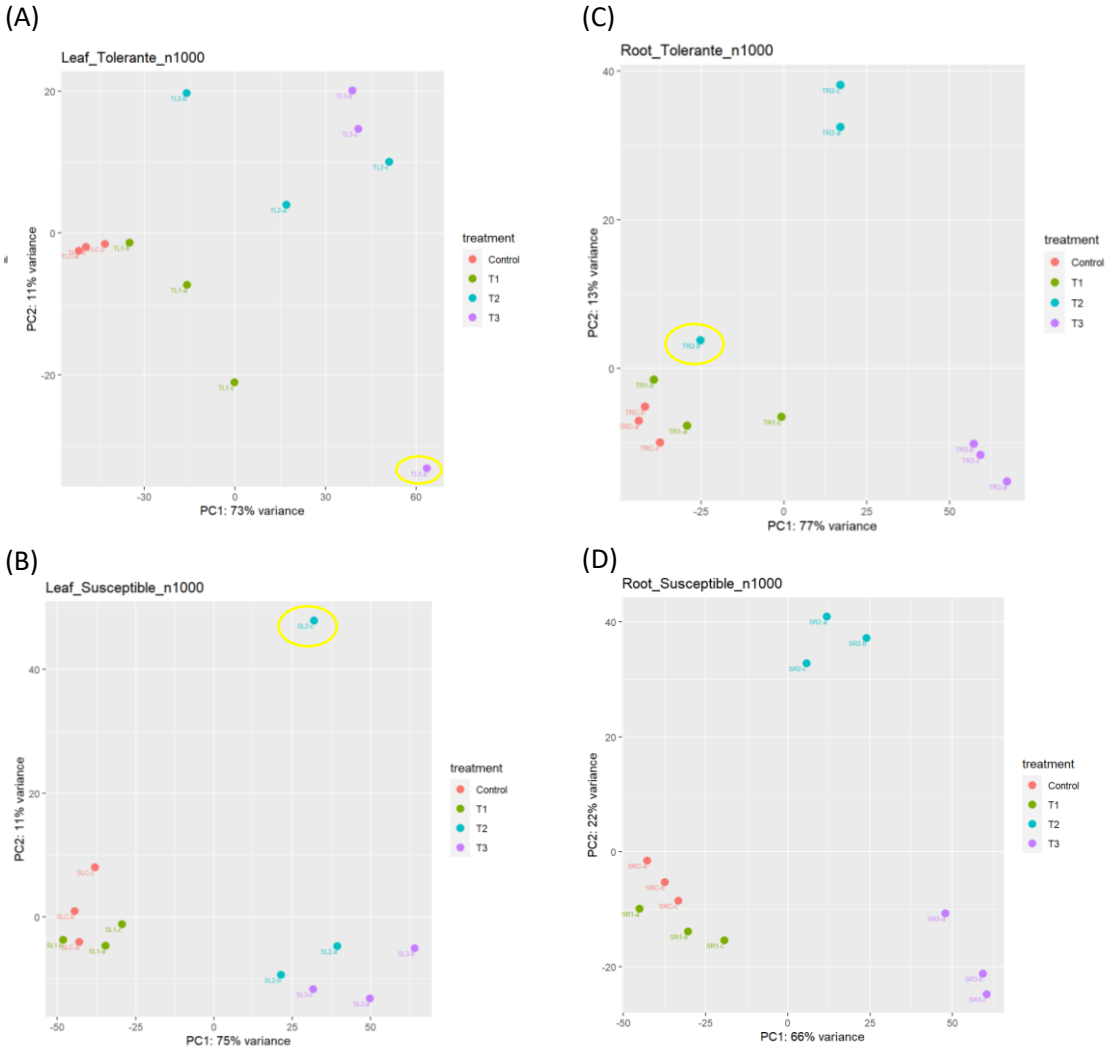
(C)



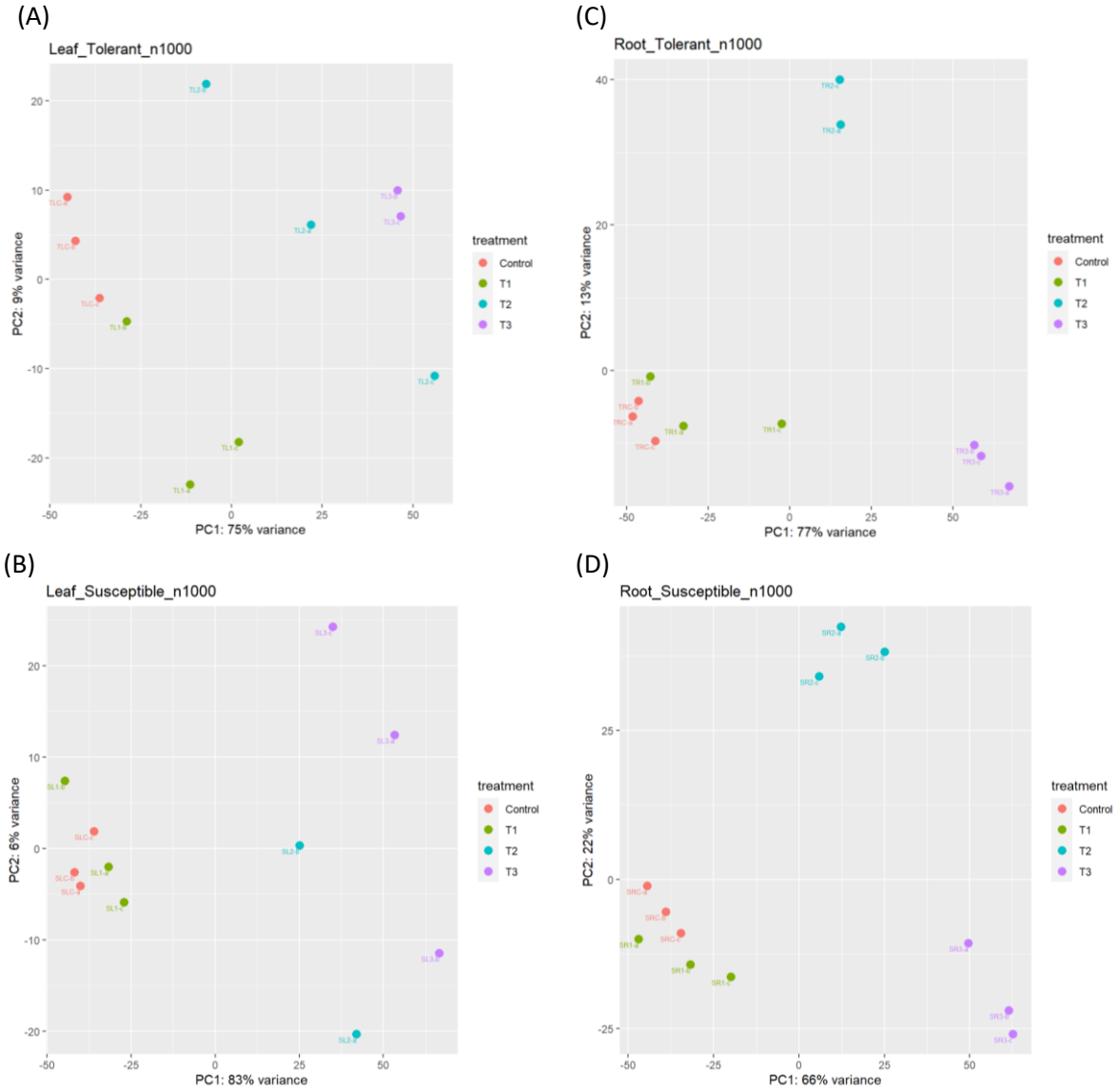
(D)



Appendix-Figure 2.1 PCA plot of all the 48 sequenced samples. The PCAs were constructed with all the expressed genes in leaf (A) and root (C), and with the 1000 most variable expressed genes in leaf (B) and root (D).

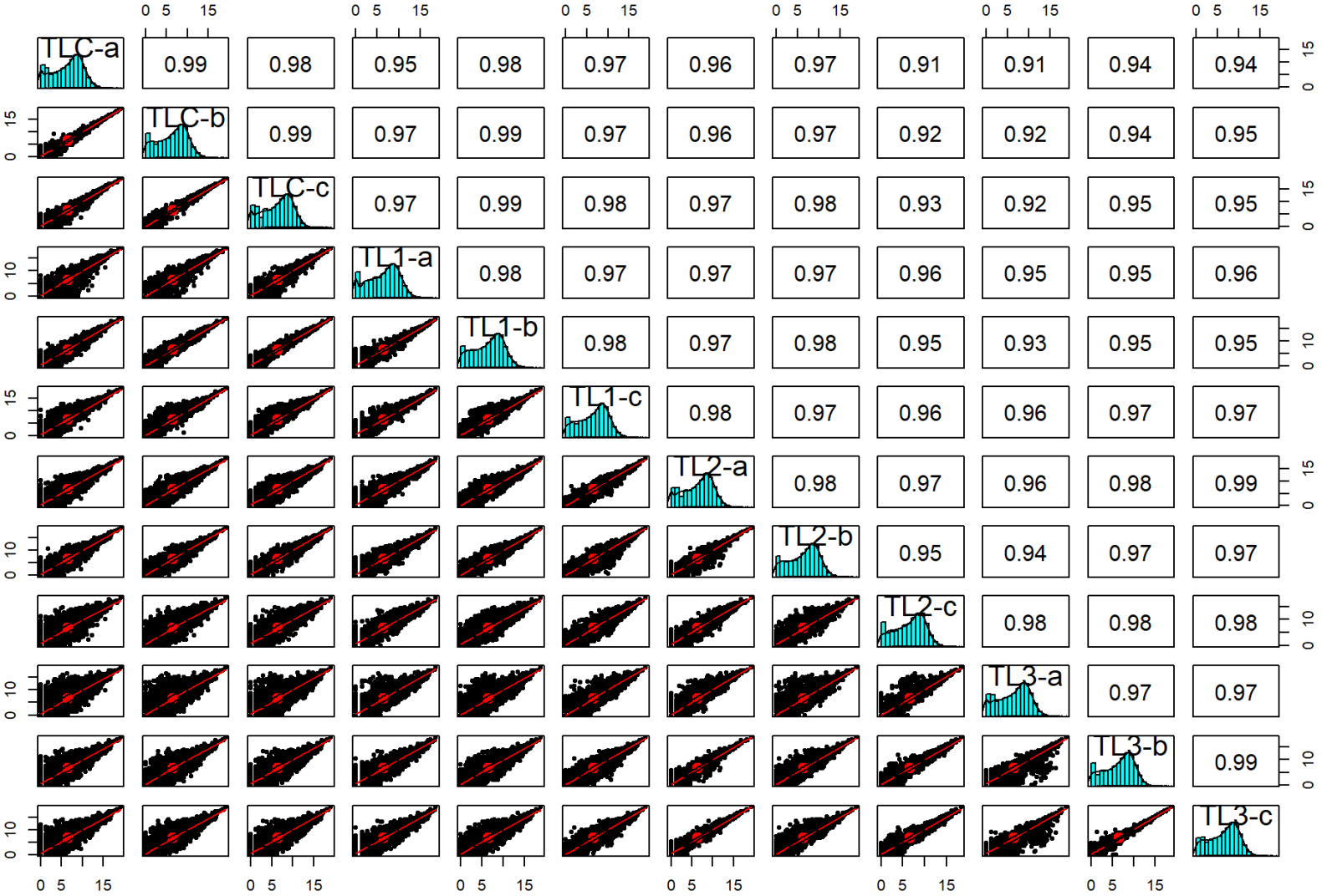


Appendix-Figure 2.2 PCA plot of the all the 48 sequenced samples separated by tolerant and susceptible in leaf (A and B) and root (C and D) samples

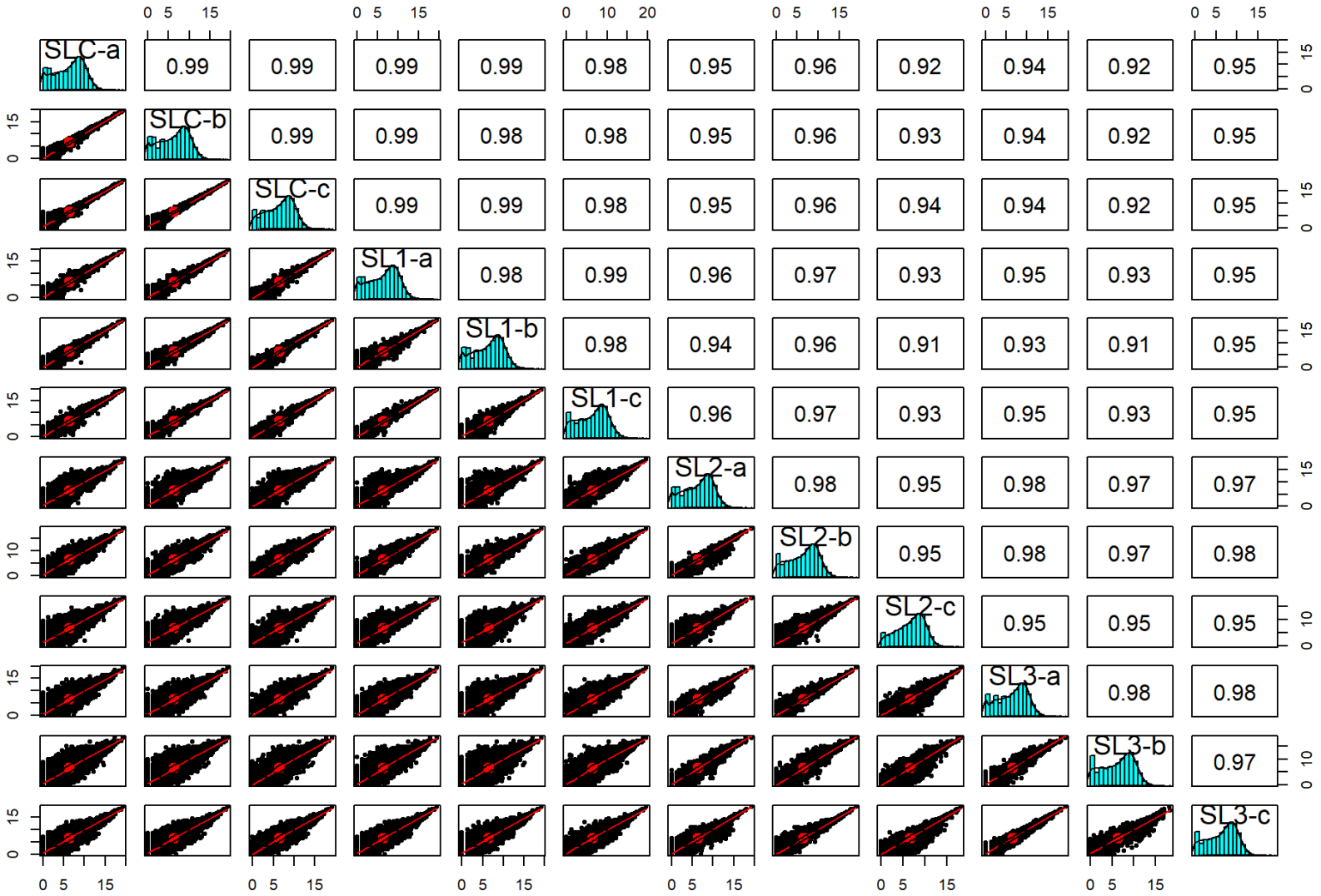


Appendix-Figure 2.3 PCA plot after removing replicates that do not appear to be real biological replicates.

(A)


TLC-a
TLC-b
TLC-c
TL1-a
TL1-b
TL1-c
TL2-a
TL2-b
TL2-c
TL3-a
TL3-b
TL3-c
0.99 0.98 0.95 0.98 0.97 0.96 0.97 0.91 0.91 0.94 0.94
0.99 0.97 0.99 0.97 0.96 0.97 0.92 0.92 0.94 0.95
0.97 0.99 0.98 0.97 0.98 0.93 0.92 0.95 0.95
0.98 0.97 0.97 0.97 0.96 0.95 0.95 0.96
0.98 0.97 0.98 0.95 0.93 0.95 0.95
0.98 0.97 0.96 0.96 0.97 0.97
0.98 0.97 0.96 0.98 0.99
0.95 0.94 0.97 0.97
0.98 0.98 0.98
0.97 0.97
0.99
0 5 15
0 10
0 15

(B)


SLC-a
SLC-b
SLC-c
SL1-a
SL1-b
SL1-c
SL2-a
SL2-b
SL2-c
SL3-a
SL3-b
SL3-c
0.99 0.99 0.99 0.99 0.98 0.95 0.96 0.92 0.94 0.92 0.95
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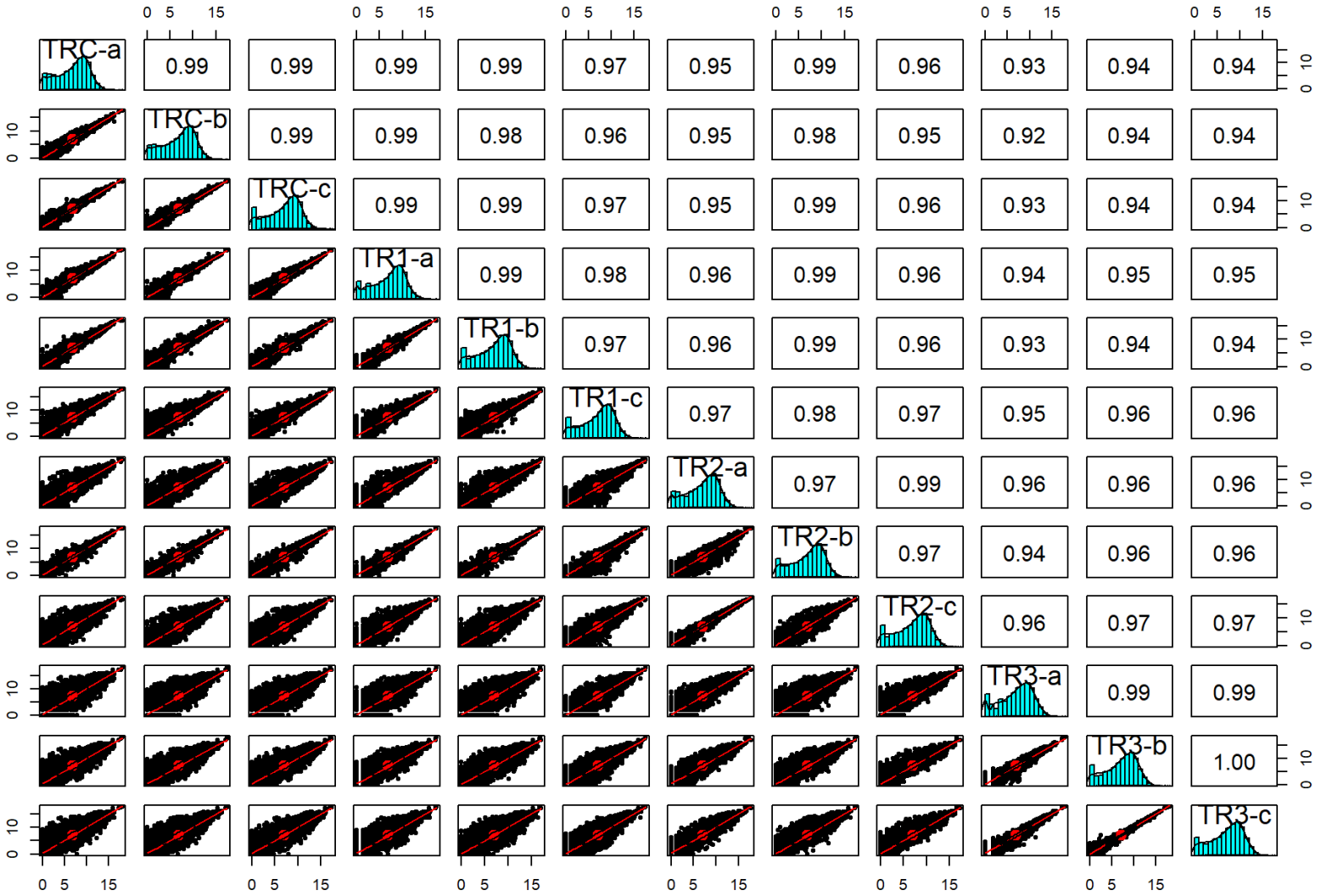
0.98 0.98
0.97

(C)


TRC-a
TRC-b
TRC-c
TR1-a
TR1-b
TR1-c
TR2-a
TR2-b
TR2-c
TR3-a
TR3-b
TR3-c
0 5 15
0 10
0.99 0.99 0.99 0.99 0.97 0.95 0.99 0.96 0.93 0.94 0.94
0.99 0.99 0.98 0.96 0.95 0.98 0.95 0.92 0.94 0.94
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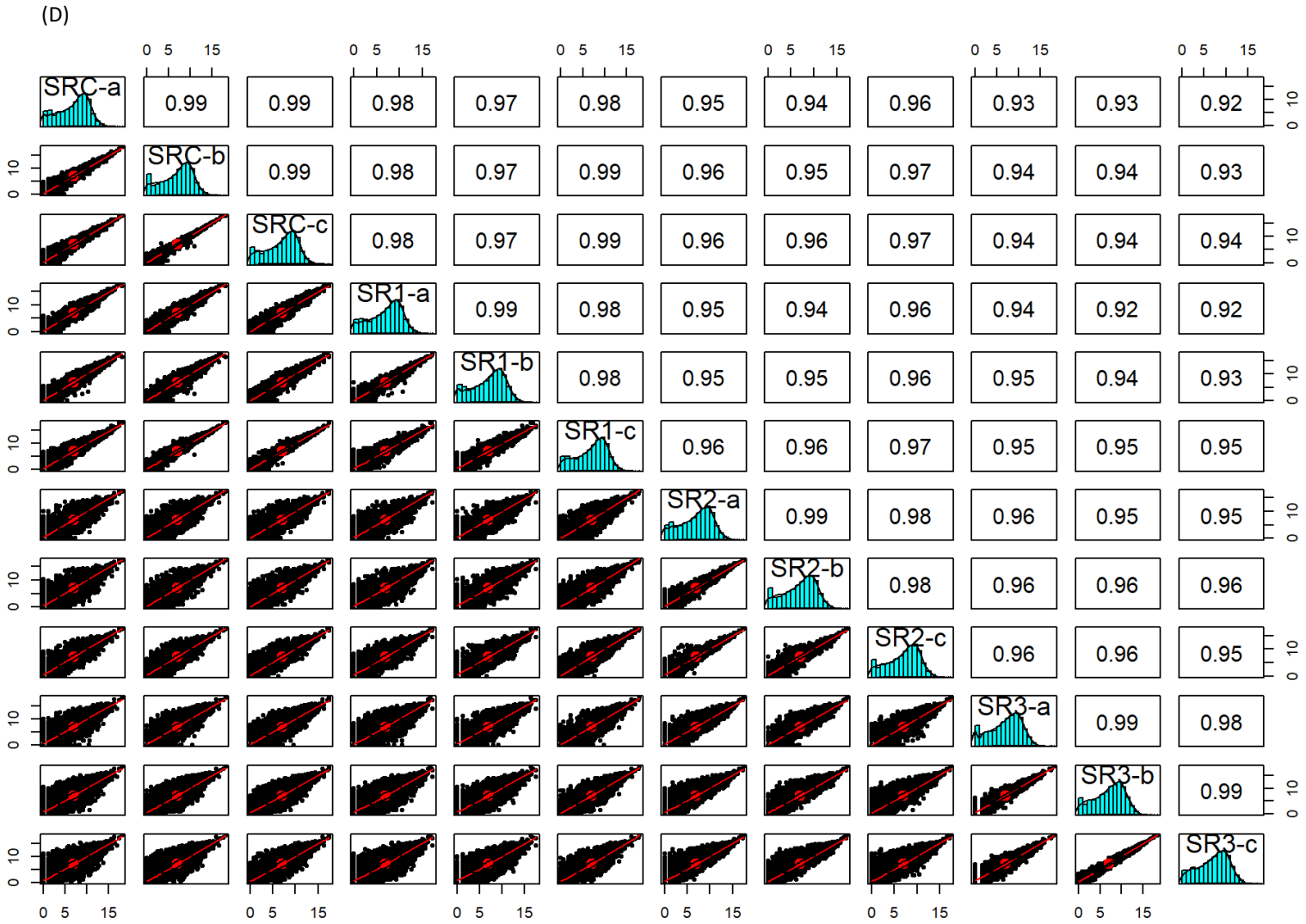
0.99 0.99
1.00



Appendix-Figure 2.4 Sample-wise correlation of the transcriptome data from tolerant leaf (A), susceptible leaf (B), tolerant root (C), and susceptible root (D).

Appendix-Table 2.2 Number of DEGs in leaf and root across the three time points. The table shows the number of up-, down-regulated, and the total number of DEGs at each time point in each tissue and variety.

Time point / Tissue	Up-regulated DEGs		Downregulated DEGs		Total	
	Tolerant	Susceptible	Tolerant	Susceptible	Tolerant	Susceptible
Early response (T1)						
Leaf	389	31	102	7	491	38
Root	552	434	35	44	587	478
Late response (T2)						
Leaf	2,471	1,875	1,417	1,059	3,888	2,934
Root	2,197	1,628	1,800	1,588	3,997	3,216
Recovery phase (T3)						
Leaf	3,048	2,741	2,411	1,996	5,459	4,737
Root	3,587	3,415	2,493	1,953	6,080	5,368

Appendix-Table 2.3 Variety-specific and common DEGs between the susceptible and the tolerant varieties. The table shows the number of up-, down-regulated, and the total number of DEGs at each time point in each tissue and variety.

Time point / Tissue	Up-regulated DEGs			Downregulated DEGs			Total		
	Susceptible unique	Common	Tolerant unique	Susceptible unique	Common	Tolerant unique	Susceptible unique	Common	Tolerant unique
Early response (T1)									
Leaf	5	26	363	4	3	99	9	29	462
Root	205	229	323	32	3	41	237	232	364
Late response (T2)									
Leaf	722	1,153	1318	774	643	416	1496	1796	1734
Root	543	1,085	1112	736	852	948	1279	1937	2060
Recovery phase (T3)									
Leaf	1,062	1,986	755	1,118	1,293	703	2180	3279	1458
Root	858	2,557	1030	1,113	1,380	573	1971	3937	1603

Appendix-Table 2.4 20 most up or down-regulated genes in the early response to drought (T_1) of only the tolerant variety, in the root. Differentially Expressed Genes (DEGs) are defined by log2FC >1 and padj. < 0.05. An asterisk denotes DEGs whose expression is significantly higher (or lower) in the tolerant than the susceptible for the upregulated (or downregulated) genes in T_1. In grey are the padj. values > 0.05. The table also shows the expression of the DEGs in T_2 and T_3, and in the susceptible variety.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Root. Tolerant						Root. Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj
Upregulated genes													
Cell wall modification													
Soltu.DM.08G001190	expansin-like B1	6.87	2.80E-02	4.09	1.58E-01	7.69	7.63E-04	2.34	NA	3.18	1.74E-01	4.51	2.23E-02
Lignin synthesis													
*Soltu.DM.04G028320**	Laccase	5.14	4.34E-02	1.72	5.12E-01	5.39	3.17E-03	2.51	NA	5.24	2.02E-02	6.89	6.24E-04
Soltu.DM.06G032730	Peroxidase superfamily protein	4.93	5.92E-03	2.45	1.78E-01	1.88	2.44E-01	0.83	7.25E-01	1.19	5.46E-01	2.10	1.70E-01
Terpene biosynthesis													
Soltu.DM.01G040960	terpene synthase	5.17	1.01E-02	2.92	1.37E-01	4.81	2.03E-03	0.53	NA	0.79	7.16E-01	-0.68	7.11E-01
Soltu.DM.01G040950	terpene synthase	4.89	2.32E-02	3.52	7.27E-02	4.91	2.28E-03	1.51	NA	1.95	3.26E-01	-1.27	5.31E-01
Soltu.DM.01G040930	terpene synthase	4.77	3.72E-03	3.51	2.51E-02	4.57	4.50E-04	0.55	7.96E-01	-1.57	3.17E-01	-2.46	5.51E-02
UDP-glycosyltransferases													
*Soltu.DM.09G031020**	UDP-Glycosyltransferase superfamily protein	5.58	3.27E-02	5.21	1.61E-02	6.20	1.04E-03	-2.43	NA	2.67	1.97E-01	2.10	2.77E-01
Soltu.DM.04G011110	UDP-glycosyltransferase 73B4	4.98	2.76E-02	4.03	4.08E-02	2.85	1.58E-01	-0.64	NA	-3.60	5.78E-04	0.21	8.72E-01
Others													
Soltu.DM.03G003070	serine-type endopeptidase inhibitors	6.45	2.50E-04	0.22	9.39E-01	1.86	2.93E-01	0.49	NA	-0.15	9.55E-01	-0.31	8.70E-01
*Soltu.DM.06G018620**	serine-type endopeptidase inhibitors	5.33	3.28E-03	-1.40	5.67E-01	0.63	7.59E-01	0.26	NA	-1.87	3.64E-01	0.40	8.37E-01
Soltu.DM.12G001820	myb domain protein	6.44	7.32E-03	4.03	7.46E-02	9.26	2.20E-07	2.67	1.52E-01	1.39	4.70E-01	6.03	5.71E-06
Soltu.DM.05G023310	myb domain protein	5.21	1.26E-02	5.68	8.26E-04	6.37	3.25E-05	1.23	NA	3.10	3.82E-03	4.27	9.49E-06
*Soltu.DM.02G006200**	Dehydrin domain containing protein	5.27	1.99E-02	8.54	8.88E-07	10.35	5.99E-11	0.64	8.50E-01	9.30	5.81E-10	9.66	3.98E-11
Soltu.DM.04G027470	Cytochrome P450 superfamily protein	5.40	4.92E-04	6.08	1.62E-05	7.59	4.67E-10	1.11	5.40E-01	7.06	1.80E-10	7.25	2.10E-11
Soltu.DM.01G005280	cytochrome P450, family 71, subfamily B, polypeptide	5.06	5.71E-03	5.96	4.37E-05	6.02	1.22E-05	0.94	NA	1.53	1.27E-03	1.04	2.97E-02
Soltu.DM.02G027440	phytosulfokine 6 precursor	5.17	2.25E-03	5.08	8.85E-04	4.62	7.35E-04	0.78	6.72E-01	1.47	3.10E-01	3.46	1.96E-03

Soltu.DM.01G047920	Cupredoxin superfamily protein	5.00	3.08E-02	2.92	1.79E-01	3.99	2.41E-02	1.75	NA	1.02	6.61E-01	3.81	1.63E-02
Soltu.DM.01G047870	Cupredoxin superfamily protein	4.87	3.28E-02	2.64	2.25E-01	2.64	1.53E-01	0.94	7.34E-01	0.68	7.99E-01	3.04	8.02E-02
Soltu.DM.01G039820	Protein kinase family protein	4.88	3.24E-02	10.42	9.32E-11	10.03	3.61E-11	-0.07	9.82E-01	5.23	1.61E-06	4.73	9.00E-06
Soltu.DM.03G015580	hypothetical protein	6.49	1.92E-02	1.61	6.54E-01	7.43	2.89E-04	2.51	NA	-0.75	8.09E-01	5.11	2.82E-03
Downregulated genes													
Circadian Clock													
*Soltu.DM.10G000080**	circadian clock associated / Homeodomain-like superfamily protein	-1.92	1.24E-03	-4.90	1.40E-12	-3.96	3.85E-14	-0.57	4.18E-01	-2.76	1.90E-08	-2.71	1.59E-08
*Soltu.DM.10G000090**	Homeodomain-like superfamily protein	-1.91	9.55E-26	-4.13	8.72E-85	-4.38	9.97E-117	-0.72	7.17E-04	-2.88	1.63E-60	-3.25	3.57E-75
Soltu.DM.10G023770	Homeodomain-like superfamily protein	-1.66	3.69E-03	-1.43	8.04E-03	-2.12	2.43E-06	-1.12	5.05E-02	-0.82	1.24E-01	-1.50	8.27E-04
Cell wall modification													
Soltu.DM.05G008140	xyloglucan:xyloglucosyl transferase	-1.50	2.30E-02	-1.46	1.27E-02	1.49	1.41E-03	-0.60	3.89E-01	-1.05	5.57E-02	1.77	1.10E-04
Others													
*Soltu.DM.01G049280**	conserved hypothetical protein	-2.75	1.60E-02	-3.37	7.92E-04	-0.76	4.67E-01	-0.84	5.18E-01	-1.46	1.58E-01	-1.98	2.44E-02
Soltu.DM.11G003320	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-1.91	1.46E-04	-2.97	9.45E-10	-2.65	1.89E-10	-0.08	9.31E-01	-0.77	1.51E-01	-1.89	4.06E-05
Soltu.DM.02G028760	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-1.57	2.51E-02	-0.49	4.96E-01	0.68	2.32E-01	-0.67	3.60E-01	-0.05	9.62E-01	-0.60	2.97E-01
Soltu.DM.02G023850	flavanone 3-hydroxylase	-2.15	5.06E-04	-2.85	1.23E-05	-0.72	1.58E-01	-0.92	NA	-0.23	7.40E-01	0.68	1.49E-01
Soltu.DM.06G008600	nodulin MtN21 /EamA-like transporter family protein	-1.83	1.37E-02	-3.95	1.08E-09	-3.67	4.08E-11	-0.67	4.18E-01	-3.93	2.94E-12	-4.09	2.31E-13
Soltu.DM.03G020100	Nodulin MtN3 family protein	-1.83	7.50E-03	2.01	4.11E-04	2.37	7.56E-07	-0.65	3.82E-01	2.93	1.13E-09	3.30	1.29E-12
*Soltu.DM.03G025460**	cytochrome P450, family 71, subfamily A, polypeptide	-1.72	1.96E-04	-4.23	5.09E-16	-4.13	6.94E-21	0.07	9.25E-01	-3.13	1.69E-13	-3.74	4.45E-17
Soltu.DM.09G017200	cytochrome P450, family 82, subfamily G, polypeptide	-1.69	1.46E-02	-1.58	1.22E-02	-0.05	9.50E-01	-0.66	NA	-1.31	7.48E-02	-1.55	2.10E-02
Soltu.DM.11G024560	alpha/beta-Hydrolases superfamily protein	-1.53	2.87E-03	-2.10	4.24E-05	-2.15	6.07E-07	-0.46	NA	-1.53	2.60E-03	-2.49	3.77E-06
Soltu.DM.02G030190	Adenosylmethionine decarboxylase family protein	-1.52	5.19E-04	-1.52	2.69E-04	0.78	4.16E-02	-0.99	2.86E-02	-0.61	1.65E-01	1.92	3.20E-08
*Soltu.DM.03G019030**	Homeodomain-like superfamily protein	-1.42	3.02E-04	-2.92	2.51E-16	-4.15	1.05E-37	-0.10	8.67E-01	-2.67	1.72E-17	-3.84	1.18E-33
Soltu.DM.09G001780	NAD(P)-binding Rossmann-fold superfamily protein	-1.41	3.57E-02	-2.88	1.07E-05	-1.98	8.46E-05	-0.85	NA	-1.33	3.69E-02	-1.96	1.33E-03
Soltu.DM.02G030180	conserved peptide upstream open reading frame	-1.37	5.17E-03	-1.47	1.02E-03	1.24	1.10E-03	-0.58	2.86E-01	-0.16	7.96E-01	2.45	5.67E-12
Soltu.DM.04G028940	STAS domain / Sulfate transporter family	-1.33	1.37E-02	-0.42	4.34E-01	0.25	6.10E-01	-0.51	NA	0.49	3.62E-01	0.29	5.62E-01

*Soltu.DM.09G029770**	terpene synthase	-1.31	4.78E-02	-2.56	1.04E-05	-2.50	3.32E-07	-0.01	9.92E-01	-1.90	1.91E-05	-1.32	2.61E-03
Soltu.DM.04G005260	dentin sialophosphoprotein-related	-1.29	2.97E-04	-2.52	3.25E-15	-3.15	3.73E-29	-0.36	4.12E-01	-1.51	2.98E-07	-1.87	4.11E-11

Appendix-Table 2.5 20 most up or down-regulated genes in the early response to drought (T_1) of only the tolerant variety, in the leaf. Differentially Expressed Genes (DEGs) are defined by log2FC >1 and padj. < 0.05. An asterisk denotes DEGs whose expression is significantly higher (or lower) in the tolerant than the susceptible for the upregulated (or downregulated) genes in T_1. In grey are the padj. values > 0.05. The table also shows the expression of the DEGs in T_2 and T_3, and in the susceptible variety.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Leaf. Tolerant						Leaf. Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj
Upregulated genes													
Response to ABA													
Soltu.DM.06G013730	protein phosphatase 2CA	6.20	8.13E-04	4.36	7.23E-03	5.45	1.53E-03	2.78	9.53E-01	3.65	4.56E-02	2.64	8.99E-02
Soltu.DM.03G012480	protein phosphatase 2CA	4.56	3.01E-03	6.39	1.36E-07	7.33	4.49E-08	2.10	1.00E+00	6.16	8.93E-06	6.57	2.28E-08
Transport													
Soltu.DM.06G010580	Transmembrane amino acid transporter family protein	6.60	9.32E-04	9.51	2.52E-09	10.06	3.87E-09	-0.03	1.00E+00	4.00	1.23E-02	5.61	1.11E-05
Soltu.DM.10G018680	lipid transfer protein	5.29	2.22E-03	5.62	7.31E-05	6.05	6.10E-05	0.16	1.00E+00	1.95	2.30E-01	2.76	2.47E-02
Soltu.DM.12G025220	sugar transporter	4.57	4.62E-03	3.61	8.28E-03	2.46	1.16E-01	0.50	1.00E+00	0.89	6.04E-01	0.72	5.86E-01
Soltu.DM.10G018810	lipid transfer protein	4.26	6.55E-04	4.41	2.43E-05	4.38	1.13E-04	-0.12	1.00E+00	2.28	5.03E-02	2.93	1.57E-03
Heat shock proteins													
Soltu.DM.06G013460	heat shock protein 90.1	6.22	1.39E-02	4.46	3.38E-02	1.68	5.40E-01	1.92	1.00E+00	7.50	6.70E-04	5.75	2.41E-03
Soltu.DM.06G031870	HSP20-like chaperones superfamily protein	5.45	2.74E-02	5.90	2.10E-03	4.68	3.17E-02	-0.06	1.00E+00	6.15	4.80E-03	5.51	2.59E-03
Stress responsive genes													
Soltu.DM.07G011880	heat shock transcription factor A2	4.44	2.79E-03	4.50	2.27E-04	5.71	1.43E-05	2.63	6.76E-01	4.59	8.88E-04	4.95	1.77E-05
Soltu.DM.10G003790	late embryogenesis abundant domain-containing protein / LEA domain-containing protein	4.33	2.37E-02	5.57	1.27E-04	7.28	3.02E-06	1.80	1.00E+00	3.77	2.81E-02	4.13	3.08E-03

Soltu.DM.03G004180	late embryogenesis abundant domain-containing protein / LEA domain-containing protein	4.03	2.39E-02	4.63	7.29E-04	4.94	9.04E-04	1.72	1.00E+00	3.90	1.36E-02	3.08	2.23E-02
Soltu.DM.02G024670	Dehydrin domain containing protein	5.68	1.11E-04	7.73	2.03E-10	8.61	1.84E-10	0.10	1.00E+00	3.79	1.24E-02	4.83	7.73E-05
Others													
Soltu.DM.06G026880	Octicosapeptide/Phox/Bem1p family protein	6.29	1.86E-03	4.72	6.84E-03	5.61	2.64E-03	4.24	3.05E-01	2.92	1.68E-01	2.91	8.69E-02
Soltu.DM.01G007150	CAP160 protein	5.91	3.61E-02	8.95	1.22E-05	10.45	1.01E-06	0.08	1.00E+00	4.80	1.43E-02	6.30	7.22E-05
Soltu.DM.01G027460	Expressed protein	5.90	6.02E-03	7.14	2.74E-05	8.48	4.91E-06	2.98	1.00E+00	6.95	3.06E-04	7.32	5.88E-06
Soltu.DM.03G024470	conserved hypothetical protein	5.86	6.07E-04	6.56	3.70E-06	8.03	2.09E-07	2.51	1.00E+00	5.30	1.24E-03	5.56	4.87E-05
Soltu.DM.01G035490	homeobox	5.74	8.57E-04	7.34	1.52E-07	7.87	4.11E-07	2.27	1.00E+00	6.25	1.26E-04	6.40	2.94E-06
Soltu.DM.02G033970	conserved hypothetical protein	4.79	1.21E-03	6.66	1.94E-08	8.16	2.46E-10	0.48	1.00E+00	4.39	9.96E-04	4.66	2.80E-05
Soltu.DM.10G028300	nuclear factor Y, subunit A9	4.25	1.87E-02	6.57	1.05E-06	7.92	5.93E-08	-0.87	1.00E+00	5.30	4.19E-04	6.34	3.65E-07
*Soltu.DM.08G010200**	cytochrome P450, family 71, subfamily B, polypeptide cytochrome P450, family 71, subfamily B, polypeptide cytochrome P450, family 71, subfamily A, polypeptide	4.18	1.53E-03	4.52	3.17E-05	3.50	4.35E-03	1.09	1.00E+00	3.28	2.55E-02	3.19	1.03E-02
Downregulated genes													
Soltu.DM.08G025250	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	-4.26	3.20E-02	-5.83	1.32E-04	-5.47	1.11E-03	1.12	1.00E+00	-3.36	9.62E-02	-2.47	1.29E-01
Soltu.DM.06G019440	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	-2.19	4.61E-02	-3.58	6.36E-06	-3.31	2.05E-04	-0.70	1.00E+00	-4.16	3.31E-06	-4.20	3.44E-08
Soltu.DM.04G033210	N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein	-3.14	3.82E-05	-3.09	3.11E-06	-1.91	1.33E-02	-0.11	1.00E+00	-0.34	7.69E-01	-0.58	4.63E-01
Soltu.DM.05G027180	ABC-2 type transporter family protein	-2.75	1.50E-03	-1.84	4.84E-03	0.28	7.03E-01	-1.18	NA	0.00	9.99E-01	1.96	7.24E-05
*Soltu.DM.01G024030**	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-2.46	1.34E-02	-1.43	7.01E-02	-0.38	7.09E-01	-0.56	1.00E+00	-0.16	9.01E-01	-1.51	4.02E-02
Soltu.DM.04G009990	Heavy metal transport/detoxification superfamily protein	-2.40	1.70E-02	-3.64	1.59E-06	-3.53	3.14E-05	-0.59	1.00E+00	-3.05	7.15E-04	-3.82	3.35E-07
Soltu.DM.06G027540	Kinase interacting (KIP1-like) family protein	-2.35	4.71E-02	-1.75	6.00E-02	-0.76	5.09E-01	-0.05	NA	0.18	9.14E-01	0.15	9.06E-01

Soltu.DM.03G018250	detoxifying efflux carrier	-2.27	4.14E-02	-2.06	1.70E-02	-0.87	4.13E-01	-1.29	1.00E+00	-1.36	2.06E-01	-1.55	6.52E-02
Soltu.DM.07G026560	glycosyl hydrolase 9B8	-2.05	1.21E-04	-3.98	1.52E-16	-4.12	1.58E-14	-1.18	5.17E-01	-2.86	1.97E-05	-3.10	3.44E-08
Soltu.DM.12G027980	O-acyltransferase (WSD1-like) family protein	-2.00	2.79E-02	-1.43	5.44E-02	-1.34	1.07E-01	-0.63	1.00E+00	-1.42	1.05E-01	-1.49	3.37E-02
Soltu.DM.10G024900	growth-regulating factor	-1.99	4.18E-02	-1.63	3.22E-02	-1.97	1.60E-02	-0.90	1.00E+00	-0.59	6.00E-01	-1.34	8.94E-02
Soltu.DM.02G025610	conserved hypothetical protein	-1.96	4.95E-02	0.18	8.49E-01	0.54	5.75E-01	-0.47	1.00E+00	0.90	3.70E-01	1.30	7.73E-02
Soltu.DM.10G029820	NAD(P)-binding Rossmann-fold superfamily protein	-1.96	3.85E-02	-2.51	4.32E-04	-2.63	7.74E-04	-0.34	1.00E+00	-1.16	2.37E-01	-1.56	3.94E-02
Soltu.DM.10G025190	NAD(P)-binding Rossmann-fold superfamily protein	-1.79	4.18E-02	-0.98	1.87E-01	-1.06	1.89E-01	-0.34	1.00E+00	-1.18	1.73E-01	-1.02	1.45E-01
Soltu.DM.01G033740	cytochrome P450, family 86, subfamily A, polypeptide	-1.96	2.64E-02	-1.68	1.68E-02	-1.77	2.15E-02	-0.79	1.00E+00	-2.38	2.45E-03	-1.73	9.86E-03
Soltu.DM.03G011660	3-ketoacyl-CoA synthase	-1.93	6.55E-04	-1.66	5.56E-04	-1.44	6.83E-03	-0.70	1.00E+00	-2.02	7.83E-03	-1.61	6.22E-03
*Soltu.DM.03G036380**	Glucose-methanol-choline (GMC) oxidoreductase family protein	-1.93	1.32E-02	-2.71	5.11E-06	-2.60	8.57E-05	-0.29	1.00E+00	-2.48	3.59E-04	-2.61	6.70E-06
*Soltu.DM.09G012800**	Leucine-rich repeat protein kinase family protein	-1.90	2.24E-02	-2.17	7.28E-04	-2.60	1.99E-04	0.10	1.00E+00	-2.52	4.93E-04	-3.62	1.23E-09
Soltu.DM.10G029690	F-box family protein	-1.89	7.67E-07	-3.23	2.44E-21	-2.76	2.07E-13	-0.65	9.73E-01	-1.72	2.05E-05	-1.87	4.06E-08
*Soltu.DM.09G028560**	Chalcone and stilbene synthase family protein	-1.78	3.52E-02	-0.90	2.16E-01	-0.37	6.85E-01	0.22	1.00E+00	-0.81	3.64E-01	-1.06	1.09E-01

Appendix-Table 2.6 20 most up or down-regulated genes in the late response to drought (T_2) of only the tolerant variety, in the root. Differentially Expressed Genes (DEGs) are defined by log2FC >1 and padj. < 0.05. An asterisk denotes DEGs whose expression is significantly higher (or lower) in the tolerant than the susceptible for the upregulated (or downregulated) genes in T_1. In grey are the padj. values > 0.05. The table also shows the expression of the DEGs in T_1 and T_3, and in the susceptible variety.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Root. Tolerant						Root. Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj
Upregulated genes													
Transcription factors													
Soltu.DM.12G007400	WRKY DNA-binding protein	4.12	5.28E-02	4.31	7.60E-03	7.68	7.54E-09	2.19	NA	1.11	2.01E-01	4.41	7.71E-14
Soltu.DM.07G014750	NAC domain containing protein	3.55	1.87E-02	4.43	2.67E-04	2.64	3.02E-02	-0.12	NA	1.04	3.67E-01	-0.46	7.06E-01
Soltu.DM.06G010320	DRE-binding protein 2A	2.26	1.41E-01	5.27	2.18E-07	4.68	3.60E-07	0.01	NA	1.07	7.37E-01	0.11	9.73E-01
Terpene biosynthesis													
Soltu.DM.01G040970	terpene synthase	3.47	2.54E-01	4.55	2.49E-02	5.01	4.75E-03	2.13	NA	0.68	7.97E-01	-1.29	5.74E-01
Soltu.DM.07G004480	terpene synthase	1.20	7.08E-01	5.46	2.78E-05	7.64	3.42E-11	2.01	NA	0.55	8.17E-01	1.03	5.40E-01
UDP-glycosyltransferases													
Soltu.DM.09G031020	UDP-Glycosyltransferase superfamily protein	5.58	3.27E-02	5.21	1.61E-02	6.20	1.04E-03	-2.43	NA	2.67	1.97E-01	2.10	2.77E-01
Soltu.DM.12G004300	UDP-Glycosyltransferase superfamily protein	0.15	NA	6.38	5.72E-03	7.07	4.91E-04	0.00	NA	0.11	9.80E-01	1.04	7.43E-01
Soltu.DM.05G007640	UDP-glucosyl transferase 74B1	2.60	2.00E-01	4.79	6.97E-04	5.02	2.81E-05	0.43	8.51E-01	2.41	9.11E-02	4.40	2.26E-04
Others													
Soltu.DM.04G020100	non-photochemical quenching	5.71	NA	5.62	6.58E-03	2.23	3.41E-01	0.58	NA	-2.07	4.40E-01	-1.22	5.89E-01
Soltu.DM.09G023010	Thioredoxin superfamily protein	5.56	5.05E-02	4.96	3.48E-02	1.15	7.15E-01	2.08	NA	0.05	9.84E-01	1.69	3.50E-01
Soltu.DM.02G027440	phytosulfokine 6 precursor	5.17	2.25E-03	5.08	8.85E-04	4.62	7.35E-04	0.78	6.72E-01	1.47	3.10E-01	3.46	1.96E-03
Soltu.DM.01G006840	NAD+ ADP-ribosyltransferases;NAD+ ADP-ribosyltransferases	3.31	NA	4.95	1.87E-03	5.01	7.29E-04	0.57	NA	1.41	1.91E-01	1.44	1.31E-01
Soltu.DM.12G009350	fatty acid desaturase	3.32	6.99E-02	4.79	4.29E-04	1.23	4.57E-01	-1.73	NA	0.34	8.56E-01	-3.67	3.20E-02
Soltu.DM.01G005200	beta-1,3-glucanase	3.24	2.99E-01	4.58	1.85E-02	3.63	5.19E-02	-0.54	NA	1.05	5.08E-01	0.49	7.48E-01
Soltu.DM.03G013100	1-cysteine peroxiredoxin	0.15	NA	4.30	3.26E-02	6.18	2.22E-04	0.82	NA	3.56	7.77E-02	4.30	1.43E-02
Soltu.DM.04G019150	cytochrome P450, family 76, subfamily C, polypeptide	0.15	NA	4.99	4.54E-03	5.16	1.25E-03	-0.52	NA	2.30	6.52E-02	1.51	2.25E-01
Soltu.DM.04G001920	hypothetical protein	3.80	3.17E-01	5.00	4.40E-02	4.39	5.14E-02	1.19	NA	1.94	5.13E-01	5.75	4.75E-03
Soltu.DM.06G019500	hypothetical protein	2.79	4.56E-02	4.87	6.90E-06	2.78	5.83E-03	2.09	8.80E-02	1.87	8.86E-02	1.01	3.58E-01

Soltu.DM.01G007060	hypothetical protein	0.75	NA	4.75	8.36E-03	4.99	1.95E-03	0.95	NA	0.11	9.78E-01	0.11	9.75E-01
Soltu.DM.08G024450	hypothetical protein	0.15	9.82E-01	6.26	2.94E-05	7.06	4.05E-07	0.98	NA	2.50	2.50E-01	1.80	3.95E-01
Downregulated genes													
Cell wall modification													
Soltu.DM.02G001870	Plant invertase/pectin methylesterase inhibitor superfamily	-0.95	4.12E-01	-4.04	2.07E-09	-0.63	3.85E-01	-1.10	1.83E-01	-0.94	2.14E-01	0.05	9.56E-01
Soltu.DM.12G025120	xyloglucan endotransglucosylase/hydrolase	-0.97	3.82E-01	-3.60	6.53E-08	-0.21	7.97E-01	-0.99	2.37E-01	-1.00	1.71E-01	0.13	8.72E-01
Soltu.DM.01G006590	root hair specific	-0.86	7.13E-01	-5.99	2.90E-03	-1.44	2.29E-01	-1.68	NA	-1.58	1.86E-01	-0.87	4.41E-01
Soltu.DM.04G014020	Pectin lyase-like superfamily protein	-0.39	NA	-3.54	1.74E-02	-1.52	1.05E-01	-1.00	NA	0.53	7.29E-01	-0.87	5.27E-01
Others													
Soltu.DM.10G029820	NAD(P)-binding Rossmann-fold superfamily protein	-2.33	NA	-4.72	1.81E-02	-5.00	1.91E-03	-1.71	NA	-3.18	6.91E-02	-4.09	1.41E-02
Soltu.DM.06G017710	Serine hydrolase (FSH1) domain containing protein	-1.47	NA	-4.37	2.20E-02	-1.57	1.88E-01	-0.26	NA	-0.09	9.44E-01	-0.24	7.89E-01
Soltu.DM.10G004610	HXXXD-type acyl-transferase family protein	-1.59	2.45E-01	-5.87	9.92E-04	-1.79	3.81E-02	-1.10	NA	-0.01	9.94E-01	-0.21	8.34E-01
Soltu.DM.01G005700	HXXXD-type acyl-transferase family protein	-0.80	3.60E-01	-3.99	1.01E-09	-1.09	3.18E-02	-0.64	3.68E-01	-0.84	1.62E-01	-0.56	3.19E-01
Soltu.DM.01G006560	Peroxidase superfamily protein	-0.75	6.61E-01	-3.81	2.02E-04	-0.51	6.07E-01	-1.18	2.70E-01	-0.99	3.10E-01	0.16	8.75E-01
Soltu.DM.03G007520	Protein kinase superfamily protein	-0.67	7.88E-01	-4.68	1.89E-02	-2.35	8.61E-02	-0.36	NA	-0.23	8.70E-01	-0.69	4.97E-01
Soltu.DM.03G025430	cytochrome P450, family 71, subfamily A, polypeptide	-0.43	NA	-3.85	4.54E-02	-1.40	2.16E-01	-0.28	NA	-0.91	5.15E-01	-1.53	1.93E-01
Soltu.DM.08G026520	Subtilase family protein	-0.30	NA	-4.68	2.85E-02	-0.17	9.22E-01	-0.74	NA	-2.69	9.15E-02	-1.19	3.71E-01
Soltu.DM.01G000610	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	0.30	NA	-3.83	3.90E-02	-2.46	3.11E-02	0.08	NA	-0.69	6.04E-01	-0.59	6.01E-01
Soltu.DM.09G009180	wall-associated kinase	-0.29	NA	-4.70	1.59E-02	-1.14	3.29E-01	0.53	NA	-0.66	6.18E-01	-1.90	9.59E-02
Soltu.DM.01G028400	Cysteine/Histidine-rich C1 domain family protein	0.05	9.81E-01	-4.42	7.31E-05	-2.97	1.84E-05	-0.15	NA	-2.62	9.54E-02	-2.73	4.11E-02
Soltu.DM.06G001160	Protein of unknown function, DUF547	-0.47	6.91E-01	-3.61	1.14E-08	-0.75	1.99E-01	-0.24	7.94E-01	-0.39	6.00E-01	0.12	8.66E-01
Soltu.DM.10G005090	Organ specific protein domain containing protein	-0.02	9.93E-01	-4.78	1.35E-02	-1.56	1.61E-01	-0.28	NA	-0.01	9.94E-01	-0.83	4.54E-01
Soltu.DM.02G009560	conserved hypothetical protein	-2.38	NA	-3.74	1.80E-02	-1.91	6.25E-02	-2.14	NA	-1.81	1.80E-01	-2.76	3.60E-02
Soltu.DM.01G046840	conserved hypothetical protein	-0.56	NA	-5.42	4.74E-03	-1.02	3.39E-01	-0.45	NA	-1.89	1.74E-01	-0.87	4.56E-01
SoltuDM.06G003740	hypothetical protein	0.23	NA	-3.99	2.72E-02	-1.25	1.81E-01	-0.42	NA	-1.44	2.17E-01	-1.69	1.03E-01

Appendix-Table 2.7 20 most up or down-regulated genes in the late response to drought (T_2) of only the tolerant variety, in the leaf. Differentially Expressed Genes (DEGs) are defined by log2FC >1 and padj. < 0.05. An asterisk denotes DEGs whose expression is significantly higher (or lower) in the tolerant than the susceptible for the upregulated (or downregulated) genes in T_1. In grey are the padj. values > 0.05. The table also shows the expression of the DEGs in T_1 and T_3, and in the susceptible variety.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Leaf.Tolerant						Leaf.Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj
Upregulated genes													
Transport													
Soltu.DM.03G008090	Transmembrane amino acid transporter family protein	2.03	NA	6.86	7.53E-05	6.73	1.71E-04	-0.02	NA	3.48	1.28E-01	4.83	6.30E-03
Soltu.DM.10G018680	lipid transfer protein	5.29	2.22E-03	5.62	7.31E-05	6.05	6.10E-05	0.16	1.00E+00	1.95	2.30E-01	2.76	2.47E-02
Heat shock proteins													
Soltu.DM.04G028280	heat shock protein 18.2	1.45	7.27E-01	5.92	1.73E-02	7.63	2.70E-03	0.50	1.00E+00	4.12	1.45E-01	7.97	1.92E-04
Others													
Soltu.DM.06G018480	Late embryogenesis abundant protein, group 1 protein	4.16	NA	6.13	1.46E-02	6.56	1.28E-02	-2.84	NA	-2.88	4.42E-01	-0.30	9.28E-01
Soltu.DM.03G030490	late embryogenesis abundant protein-related / LEA protein-related	-0.21	NA	5.07	1.52E-02	6.67	1.25E-03	0.00	NA	4.49	5.95E-02	6.89	2.69E-04
Soltu.DM.03G010080	Late Embryogenesis Abundant 4-5	1.50	NA	5.06	1.09E-02	6.10	2.12E-03	-0.98	NA	3.60	1.25E-01	5.76	1.62E-03
Soltu.DM.04G002580	Heavy metal transport/detoxification superfamily protein	3.29	NA	5.70	8.91E-03	4.31	7.27E-02	0.01	NA	2.83	3.33E-01	4.37	4.45E-02
Soltu.DM.09G027510	AWPM-19-like family protein	1.70	NA	5.69	5.45E-03	5.74	6.86E-03	-1.24	NA	2.62	2.12E-01	3.80	1.78E-02
Soltu.DM.06G033600	nuclear transport factor 2 (NTF2) family protein / RNA recognition motif (RRM)-containing protein	4.47	2.26E-01	5.66	4.26E-02	7.69	6.73E-03	2.20	NA	5.14	9.07E-02	6.12	1.27E-02
Soltu.DM.09G021850	Pollen Ole e 1 allergen and extensin family protein	1.49	7.27E-01	5.63	2.38E-02	7.16	4.71E-03	0.39	1.00E+00	0.53	8.75E-01	-0.20	9.41E-01
Soltu.DM.08G017870	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein	3.86	5.82E-02	5.55	1.57E-04	7.03	1.46E-06	-0.51	NA	-1.14	4.38E-01	1.88	3.84E-02
Soltu.DM.08G017880	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein	3.84	NA	5.17	2.55E-03	6.63	8.19E-05	0.47	NA	0.18	9.35E-01	3.41	1.22E-03
Soltu.DM.08G028850	WRKY family transcription factor	3.59	2.99E-04	5.36	2.17E-11	5.17	6.74E-09	1.84	6.23E-01	0.30	8.34E-01	5.05	3.10E-11
Soltu.DM.10G006090	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	5.01	NA	5.30	2.44E-02	6.82	4.02E-03	-0.84	NA	1.27	7.25E-01	1.80	4.75E-01
Soltu.DM.02G014830	Protein of unknown function (DUF1264)	2.97	NA	5.37	1.01E-02	6.14	3.73E-03	-1.80	NA	3.87	9.10E-02	5.55	2.49E-03

Soltu.DM.10G023160	Protein of unknown function (DUF668)	1.93	2.60E-01	5.20	1.12E-05	6.33	8.47E-07	-0.75	1.00E+00	1.12	5.42E-01	4.82	2.72E-05
Soltu.DM.08G029210	hypothetical protein	3.91	1.07E-01	7.69	1.48E-06	7.45	6.28E-06	0.22	NA	2.74	5.67E-02	4.29	1.61E-04
Soltu.DM.01G007060	hypothetical protein	3.89	NA	6.28	2.04E-03	6.78	1.28E-03	-0.98	NA	-1.01	8.06E-01	-1.07	7.26E-01
Soltu.DM.12G009200	conserved hypothetical protein	2.96	NA	6.80	3.58E-03	6.08	1.53E-02	-2.03	NA	3.83	1.57E-01	5.17	1.55E-02
Soltu.DM.03G031190	hypothetical protein	4.07	NA	5.13	1.75E-03	5.56	5.98E-04	-1.87	NA	1.78	NA	2.48	4.08E-02
Downregulated genes													
Cell wall modification													
Soltu.DM.02G024320	Pectin lyase-like superfamily protein	-2.69	1.17E-01	-3.36	9.67E-03	-1.41	3.67E-01	-0.01	1.00E+00	-1.62	3.40E-01	-1.80	1.67E-01
Others													
Soltu.DM.08G025250	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	-4.26	3.20E-02	-5.83	1.32E-04	-5.47	1.11E-03	1.12	1.00E+00	-3.36	9.62E-02	-2.47	1.29E-01
Soltu.DM.10G020770	myb domain protein	-3.02	8.13E-02	-5.00	3.93E-04	-3.13	3.01E-02	0.37	NA	-0.96	6.25E-01	-2.47	7.36E-02
Soltu.DM.11G019840	cytochrome P450, family 716, subfamily A, polypeptide	-1.21	2.29E-01	-4.92	2.35E-04	-3.50	6.13E-04	-0.36	1.00E+00	-0.87	3.16E-01	-2.13	9.00E-04
Soltu.DM.06G015970	Plant stearoyl-acyl-carrier-protein desaturase family protein	-2.94	NA	-4.65	3.03E-02	-0.99	6.66E-01	0.15	NA	-0.77	NA	-0.85	6.47E-01
Soltu.DM.04G019810	GDSL-like Lipase/Acylhydrolase superfamily protein	-3.66	NA	-4.60	4.24E-02	-1.05	7.06E-01	1.28	NA	-0.52	8.90E-01	-2.11	4.14E-01
Soltu.DM.10G015940	SAUR-like auxin-responsive protein family	-1.07	NA	-4.52	1.08E-04	-3.95	2.64E-04	-0.33	NA	-3.65	6.33E-02	-4.15	7.28E-03
Soltu.DM.03G011570	HXXXD-type acyl-transferase family protein	-0.89	NA	-4.36	2.13E-03	-2.26	3.25E-02	0.12	NA	-0.81	5.37E-01	-2.40	1.99E-02
Soltu.DM.07G021960	protodermal factor	-4.01	7.11E-02	-3.95	2.11E-02	-2.21	2.87E-01	0.37	1.00E+00	-2.58	2.36E-01	-2.66	1.22E-01
Soltu.DM.06G032220	Undecaprenyl pyrophosphate synthetase family protein	-1.71	NA	-3.57	1.17E-02	-0.55	7.57E-01	1.28	NA	-0.54	8.25E-01	-0.03	9.88E-01
Soltu.DM.01G035840	RING/U-box superfamily protein	-1.48	NA	-3.44	7.24E-03	-1.95	7.04E-02	0.19	NA	-0.87	4.95E-01	-1.94	4.84E-02
Soltu.DM.09G020340	heat shock transcription factor A6B	1.18	4.38E-01	-3.38	1.73E-03	-5.26	7.58E-06	1.13	1.00E+00	-0.12	9.49E-01	-2.90	5.61E-03
Soltu.DM.10G000470	terpene synthase	-0.90	NA	-3.35	4.56E-02	-0.79	5.44E-01	-0.32	NA	-1.46	7.03E-02	-3.07	3.26E-05
Soltu.DM.02G029200	NAD(P)-binding Rossmann-fold superfamily protein	-2.00	NA	-3.26	4.61E-03	-2.11	6.92E-02	0.30	NA	-2.29	1.80E-01	-1.73	1.65E-01
Soltu.DM.01G044230	Chloroplast-targeted copper chaperone protein	-2.55	NA	-3.18	1.59E-02	-2.33	9.27E-02	-0.27	NA	-3.82	9.20E-02	-2.85	6.23E-02
Soltu.DM.04G028960	sulfate transporter 1;3	-0.78	NA	-3.17	3.35E-02	-2.02	1.09E-01	0.36	NA	-0.08	9.55E-01	-1.35	1.48E-01
Soltu.DM.03G035650	Protein of unknown function (DUF1677)	-0.87	4.75E-01	-3.32	1.64E-04	-5.20	1.90E-06	-0.63	1.00E+00	-1.21	2.80E-01	-3.71	1.16E-05
Soltu.DM.05G015980	hypothetical protein	-0.93	NA	-3.75	3.05E-02	-1.81	2.94E-01	0.19	NA	0.54	5.97E-01	0.41	6.15E-01
Soltu.DM.07G027150	conserved hypothetical protein	0.63	6.30E-01	-3.21	1.40E-02	-1.55	1.79E-01	-0.40	1.00E+00	-0.19	8.91E-01	0.09	9.30E-01

Soltu.DM.08G020230	hypothetical protein	-1.79	NA	-3.16	9.65E-03	-2.40	4.81E-02	-0.49	NA	0.58	7.48E-01	-0.03	9.87E-01

Appendix-Table 2.8 20 most up or down-regulated genes in the recovery phase to drought (T_3) of only the tolerant variety, in the root. Differentially Expressed Genes (DEGs) are defined by log2FC >1 and padj. < 0.05. An asterisk denotes DEGs whose expression is significantly higher (or lower) in the tolerant than the susceptible for the upregulated (or downregulated) genes in T_1. In grey are the padj. values > 0.05. The table also shows the expression of the DEGs in T_1 and T_3, and in the susceptible variety.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Root. Tolerant						Root. Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj
UPREGULATED DEGs													
Terpene biosynthesis													
Soltu.DM.07G004480	terpene synthase	1.20	7.08E-01	5.46	2.78E-05	7.64	3.42E-11	2.01	NA	0.55	8.17E-01	1.03	5.40E-01
Soltu.DM.01G040970	terpene synthase	3.47	2.54E-01	4.55	2.49E-02	5.01	4.75E-03	2.13	NA	0.68	7.97E-01	-1.29	5.74E-01
Soltu.DM.01G040990	terpene synthase	5.23	NA	3.03	2.30E-01	4.99	9.13E-03	1.86	NA	0.55	8.30E-01	-0.43	8.48E-01
Soltu.DM.01G040950	terpene synthase	4.89	2.32E-02	3.52	7.27E-02	4.91	2.28E-03	1.51	NA	1.95	3.26E-01	-1.27	5.31E-01
UDP-glycosyltransferases													
Soltu.DM.12G004300	UDP-Glycosyltransferase superfamily protein	0.15	NA	6.38	5.72E-03	7.07	4.91E-04	0.00	NA	0.11	9.80E-01	1.04	7.43E-01
Soltu.DM.09G031020	UDP-Glycosyltransferase superfamily protein	5.58	3.27E-02	5.21	1.61E-02	6.20	1.04E-03	-2.43	NA	2.67	1.97E-01	2.10	2.77E-01
Others													
Soltu.DM.03G001060	Major facilitator superfamily protein	0.15	NA	5.63	NA	5.91	1.83E-02	0.00	NA	2.35	5.20E-01	2.53	4.03E-01
Soltu.DM.10G017410	CYCLIN D3;2	0.99	NA	3.18	7.92E-02	5.26	3.36E-04	-1.15	NA	0.02	9.88E-01	0.27	7.86E-01
Soltu.DM.04G019150	cytochrome P450, family 76, subfamily C, polypeptide	0.15	NA	4.99	4.54E-03	5.16	1.25E-03	-0.52	NA	2.30	6.52E-02	1.51	2.25E-01
Soltu.DM.03G026160	Raffinose synthase family protein	1.93	NA	0.07	NA	5.14	2.75E-03	-0.02	NA	-1.82	NA	2.00	1.76E-01
Soltu.DM.07G011740	basic helix-loop-helix (bHLH) DNA-binding family protein	3.16	1.07E-02	5.13	2.24E-07	5.03	1.66E-08	1.69	NA	2.61	2.42E-03	0.84	3.92E-01
Soltu.DM.01G006840	NAD+ ADP-ribosyltransferases;NAD+ ADP-ribosyltransferases	3.31	NA	4.95	1.87E-03	5.01	7.29E-04	0.57	NA	1.41	1.91E-01	1.44	1.31E-01
Soltu.DM.01G007150	CAP160 protein	-0.74	NA	3.27	5.43E-02	4.98	4.54E-04	-1.40	NA	1.19	5.59E-01	1.69	2.79E-01
Soltu.DM.01G006870	DUF4228 domain containing protein	4.02	NA	3.22	NA	5.41	2.38E-02	2.38	NA	0.11	NA	3.59	1.67E-01
Soltu.DM.12G029320	Protein of unknown function (DUF1645)	3.60	8.34E-03	2.37	7.15E-02	5.03	7.76E-07	1.69	2.46E-01	-1.08	4.55E-01	1.67	1.41E-01
Soltu.DM.03G037250	Protein of unknown function (DUF581)	3.20	3.56E-01	0.98	7.46E-01	7.06	1.41E-04	0.30	9.36E-01	-2.43	3.16E-01	3.68	5.53E-02

Soltu.DM.08G024450	hypothetical protein	0.15	9.82E-01	6.26	2.94E-05	7.06	4.05E-07	0.98	NA	2.50	2.50E-01	1.80	3.95E-01
Soltu.DM.03G028750	hypothetical protein	1.16	6.71E-01	3.38	3.40E-03	5.92	1.41E-09	1.30	NA	1.62	2.05E-01	2.04	5.34E-02
Soltu.DM.07G002060	hypothetical protein	0.15	NA	2.84	NA	5.40	1.67E-02	-1.84	NA	1.18	NA	1.53	5.65E-01
Soltu.DM.01G007060	hypothetical protein	0.75	NA	4.75	8.36E-03	4.99	1.95E-03	0.95	NA	0.11	9.78E-01	0.11	9.75E-01
Downregulated genes													
Cell wall modification													
Soltu.DM.08G004820	cellulose synthase like G3	0.22	9.44E-01	-1.84	1.91E-01	-5.54	6.21E-04	0.03	NA	-1.45	5.93E-01	0.60	7.65E-01
Terpene biosynthesis													
Soltu.DM.09G029860	terpene synthase	-1.90	NA	-4.80	NA	-3.71	3.76E-02	-0.28	NA	-0.69	NA	-2.22	2.09E-01
Transporters													
Soltu.DM.11G023240	nitrate transporter 2.4	0.51	8.74E-01	-3.38	4.74E-02	-4.02	7.30E-03	-0.23	NA	-0.46	7.98E-01	-0.19	9.01E-01
Soltu.DM.08G016100	lipid transfer protein	0.90	NA	-0.15	9.40E-01	-3.97	1.96E-02	0.36	NA	-0.69	6.54E-01	-0.16	9.08E-01
Others													
Soltu.DM.06G022680	Glucose-methanol-choline (GMC) oxidoreductase family protein	-0.98	NA	-2.95	NA	-5.24	2.21E-03	-1.34	NA	1.20	5.98E-01	1.00	6.15E-01
Soltu.DM.11G010690	gibberellin 20-oxidase	0.05	NA	-2.53	NA	-5.23	2.26E-03	0.18	NA	-3.77	6.33E-02	-0.46	7.98E-01
Soltu.DM.11G010680	gibberellin 20 oxidase	-0.61	NA	-3.73	NA	-5.04	1.31E-03	-1.10	NA	-3.52	5.27E-02	-2.27	1.04E-01
Soltu.DM.06G014710	C2H2 and C2HC zinc fingers superfamily protein	-2.55	NA	-2.61	NA	-4.89	2.02E-03	-0.15	NA	-1.12	NA	-0.52	7.48E-01
Soltu.DM.06G002200	Tetratricopeptide repeat (TPR)-like superfamily protein	-0.04	9.90E-01	-1.35	3.64E-01	-4.78	4.34E-05	2.28	NA	-0.04	9.83E-01	-2.22	1.10E-01
Soltu.DM.11G006060	pleiotropic drug resistance	0.15	NA	-3.38	5.20E-02	-4.72	4.07E-03	0.24	NA	0.87	6.91E-01	1.43	3.71E-01
Soltu.DM.09G031110	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	0.28	NA	-1.34	2.03E-01	-4.21	3.05E-03	0.47	NA	-1.38	2.18E-01	-0.26	7.92E-01
Soltu.DM.08G018250	RING/U-box superfamily protein	-1.27	NA	-0.98	NA	-4.10	1.84E-02	-0.36	NA	-1.72	NA	-1.78	3.12E-01
Soltu.DM.02G029250	Peroxidase superfamily protein	0.78	5.91E-01	-2.24	9.21E-03	-4.05	2.87E-07	0.67	NA	-2.13	2.35E-01	-3.01	6.60E-02
Soltu.DM.02G031870	Galactose mutarotase-like superfamily protein	-0.34	NA	-2.66	2.14E-01	-3.98	1.88E-02	-0.19	NA	-0.78	5.57E-01	-0.99	3.73E-01
Soltu.DM.03G001640	Leucine-rich repeat transmembrane protein kinase	-0.33	NA	-0.45	NA	-3.82	2.55E-02	-1.61	NA	-0.44	8.01E-01	0.13	9.29E-01
Soltu.DM.06G034500	3-ketoacyl-CoA synthase	-0.77	4.94E-01	-2.19	8.25E-03	-3.78	5.68E-05	-0.60	NA	-2.00	1.26E-02	-1.14	9.32E-02
Soltu.DM.06G027680	Protein of unknown function (DUF674)	0.23	NA	-3.06	NA	-4.43	2.74E-02	2.61	NA	-0.85	NA	-0.86	7.78E-01
Soltu.DM.08G007260	hypothetical protein	-0.54	5.82E-01	-2.16	4.43E-03	-5.27	5.85E-05	-0.17	NA	-0.38	4.12E-01	-0.64	8.68E-02

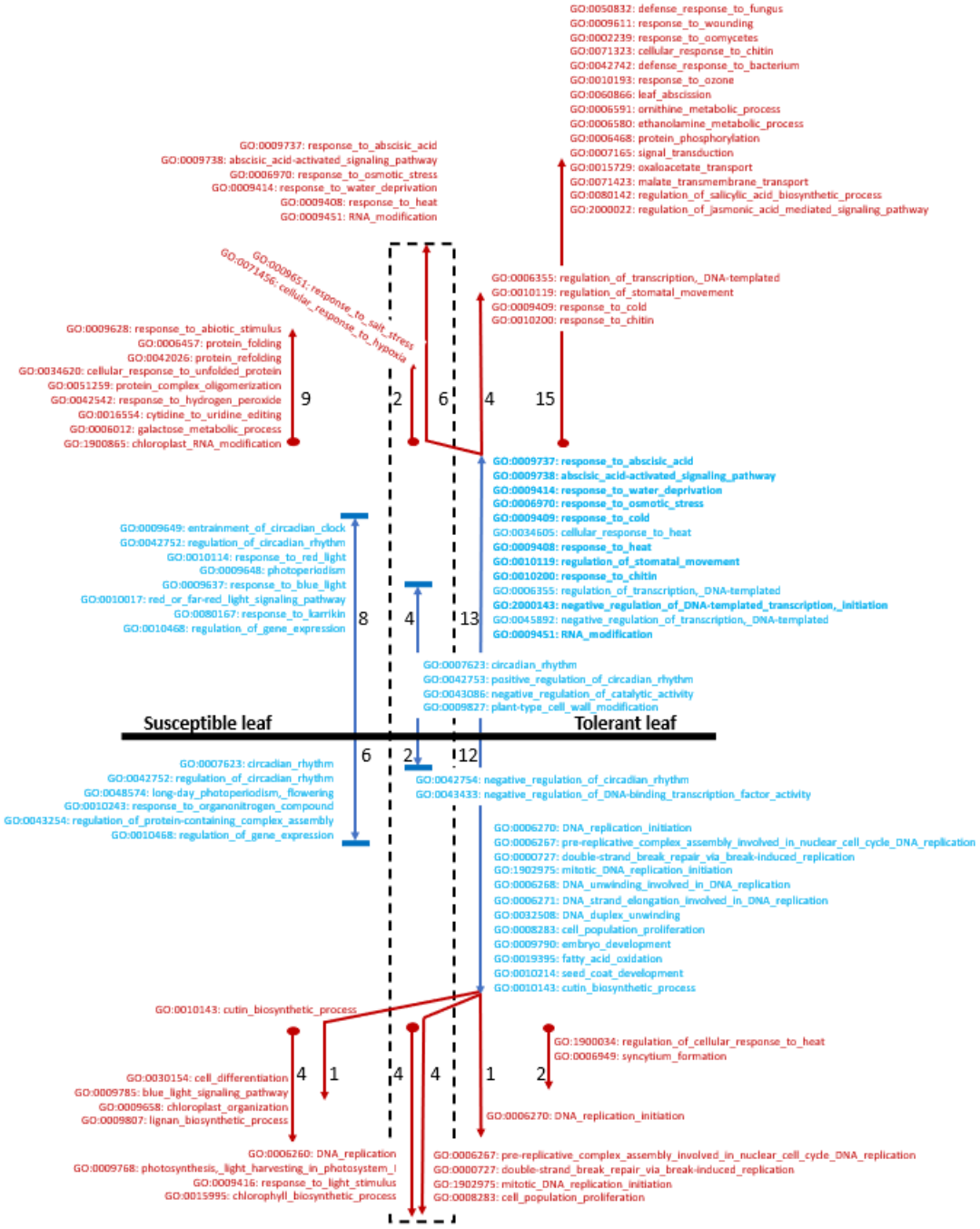
Soltu.DM.03G002880	hypothetical protein	-0.74	NA	-3.86	6.40E-02	-5.11	2.13E-03	0.00	NA	-2.38	1.50E-01	-1.88	1.77E-01
Soltu.DM.04G000920	hypothetical protein	-0.63	NA	0.17	NA	-3.96	1.55E-02	-0.41	NA	-0.51	7.61E-01	-0.43	7.53E-01

Appendix-Table 2.9 20 most up or down-regulated genes in the recovery phase to drought (T_3) of only the tolerant variety, in the leaf. Differentially Expressed Genes (DEGs) are defined by log2FC >1 and padj. < 0.05. An asterisk denotes DEGs whose expression is significantly higher (or lower) in the tolerant than the susceptible for the upregulated (or downregulated) genes in T_1. In grey are the padj. values > 0.05. The table also shows the expression of the DEGs in T_1 and T_3, and in the susceptible variety.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Leaf.Tolerant						Leaf.Susceptible					
		T1		T2		T3		T1		T2		T3	
		LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj	LFC	padj
Upregulated genes													
Response to ABA													
Soltu.DM.06G013730	protein phosphatase 2CA	6.20	8.13E-04	4.36	7.23E-03	5.45	1.53E-03	2.78	9.53E-01	3.65	4.56E-02	2.64	8.99E-02
Soltu.DM.02G009240	ABI five binding protein	0.26	NA	2.58	2.04E-01	5.27	4.34E-03	-0.02	NA	1.33	NA	0.85	7.75E-01
Soltu.DM.08G016370	ABC-2 and Plant PDR ABC-type transporter family protein	1.87	1.10E-01	4.25	1.06E-07	4.81	5.62E-08	-2.34	2.94E-01	-1.50	1.88E-01	-2.63	2.66E-03
Others													
Soltu.DM.09G021850	Pollen Ole e 1 allergen and extensin family protein	1.49	7.27E-01	5.63	2.38E-02	7.16	4.71E-03	0.39	1.00E+00	0.53	8.75E-01	-0.20	9.41E-01
Soltu.DM.10G006090	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	5.01	NA	5.30	2.44E-02	6.82	4.02E-03	-0.84	NA	1.27	7.25E-01	1.80	4.75E-01
Soltu.DM.06G018480	Late embryogenesis abundant protein, group 1 protein	4.16	NA	6.13	1.46E-02	6.56	1.28E-02	-2.84	NA	-2.88	4.42E-01	-0.30	9.28E-01
Soltu.DM.06G012920	Integrase-type DNA-binding superfamily protein	3.63	NA	0.79	NA	6.16	2.05E-03	1.67	NA	2.02	NA	2.79	1.60E-01
Soltu.DM.10G024340	SAUR-like auxin-responsive protein family	0.77	NA	4.88	NA	5.89	3.32E-03	0.78	NA	2.43	NA	3.07	1.13E-01
Soltu.DM.10G015450	redox responsive transcription factor	4.15	NA	1.69	NA	5.67	1.66E-02	1.73	NA	-0.14	NA	2.94	2.00E-01
Soltu.DM.06G026880	Octicosapeptide/Phox/Bem1p family protein	6.29	1.86E-03	4.72	6.84E-03	5.61	2.64E-03	4.24	3.05E-01	2.92	1.68E-01	2.91	8.69E-02
Soltu.DM.05G000390	Glycosyltransferase family 61 protein	2.14	NA	3.91	NA	5.38	1.23E-03	-0.59	NA	-0.79	NA	0.05	9.72E-01
Soltu.DM.12G026290	Integrase-type DNA-binding superfamily protein	3.71	NA	2.95	1.24E-01	5.10	4.25E-03	1.02	NA	-0.17	9.50E-01	1.95	1.85E-01
Soltu.DM.02G006760	homeobox protein	2.59	NA	3.98	7.93E-04	5.09	1.14E-05	-1.19	NA	1.87	4.52E-02	1.02	2.44E-01
Soltu.DM.10G015440	redox responsive transcription factor	2.70	NA	2.37	NA	5.06	2.21E-02	0.63	NA	0.96	NA	2.71	2.10E-01
Soltu.DM.03G005570	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein	3.65	3.27E-02	4.57	3.91E-04	5.04	3.37E-04	0.67	1.00E+00	1.69	3.36E-01	2.51	5.37E-02

Soltu.DM.04G021930	Cupredoxin superfamily protein	1.80	NA	3.40	6.37E-03	4.87	6.89E-05	-0.63	NA	-3.88	6.03E-02	1.77	6.04E-02
Soltu.DM.09G026180	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	2.81	2.93E-02	4.00	2.95E-05	4.73	3.89E-06	-0.41	1.00E+00	0.43	8.00E-01	-1.91	1.33E-01
Soltu.DM.06G000880	trehalose phosphate synthase	2.88	NA	4.02	1.87E-02	4.69	4.83E-03	-0.73	NA	1.50	2.89E-01	1.59	1.57E-01
Soltu.DM.01G007060	hypothetical protein	3.89	NA	6.28	2.04E-03	6.78	1.28E-03	-0.98	NA	-1.01	8.06E-01	-1.07	7.26E-01
Soltu.DM.12G009000	conserved hypothetical protein	1.71	NA	2.73	2.37E-01	5.21	1.26E-02	-1.60	NA	3.46	8.88E-02	2.79	1.15E-01
Downregulated genes													
Soltu.DM.10G015950	Putative S-adenosyl-L-methionine-dependent methyltransferase domain containing protein	-0.82	NA	-2.72	1.30E-02	-6.61	3.93E-04	-0.36	NA	-0.04	NA	-1.29	4.12E-01
Soltu.DM.08G025250	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	-4.26	3.20E-02	-5.83	1.32E-04	-5.47	1.11E-03	1.12	1.00E+00	-3.36	9.62E-02	-2.47	1.29E-01
Soltu.DM.06G027760	purple acid phosphatase	2.56	1.14E-01	-0.33	8.41E-01	-5.06	1.70E-02	2.20	NA	2.45	1.09E-01	0.04	9.81E-01
Soltu.DM.01G050520	SAUR-like auxin-responsive protein family	-0.24	NA	-1.47	3.12E-01	-4.76	2.34E-02	-0.77	NA	-2.78	NA	-3.35	7.73E-02
Soltu.DM.10G015900	SAUR-like auxin-responsive protein family	-0.21	NA	-0.81	4.63E-01	-3.42	5.21E-03	-0.12	NA	-1.18	4.67E-01	-2.33	7.45E-02
Soltu.DM.11G001480	SAUR-like auxin-responsive protein family	-0.21	8.32E-01	-1.19	6.18E-02	-3.26	1.41E-05	0.28	NA	-2.12	4.12E-02	-1.20	1.12E-01
Soltu.DM.10G015910	SAUR-like auxin-responsive protein family	0.39	NA	-2.07	NA	-4.19	3.61E-02	0.82	NA	-2.23	NA	-2.71	1.42E-01
Soltu.DM.07G008910	ATP synthase subunit	-1.75	NA	-1.08	NA	-4.29	4.16E-02	1.02	NA	-1.13	NA	0.43	8.02E-01
Soltu.DM.10G029740	NAD(P)-binding Rossmann-fold superfamily protein	-2.51	NA	-2.34	3.63E-02	-4.26	1.80E-03	-1.30	NA	-1.65	3.07E-01	-2.49	5.31E-02
Soltu.DM.04G027510	Cytochrome P450 superfamily protein	0.29	NA	-1.91	2.18E-02	-3.90	2.31E-04	0.93	NA	-0.06	9.88E-01	-0.11	9.71E-01
Soltu.DM.S000200	cytochrome P450, family 71, subfamily B, polypeptide	-0.17	9.04E-01	0.19	8.49E-01	-3.42	1.55E-03	0.33	NA	-2.24	3.41E-02	-0.70	4.13E-01
Soltu.DM.06G011930	response regulator	-1.01	4.72E-01	-2.17	3.55E-02	-3.89	5.04E-04	-0.86	NA	-2.07	2.20E-01	-2.68	5.07E-02
Soltu.DM.07G003960	MATE efflux family protein	0.60	NA	-1.57	1.62E-01	-3.74	4.11E-02	0.33	NA	-0.38	7.15E-01	-0.88	2.17E-01
Soltu.DM.09G020330	heat shock transcription factor A7A	1.03	5.08E-01	-2.57	3.56E-02	-3.73	7.33E-03	1.09	1.00E+00	1.12	4.61E-01	-0.21	8.83E-01
Soltu.DM.09G000820	receptor lectin kinase	-0.49	NA	-1.67	6.77E-02	-3.52	4.28E-03	-0.08	NA	-0.56	NA	-0.78	6.04E-01
Soltu.DM.01G038730	SGNH hydrolase-type esterase superfamily protein	-0.77	4.32E-01	-1.44	8.89E-02	-3.44	9.53E-03	0.09	NA	0.40	5.66E-01	0.13	8.31E-01
Soltu.DM.09G023780	Chaperone DnaJ-domain superfamily protein	1.79	3.23E-01	-2.75	4.94E-02	-3.43	2.28E-02	1.81	1.00E+00	0.32	8.91E-01	-1.82	1.95E-01
Soltu.DM.04G007930	Thioredoxin superfamily protein	0.10	NA	-0.29	8.14E-01	-3.42	8.05E-03	0.50	NA	-1.95	1.51E-01	-0.43	7.08E-01
Soltu.DM.12G014530	HXXXD-type acyl-transferase family protein	-0.59	5.80E-01	-2.13	4.17E-03	-3.38	3.57E-05	-0.26	1.00E+00	-2.95	1.66E-03	-1.46	5.32E-02
Soltu.DM.11G007500	conserved hypothetical protein	0.66	NA	-1.14	2.44E-01	-3.43	1.55E-02	0.09	NA	-1.63	1.83E-01	-0.57	5.36E-01

(A)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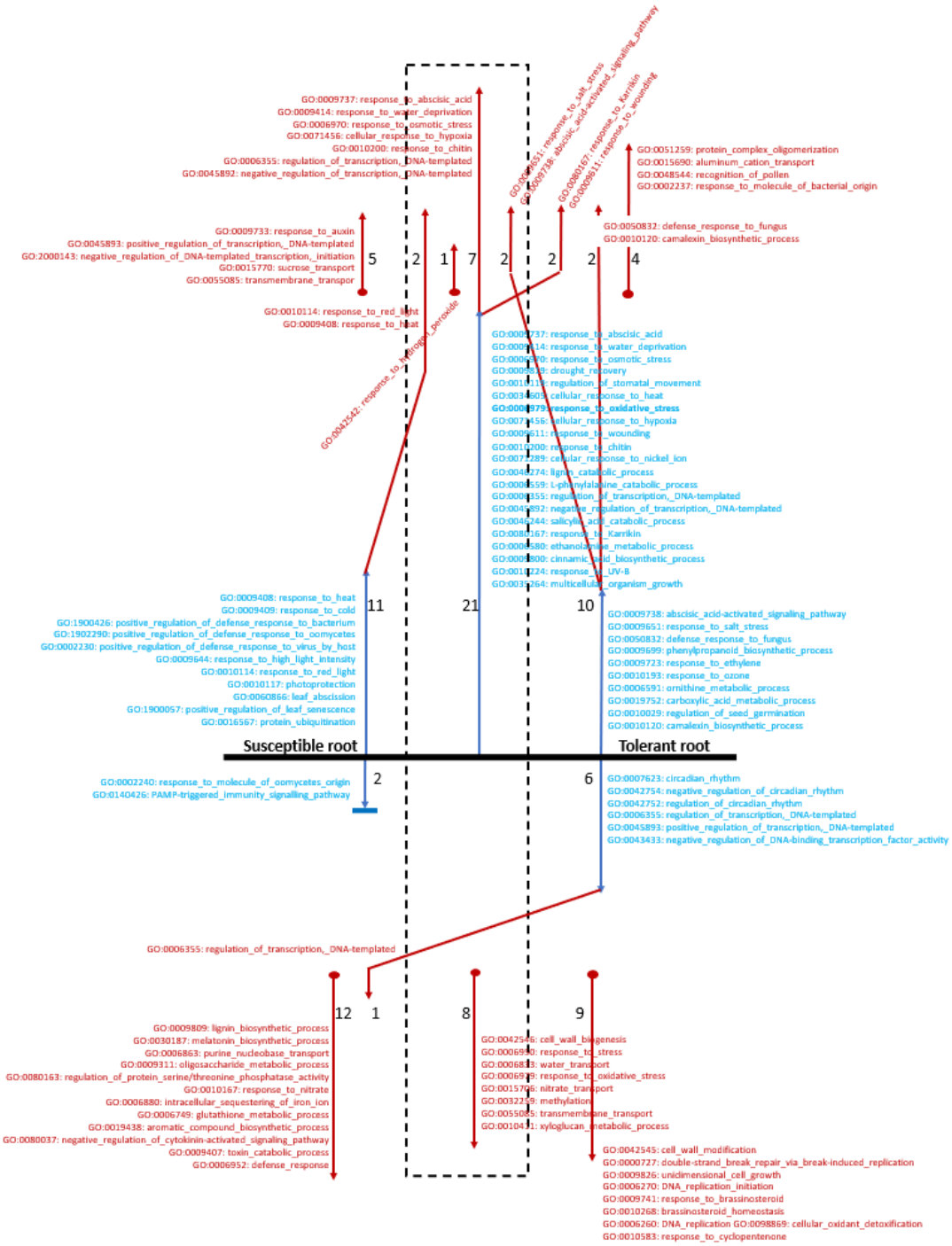
(B)



Appendix-Figure 2.5 Enriched GO terms in both varieties during drought stress. This graph shows the enriched GO terms for the leaf **(A)** and the root **(B)** tissue. The GO for up and downregulated DEGs (above and below the horizontal black line, respectively) are separated in the susceptible (left) and the tolerant (right) variety, for T_1 (light blue) and T_2 (red). Inside the dotted-black square are the common GO terms between both potato varieties.

Appendix-Table 2.10 DEGs inside the "response to abscisic acid" category (GO:0009737). The table shows the DEGs that are only expressed in the tolerant leaves (green) and only in the tolerant root (yellow), or only in both tissue (orange) but not in the susceptible during the early response to drought. The values are the log2FC, in (-) are for the genes not DE in the respective time point.

Potato genome v6.1 Gene ID	Potato genome v6.1 Gene Annotation	Leaf.Tolerant			Leaf.Susceptible			Root.Tolerant			Root.Susceptible			*A. thaliana* GeneName
		T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	
Soltu.DM.11G011430	ATP-binding casette family G25	1.48	2.26	2.74	-	1.74	1.66	-	2.84	1.45	-	1.73	-	ABCG25
Soltu.DM.05G023720	pleiotropic drug resistance	1.21	1.89	1.76	-	-	1.56	-	-	1.22	-	1.05	1.10	ABCG40
Soltu.DM.04G000490	ABI five binding protein	3.42	4.44	5.17	-	4.07	4.13	-	6.82	6.16	-	5.98	5.00	AFP1
Soltu.DM.02G030840	ABI five binding protein	3.65	3.63	5.10	-	3.99	4.67	-	2.51	1.96	-	2.43	1.78	AFP3
Soltu.DM.05G000860	ABI five binding protein	2.98	4.42	5.54	-	3.81	3.99	-	4.60	4.43	-	3.40	3.07	AFP3
Soltu.DM.08G001760	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	1.60	2.40	1.64	-	-	1.17	-	-	3.68	-	3.34	3.14	AT2G36690
Soltu.DM.02G017790	Aluminium induced protein with YGL and LRDR motifs	1.77	2.30	2.27	-	1.63	2.17	-	2.25	1.77	-	-	-	AT4G27450 / AT3G22850
Soltu.DM.01G035490	homeobox	5.74	7.34	7.87	-	6.25	6.40	-	6.01	4.19	-	3.61	2.18	ATHB-12
Soltu.DM.06G014070	homeobox	2.68	3.19	3.07	-	3.26	2.29	-	1.47	-	-	2.58	-	ATHB-7
Soltu.DM.11G022320	BEL1-like homeodomain	1.61	2.01	1.77	-	1.95	1.95	0.92	1.54	-	-	1.06	-	BLH1
Soltu.DM.06G026950	BTB and TAZ domain protein	1.74	-	-	-	-	-	0.77	1.24	1.21	0.68	0.97	1.41	BT2
Soltu.DM.02G032680	Peroxidase superfamily protein	1.41	-	-	-	-	-1.13	-	-	-	-	-	-	DOX1
Soltu.DM.06G010320	DRE-binding protein 2A	2.07	-	-	-	-	-	-	5.27	4.68	-	-	-	DREB2C
Soltu.DM.04G031660	MATE efflux family protein	2.20	2.30	-	-	-	1.60	-	3.49	1.27	1.41	3.52	2.28	DTX48
Soltu.DM.06G034820	EID1-like	2.32	-	-	-	2.39	-	3.23	5.07	2.80	2.75	4.22	2.19	EDL3
Soltu.DM.10G005000	erf domain protein	2.80	2.74	2.98	-	2.19	2.90	1.73	2.28	1.89	1.63	-	1.68	ERF4
Soltu.DM.01G050660	GAST1 protein homolog	1.82	2.03	-	-	-	-	-	-	-	-	-	-	GASA1
Soltu.DM.05G019830	G-box binding factor	3.59	4.65	5.61	-	3.94	4.77	-	2.93	3.13	-	2.67	3.07	GBF3
Soltu.DM.01G007150	CAP160 protein	5.91	8.95	10.45	-	4.80	6.30	-	-	4.98	-	-	-	LTI65
Soltu.DM.01G045280	nicotinamidase	1.09	0.95	-	-	1.05	1.21	-	-	-	-	-	-	NIC1
Soltu.DM.09G026670	phosphate transporter 1;4	1.31	1.77	2.02	-	1.02	1.02	-	-	-	-	-1.37	-0.99	PHT1-4
Soltu.DM.06G013730	protein phosphatase 2CA	6.20	4.36	5.45	-	3.65	-	-	7.72	5.90	-	8.33	6.10	PP2CA
Soltu.DM.03G012480	protein phosphatase 2CA	4.56	6.39	7.33	-	6.16	6.57	-	-	-	-	-	-	PP2CA
Soltu.DM.05G023010	protein phosphatase 2CA	2.64	2.63	2.99	-	2.80	2.45	1.51	3.37	1.22	1.75	3.08	1.19	PP2CA
Soltu.DM.03G022710	highly ABA-induced PP2C gene	3.78	5.65	6.73	-	4.93	5.05	-	7.10	6.73	-	6.21	5.43	AIP1 / PP2CA
Soltu.DM.07G012130	Protein phosphatase 2C family protein	1.47	2.28	2.54	-	2.00	2.04	-	2.91	2.41	-	2.21	1.49	ABI2
Soltu.DM.12G029330	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	3.38	4.37	4.83	-	3.55	3.38	2.95	5.18	3.29	2.65	5.08	3.29	RD26
Soltu.DM.07G024710	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	3.13	5.52	6.16	-	5.25	5.94	1.32	4.52	2.48	1.63	4.34	2.57	RD26
Soltu.DM.09G024150	Major facilitator superfamily protein	1.09	2.01	1.60	-	1.44	1.63	-	2.64	3.09	-	-	2.36	STP13
Soltu.DM.01G048750	extra-large G-protein	1.99	3.57	3.51	-	1.43	3.48	-	-	2.52	-	-	1.71	XLG1

Soltu.DM.12G006170	zinc-finger protein	1.90	2.02	3.50	-	-	1.88	2.94	-	2.15	3.29	-1.82	-	ZAT10
Soltu.DM.08G015120	carotenoid cleavage dioxygenase	1.24	1.51	1.02	-	-	-	-	-	-	-	-	-	NCED1 / NCED3
Soltu.DM.05G021330	white-brown complex homolog protein	-	-	1.96	-	-	-	1.46	1.84	2.25	-	-	1.72	ABCG11
Soltu.DM.05G021350	white-brown complex homolog protein	-	-	-	-	-	-	1.39	1.84	2.15	-	1.03	1.78	ABCG11
Soltu.DM.05G021360	white-brown complex homolog protein	-	-	-	-	-	-	1.71	1.93	2.45	-	0.98	1.75	ABCG11
Soltu.DM.05G023730	pleiotropic drug resistance	-	1.64	-	-	-	-	1.27	1.09	1.25	-	-	-	ABCG40
Soltu.DM.09G028710	pleiotropic drug resistance	-	-	-	-	-	-	1.18	0.97	1.60	-	-	1.14	ABCG40
Soltu.DM.11G004860	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	-	-	-	-	-	-	2.40	-	-	-	-	-2.89	AT2G36690
Soltu.DM.05G023310	myb domain protein	-	-	-	-	-	-	5.21	5.68	6.37	-	3.10	4.27	AtMYB78
Soltu.DM.12G001820	myb domain protein	-	-	-	-	-	-	6.44	-	9.26	-	-	6.03	AtMYB78
Soltu.DM.02G019660	cysteine-rich RLK (RECEPTOR-like protein kinase)	-	-	-	-	-	-	4.00	4.05	4.25	-	-	-	CRK29
Soltu.DM.12G023090	Receptor-like protein kinase-related family protein	-	-	-	-	-	-	2.19	-	-	-	-	-	CRRSP38
Soltu.DM.03G001930	ethylene responsive element binding factor	-	2.19	2.85	-	3.83	4.18	1.86	3.71	2.57	-	2.57	1.90	ERF4
Soltu.DM.10G020680	GRAS family transcription factor family protein	-	-	2.70	-	-	1.49	2.53	2.52	3.83	-	-	-	GAI
Soltu.DM.02G006360	galactinol synthase	-	-	5.31	-	4.50	5.08	4.74	7.13	7.43	-	4.01	5.16	GOLS1
Soltu.DM.08G010990	lipoxygenase	-	-	-	-	-	-	1.85	1.95	2.47	-	1.25	1.81	LOX1
Soltu.DM.07G017180	mitogen-activated protein kinase kinase kinase	-	2.40	3.77	-	-	3.39	1.51	4.79	3.02	-	3.59	2.25	MAPKKK17
Soltu.DM.02G008230	PATATIN-like protein	-	-	-	-	-	-	4.06	-	3.57	-	-	-	PLP3
Soltu.DM.02G006090	BURP domain-containing protein	-	-	-	-	-	-	1.89	-	-	-	-	-0.95	RD22
Soltu.DM.02G033230	Raffinose synthase family protein	-	7.15	8.14	-	4.07	5.06	1.57	6.94	8.22	-	4.98	5.57	RFS5
Soltu.DM.10G020700	GRAS family transcription factor family protein	-	2.38	3.05	-	-	2.36	1.26	1.05	3.84	-	-	1.90	RGL1
Soltu.DM.08G030040	homeobox	3.50	5.61	6.07	-	5.31	5.93	2.33	6.03	4.75	-	5.26	3.73	ATHB-7

Appendix-Table 2.11 Genes inside the "plant cell wall modification" (GO:0009827) expressed in any time point (T_1 or T_2 or T_3) in the leaf of the susceptible or the tolerant. The values in grey are for the padj. higher than 0.05 in the respective time point.

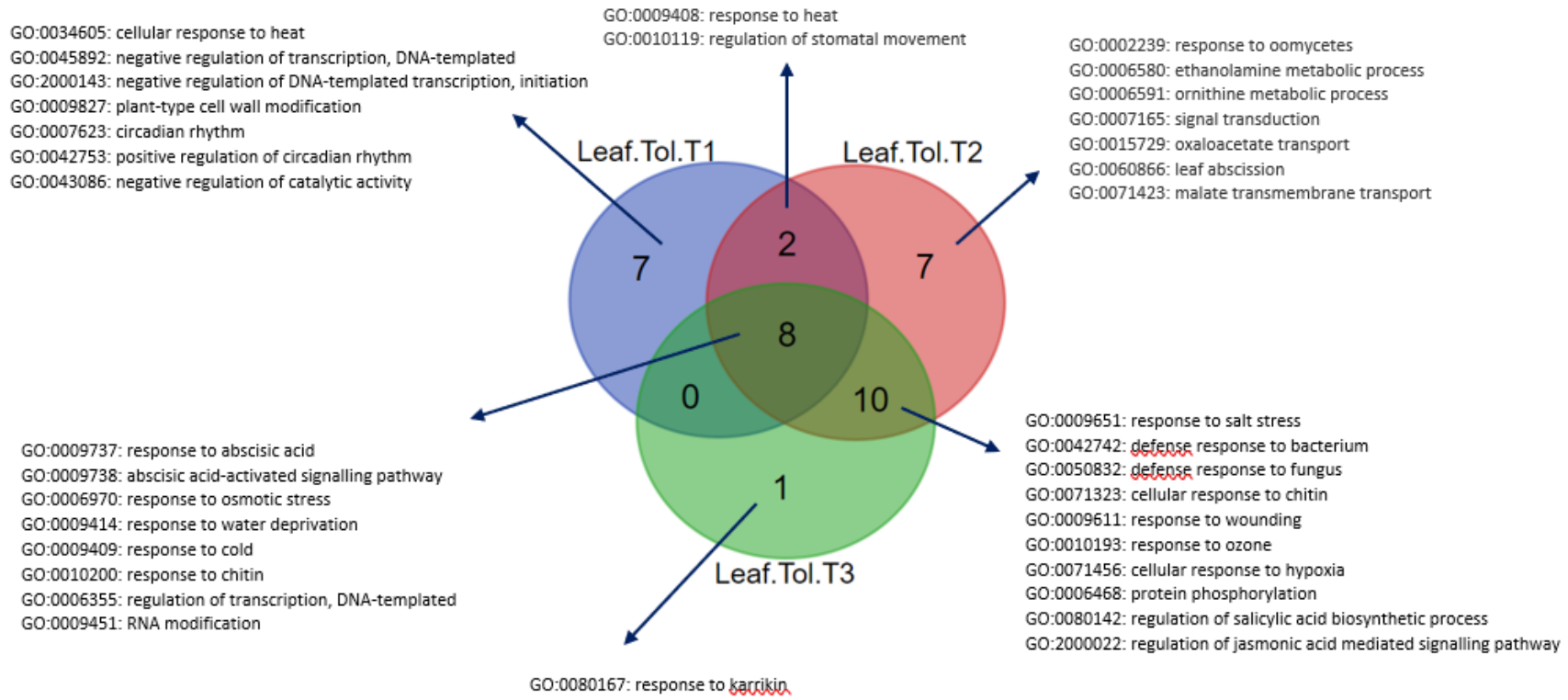
Potato genome v6.1 Gene ID	*Potato genome v6.1 Gene Annotation*	Leaf.Tolerant						Leaf.Susceptible					
		T1		T2		T3		T1		T2		T3	
		log2FC	padj	log2FC	padj	log2FC	padj	log2FC	padj	log2FC	padj	log2FC	padj
Soltu.DM.01G050980	subtilisin-like serine protease	-0.09	8.59E-01	0.08	8.36E-01	1.11	4.23E-04	-0.06	1.00E+00	0.6	9.85E-02	0.99	3.59E-04
Soltu.DM.03G015440	Plant invertase/pectin methylesterase inhibitor superfamily protein	2.4	4.25E-05	5.00	4.61E-26	4.76	3.71E-19	2.16	2.28E-03	5.49	5.13E-24	5.45	1.53E-29
Soltu.DM.03G015460	Plant invertase/pectin methylesterase inhibitor superfamily protein	3.42	2.48E-02	6.86	1.03E-10	6.66	1.05E-09	3.35	6.82E-01	6.18	3.98E-05	6.75	1.24E-06
Soltu.DM.03G015480	Plant invertase/pectin methylesterase inhibitor superfamily protein	1.82	2.79E-03	3.73	4.64E-16	3.24	4.53E-10	2.8	8.72E-05	6.3	2.40E-27	6.11	2.16E-30
Soltu.DM.03G015490	Plant invertase/pectin methylesterase inhibitor superfamily protein	3.21	2.41E-04	5.74	1.57E-16	5.6	6.63E-13	2.48	5.33E-02	5.76	2.62E-13	6.46	5.47E-21
Soltu.DM.03G015500	Plant invertase/pectin methylesterase inhibitor superfamily protein	3.17	3.32E-05	4.51	1.20E-12	3.71	1.76E-07	1.78	4.60E-01	5.16	1.01E-12	5.71	6.09E-19
Soltu.DM.03G015510	Plant invertase/pectin methylesterase inhibitor superfamily protein	2.9	5.30E-09	5.13	8.02E-32	4.56	2.29E-20	1.98	3.29E-03	5.55	1.59E-28	6.08	2.12E-42
Soltu.DM.03G015520	Plant invertase/pectin methylesterase inhibitor superfamily protein	1.55	1.67E-01	2.8	3.39E-04	1.06	2.92E-01	1.26	1.00E+00	4.18	1.82E-06	4.88	1.27E-10
Soltu.DM.10G026840	Plant protein of unknown function (DUF828)	-1.00	2.72E-02	-1.4	4.19E-05	-1.00	1.01E-02	-0.29	1.00E+00	-0.74	9.27E-02	-1.44	1.94E-05

Appendix-Table 2.12 Genes inside the GO terms related to the cell wall ("xyloglucan metabolic process" (GO:0010411), "cell wall biogenesis" (GO:0042546), and "cell wall modification" (GO:0042545) that were enriched in T_2 in the tolerant variety. The values are the log2FC, in (-) are for the genes not DE in the respective time point.

Potato genome v6.1	Potato genome v6.1 Gene Annotation	Root.Tolerant			Root.Susceptible		
		T1	T2	T3	T1	T2	T3
Soltu.DM.02G021800	Plant invertase/pectin methylesterase inhibitor superfamily	-	-3.70	-1.34	-	-2.88	-1.58
Soltu.DM.03G015350	Plant invertase/pectin methylesterase inhibitor superfamily	-	-3.85	-	-	-2.73	-
Soltu.DM.01G039670	Plant invertase/pectin methylesterase inhibitor superfamily	-	-5.30	-	-	-2.50	-1.90
Soltu.DM.03G015370	Plant invertase/pectin methylesterase inhibitor superfamily	-0.83	-2.42	-0.67	-	-1.41	-0.62
Soltu.DM.02G001870	Plant invertase/pectin methylesterase inhibitor superfamily	-	-4.04	-	-	-	-
Soltu.DM.03G011600	Plant invertase/pectin methylesterase inhibitor superfamily	-	-1.85	-1.02	-	-	-1.16
Soltu.DM.09G023650	Plant invertase/pectin methylesterase inhibitor superfamily	-	-1.45	1.49	-	-	1.33
Soltu.DM.06G012610	Plant invertase/pectin methylesterase inhibitor superfamily	-	-1.71	-	-	-	-
Soltu.DM.05G019240	Plant invertase/pectin methylesterase inhibitor superfamily	-	-1.70	-2.27	-	-	-2.72
Soltu.DM.09G023660	Plant invertase/pectin methylesterase inhibitor superfamily	-	-1.74	-	-	-	-
Soltu.DM.06G013790	Plant invertase/pectin methylesterase inhibitor superfamily	-	-	-0.63	-	-0.59	-1.06
Soltu.DM.01G031690	Plant invertase/pectin methylesterase inhibitor superfamily	-	-	-	-	-	-1.15
Soltu.DM.11G008250	Plant invertase/pectin methylesterase inhibitor superfamily	-	-	1.73	-	-	1.27
Soltu.DM.01G025220	Pectin lyase-like superfamily protein	-	-	-1.29	-	-1.76	-2.23
Soltu.DM.08G024910	Pectin lyase-like superfamily protein	-	-	-1.54	-	-	-1.58
Soltu.DM.12G025490	Pectin lyase-like superfamily protein	-	-	-	-	-	-1.75
Soltu.DM.07G014040	Pectin lyase-like superfamily protein	-	-1.74	-	-	-	-
Soltu.DM.03G037420	pectin methylesterase	-	-	2.13	-	-	2.84
Soltu.DM.07G006430	pectin methylesterase	-	1.30	1.84	-	1.34	1.85
Soltu.DM.01G049540	Pectinesterase	-	-1.55	-	-	-0.64	-
Soltu.DM.07G002190	Xyloglucan endotransglucosylase/hydrolase family protein	-	3.78	4.10	1.16	4.58	4.84
Soltu.DM.12G020770	Xyloglucan endotransglucosylase/hydrolase family protein	-	1.62	6.65	-	-	5.68
Soltu.DM.12G028730	xyloglucan endotransglucosylase/hydrolase	-	-2.89	-	-	-2.68	-
Soltu.DM.09G030000	xyloglucan endotransglucosylase/hydrolase	-	-2.64	-	-	-2.38	-
Soltu.DM.03G018880	xyloglucan endotransglucosylase/hydrolase	-	-1.46	-2.53	-	-2.27	-3.19
Soltu.DM.07G021990	xyloglucan endotransglucosylase/hydrolase	-	-3.09	0.87	-	-1.69	0.92
Soltu.DM.03G002910	xyloglucan endotransglucosylase/hydrolase	-	-2.21	-	-	-1.63	-
Soltu.DM.12G025130	xyloglucan endotransglucosylase/hydrolase	-	-3.25	-	-	-1.59	-
Soltu.DM.10G000430	xyloglucan endotransglucosylase/hydrolase	-	-1.87	-2.14	-	-1.57	-2.04
Soltu.DM.11G020640	xyloglucan endotransglucosylase/hydrolase	-	-1.69	-2.38	-	-1.56	-3.02
Soltu.DM.07G018430	xyloglucan endotransglucosylase/hydrolase	-	-	-	-	-1.55	-
Soltu.DM.10G000420	xyloglucan endotransglucosylase/hydrolase	-	-1.98	-1.68	-	-1.25	-1.35
Soltu.DM.09G005280	xyloglucan endotransglucosylase/hydrolase	-	-2.07	-2.34	-	-1.48	-1.26
Soltu.DM.12G025120	xyloglucan endotransglucosylase/hydrolase	-	-3.60	-	-	-	-
Soltu.DM.07G022400	xyloglucan endotransglucosylase/hydrolase	1.59	-	5.96	-	-	5.25
Soltu.DM.12G028740	xyloglucan endotransglucosylase/hydrolase	-	-1.88	1.87	-	-	1.50
Soltu.DM.02G019780	xyloglucan endotransglucosylase/hydrolase	-	-2.20	-	-	-	-
Soltu.DM.07G002180	xyloglucan endotransglucosylase/hydrolase	-	-1.02	1.08	-	-	1.38
Soltu.DM.01G029240	xyloglucan endotransglucosylase/hydrolase	-	1.46	1.35	-	1.46	1.74
Soltu.DM.07G005220	xyloglucan endotransglucosylase/hydrolase	-	-	-	-	1.51	3.07
Soltu.DM.11G019660	xyloglucan endotransglucosylase/hydrolase	-	1.91	1.74	-	1.90	1.30

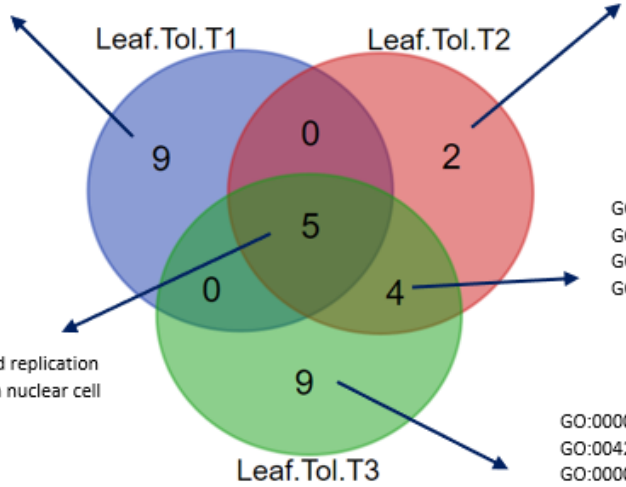
Soltu.DM.11G015160	xyloglucan endotransglucosylase/hydrolase	-	-1.91	-1.20	-	-0.87	-0.92
Soltu.DM.12G025140	xyloglucan endotransglucosylase/hydrolase	-	-1.92	1.57	-	-	2.01
Soltu.DM.07G022000	xyloglucan endotransglucosylase/hydrolase	-	-	6.53	-	-	5.69
Soltu.DM.12G002500	xyloglucan endotransglucosylase/hydrolase	-	-	-	-	-	2.15
Soltu.DM.01G039330	xyloglucan endotransglucosylase/hydrolase	-	-1.08	-	-	-	-
Soltu.DM.08G021810	xyloglucan endotransglucosylase/hydrolase	-	-	1.00	-	-	1.63
Soltu.DM.04G003720	xyloglucan endotransglucosylase/hydrolase	-	-	-	-	-	1.00
Soltu.DM.05G008140	xyloglucan:xyloglucosyl transferase	-1.50	-1.46	1.49	-	-	1.77
Soltu.DM.03G029870	xyloglucan xylosyltransferase	-	-0.41	1.78	-	-	1.50
Soltu.DM.03G015040	xyloglucan endotransglycosylase	-	-1.94	4.00	-1.28	-3.31	3.57
Soltu.DM.03G015050	xyloglucan endotransglycosylase	-	-	5.36	-	-2.59	4.83
Soltu.DM.03G015060	xyloglucan endotransglycosylase	-	-2.04	4.77	-	-2.52	4.32
Soltu.DM.03G015070	xyloglucan endotransglycosylase	-	-1.63	4.61	-	-2.41	3.98
Soltu.DM.05G018970	xyloglucan endotransglycosylase	-	-	1.99	-	-	1.83
Soltu.DM.09G023860	cellulose synthase-like D5	-	-1.00	-0.84	-	-	-1.93
Soltu.DM.05G000310	Exostosin family protein	-	-	-	-	-1.48	-1.42
Soltu.DM.04G036240	glyoxal oxidase-related protein	-	-2.17	-1.49	-	-1.31	-1.24
Soltu.DM.04G036230	glyoxal oxidase-related protein	-	-1.48	-	-	-	-
Soltu.DM.04G036250	glyoxal oxidase-related protein	-	-2.09	-	-	-	-
Soltu.DM.04G034500	OBF binding protein	-	-	-1.29	-	-0.85	-1.67
Soltu.DM.02G022030	Domain of unknown function (DUF23)	-	-	1.07	-	-0.76	0.68
Soltu.DM.08G030280	Pyrophosphorylase	-	-0.99	1.34	-	-	1.47
Soltu.DM.12G008450	UDP-D-glucose/UDP-D-galactose 4-epimerase	-0.89	-1.78	0.58	-0.71	-	2.00
Soltu.DM.01G022940	UDP-xylosyltransferase	-	-	2.26	-	0.47	2.22
Soltu.DM.05G000160	Galactosyl transferase GMA12/MNN10 family protein	-	-2.17	-1.86	-	-	-1.42
Soltu.DM.01G003570	TRICHOME BIREFRINGENCE-LIKE	-	-	1.19	-	-	-
Soltu.DM.07G025630	methylesterase PCR A	-	-	-	-	-	1.29
Soltu.DM.08G027650	root hair specific	-	1.64	2.07	-	0.98	1.80
Soltu.DM.02G009560	conserved hypothetical protein	-	-3.74	-	-	-	-2.76

(A.1) Leaf – Tolerant, GO terms form upregulated DEGs



(A.2) Leaf – Tolerant, GO terms form downregulated DEGs

GO:0006268: DNA unwinding involved in DNA replication
GO:0006271: DNA strand elongation involved in DNA replication
GO:0032508: DNA duplex unwinding
GO:0010143: cutin biosynthetic process
GO:0009790: embryo development
GO:0010214: seed coat development
GO:0019395: fatty acid oxidation
GO:0042754: negative regulation of circadian rhythm
GO:0043433: negative regulation of sequence-specific DNA binding transcription factor activity



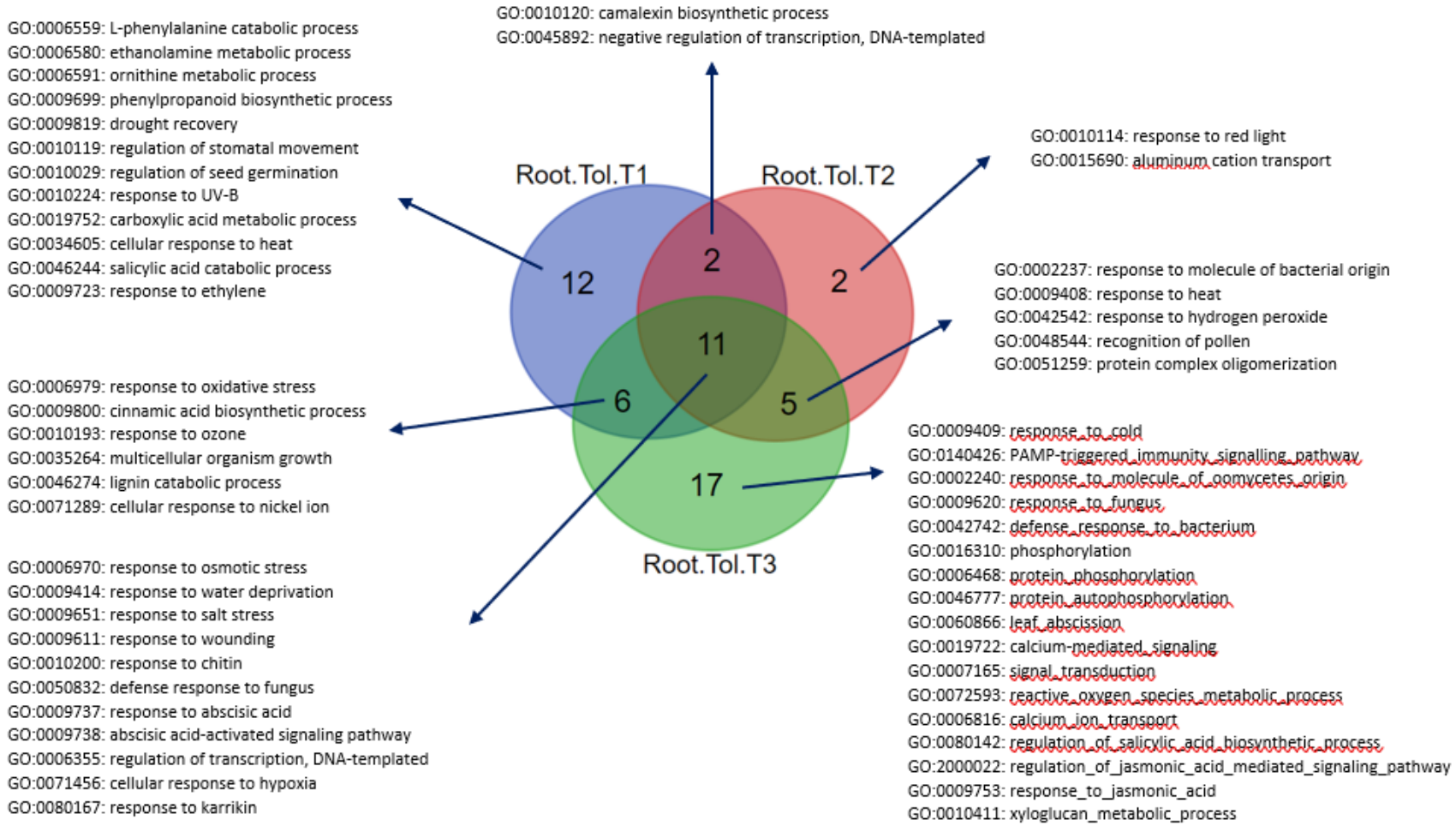
GO:0006949: syncytium formation
GO:1900034: regulation of cellular response to heat

GO:0006260: DNA replication
GO:0009416: response to light stimulus
GO:0009768: photosynthesis, light harvesting in photosystem I
GO:0015995: chlorophyll biosynthetic process

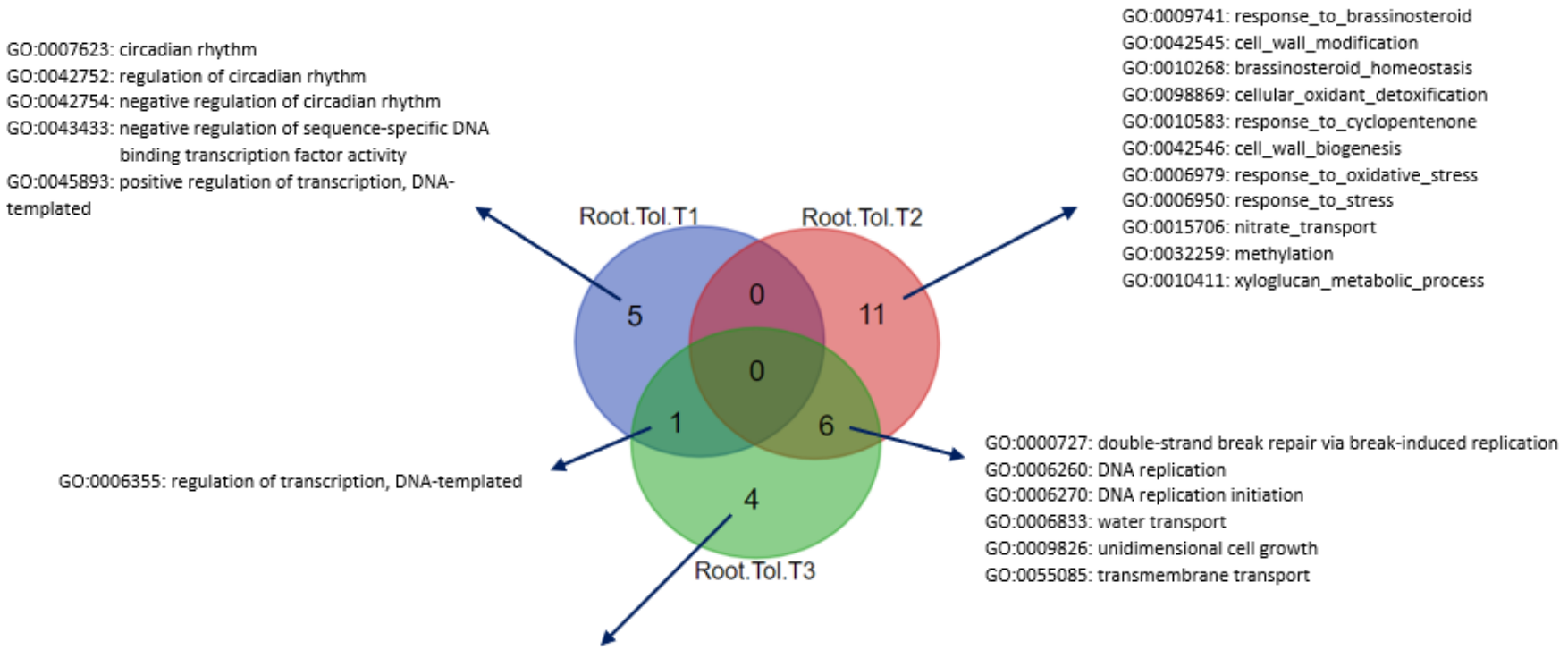
GO:0000727: double-strand break repair via break-induced replication
GO:0006267: pre-replicative complex assembly involved in nuclear cell cycle DNA replication
GO:0006270: DNA replication initiation
GO:1902975: mitotic DNA replication initiation
GO:0008283: cell population proliferation

GO:0000027: ribosomal large subunit assembly
GO:0042273: ribosomal large subunit biogenesis
GO:0000028: ribosomal small subunit assembly
GO:0006364: rRNA processing
GO:0002181: cytoplasmic translation
GO:0006412: translation
GO:0009645: response to low light intensity stimulus
GO:0010114: response to red light
GO:0019419: sulphate reduction

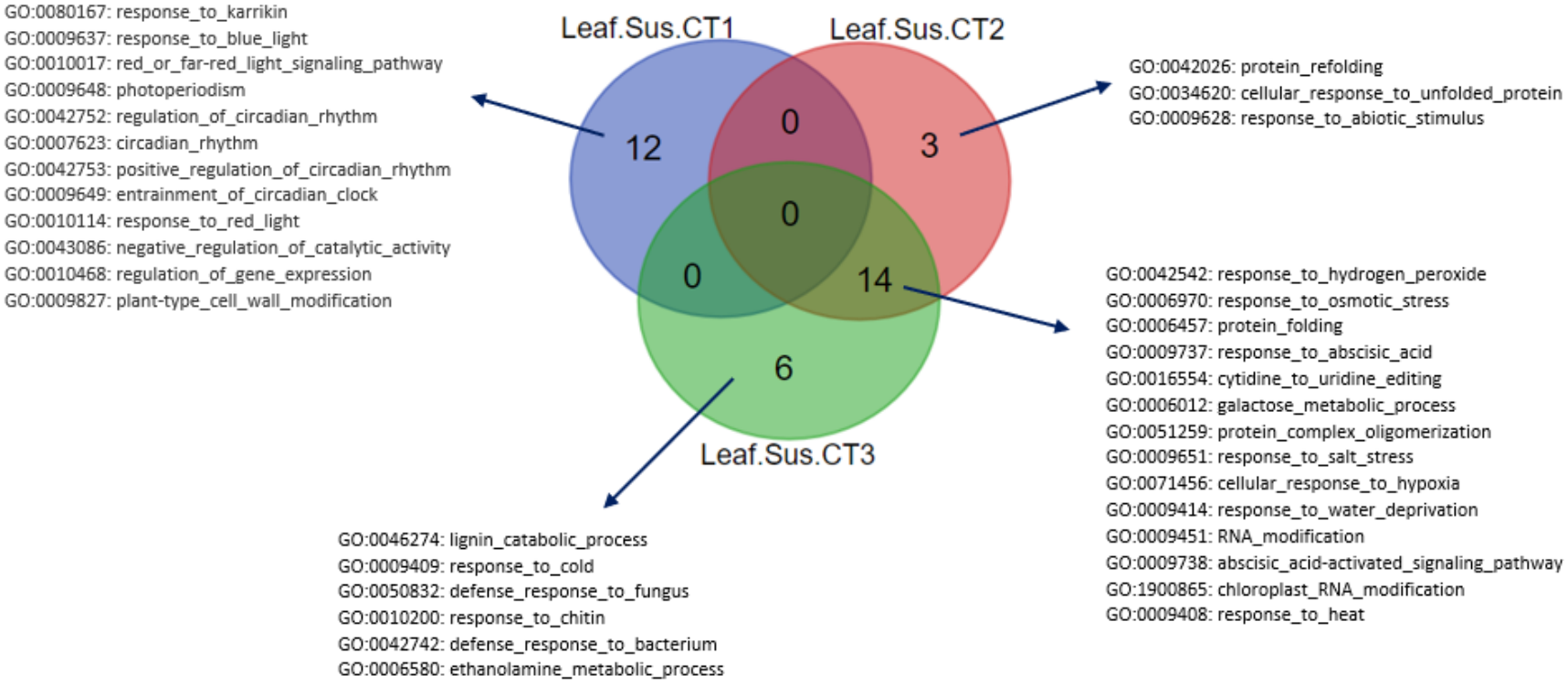
(B.1) Root – Tolerant, GO terms form upregulated DEGs



(B.2) Root – Tolerant, GO terms form downregulated DEGs

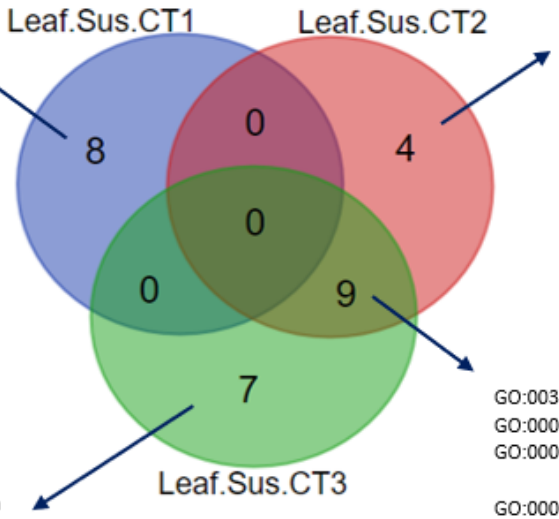


(C.1) Leaf – Susceptible, GO terms form upregulated DEGs



(C.2) Leaf – Susceptible, GO terms form downregulated DEGs

GO:0010243: response_to_organonitrogen_compound
GO:0043254: regulation_of_protein-containing_complex_assembly
GO:0043433: negative_regulation_of_DNA- binding_transcription_factor_activity
GO:0042752: regulation_of_circadian_rhythm
GO:0007623: circadian_rhythm
GO:0042754: negative_regulation_of_circadian_rhythm
GO:0048574: long-day_photoperiodism,_flowering
GO:0010468: regulation_of_gene_expression

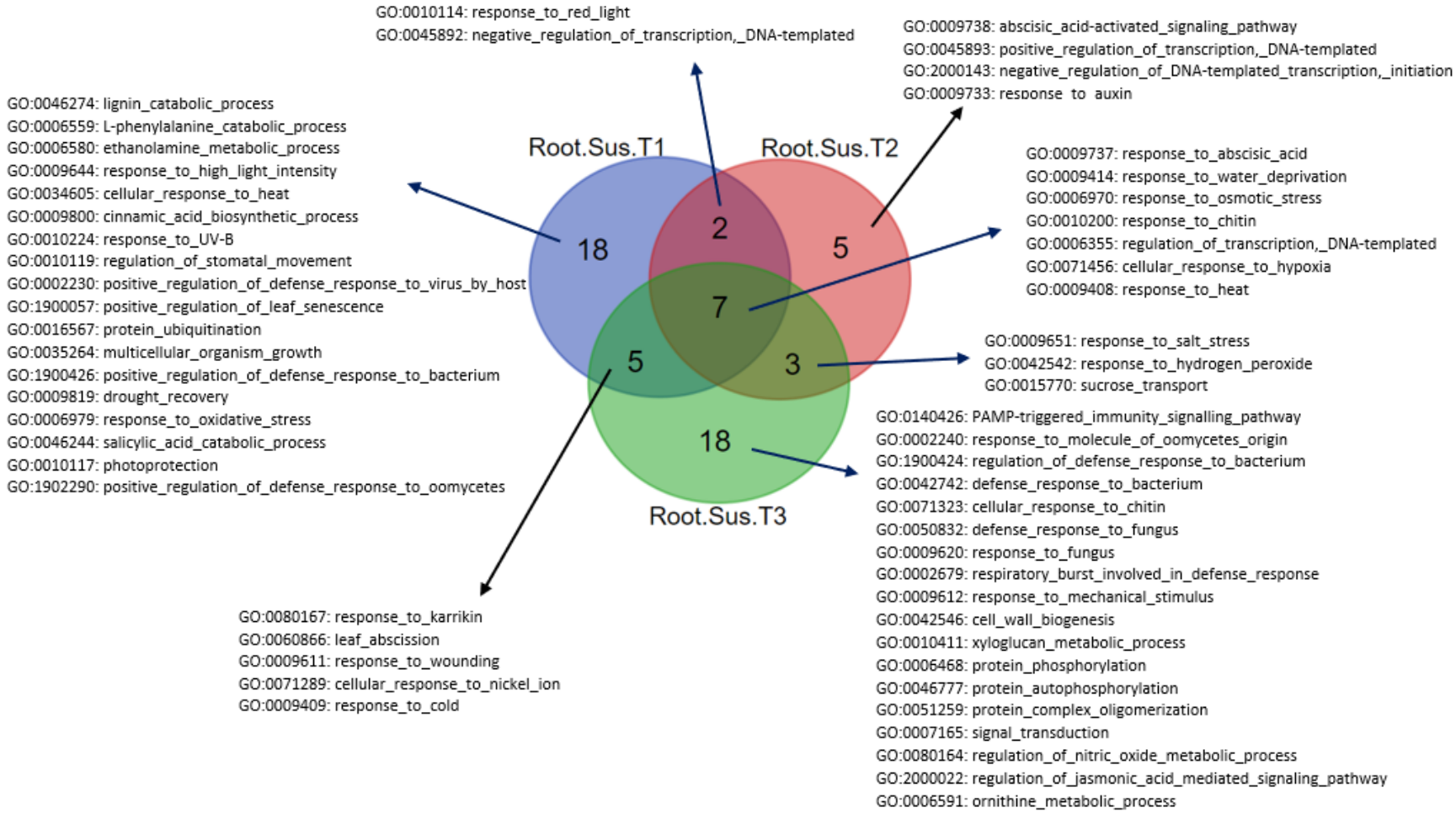


GO:0009785: blue_light_signaling_pathway
GO:0015995: chlorophyll_biosynthetic_process
GO:0009807: lignan_biosynthetic_process
GO:0010143: cutin_biosynthetic_process

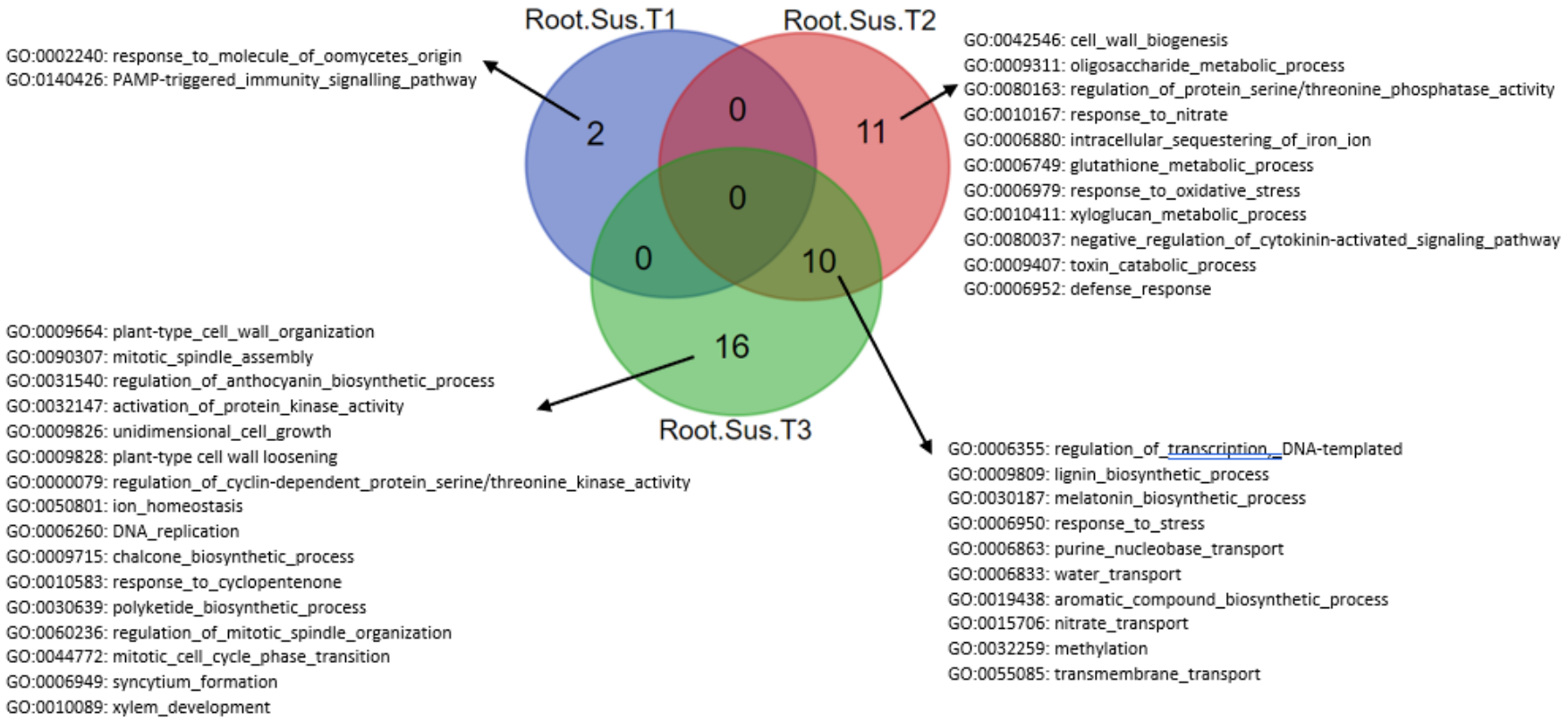
GO:0006270: DNA_replication_initiation
GO:0019419: sulfate_reduction
GO:0055085: transmembrane_transport
GO:0009826: unidimensional_cell_growth
GO:0009828: plant-type_cell_wall_loosening
GO:0006949: syncytium_formation
GO:0006833: water_transport

GO:0030154: cell_differentiation
GO:0006260: DNA_replication
GO:0006267: pre-replicative_complex_assembly
involved_in_nuclear_cell_cycle_DNA_replication
GO:0008283: cell_population_proliferation
GO:0000727: double-strand_break_repair_via_break-induced_replication
GO:1902975: mitotic_DNA_replication_initiation
GO:0009768: photosynthesis, light_harvesting_in_photosystem_I
GO:0009658: chloroplast_organization
GO:0009416: response_to_light_stimulus

(D.1) Root – Susceptible, GO terms form upregulated DEGs

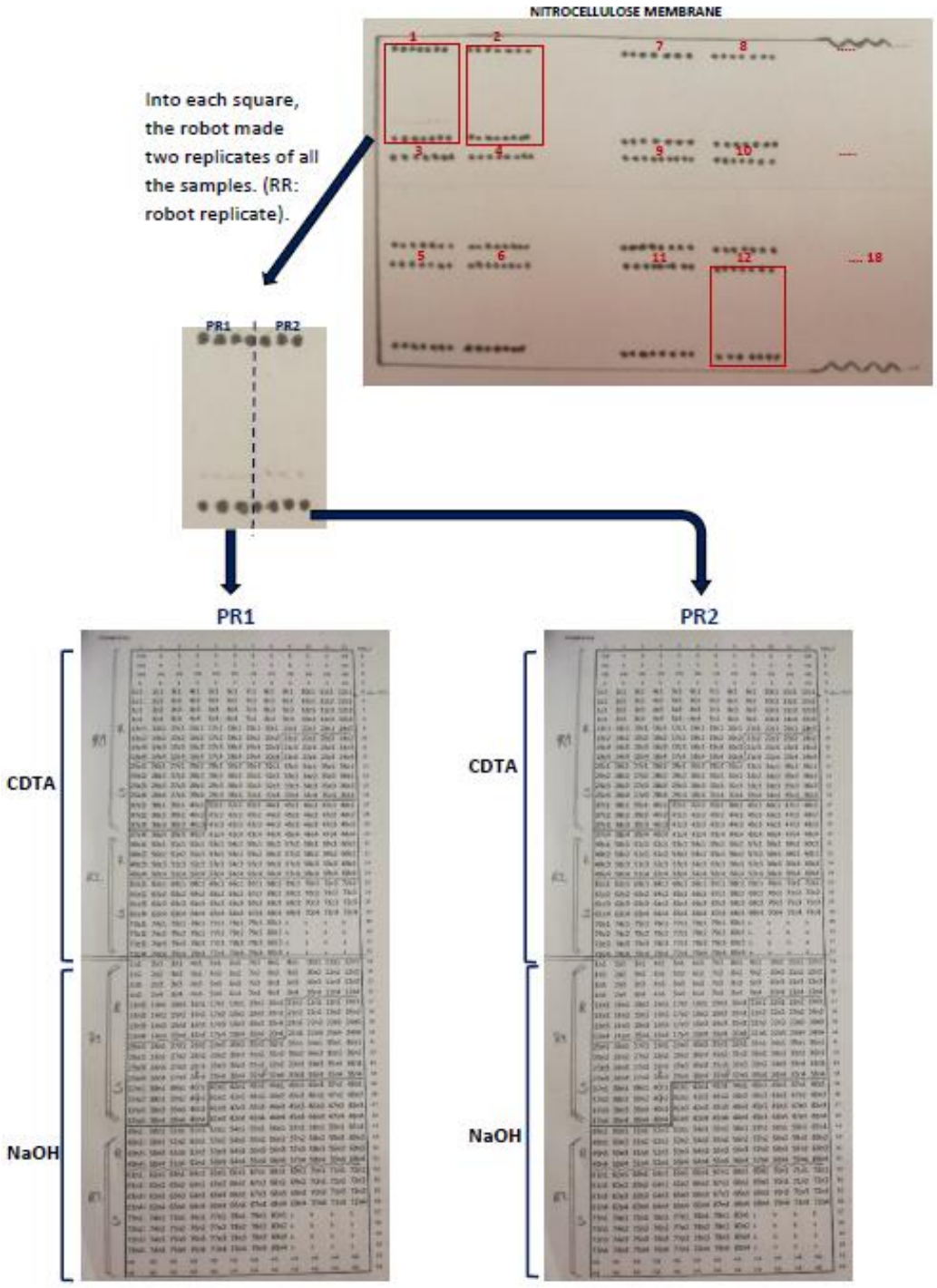


(D.2) Root – Susceptible, GO terms form downregulated DEGs



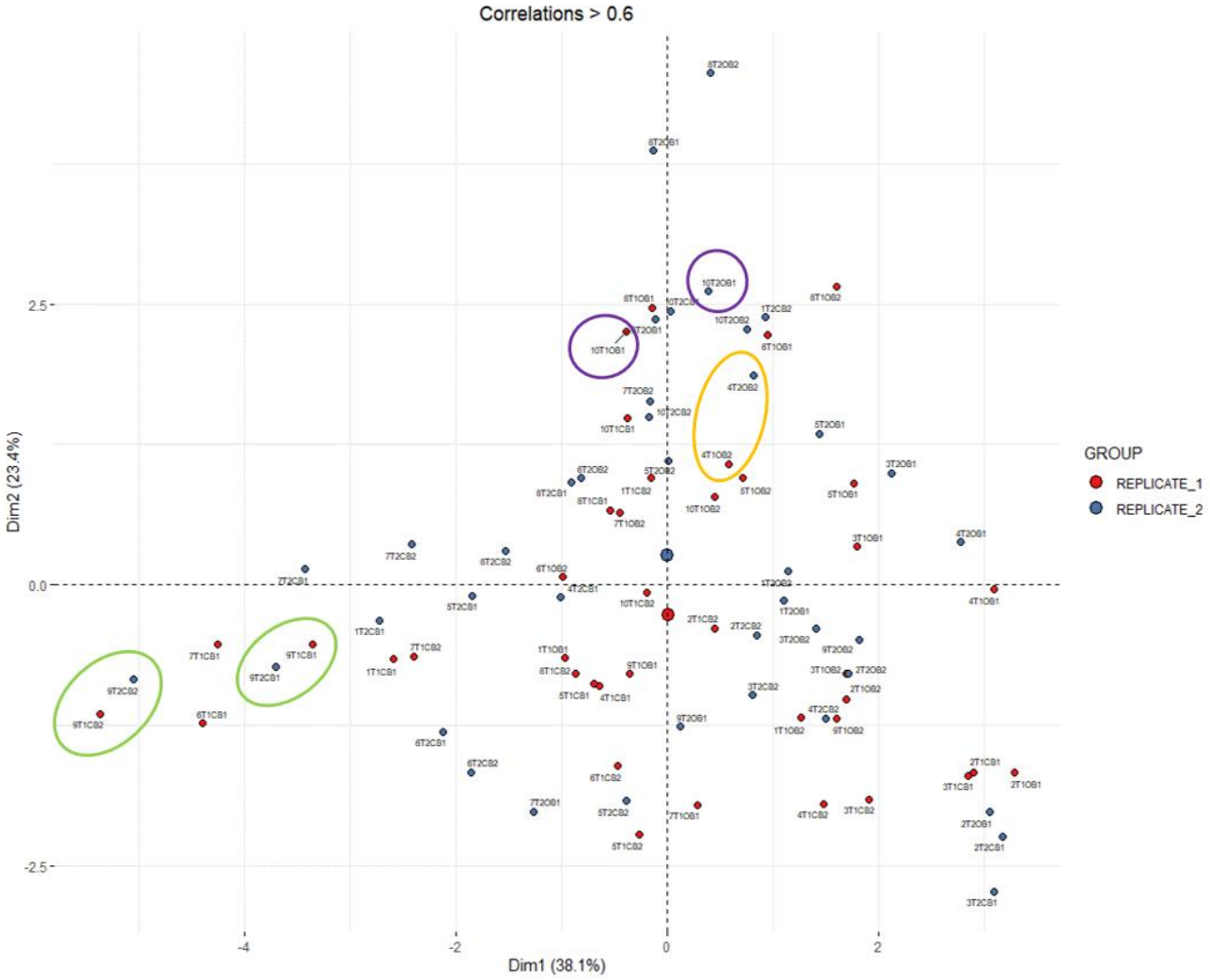
Appendix-Figure 2.6 Intersection of the enriched GO terms across the different time point.

Appendix - Chapter 3

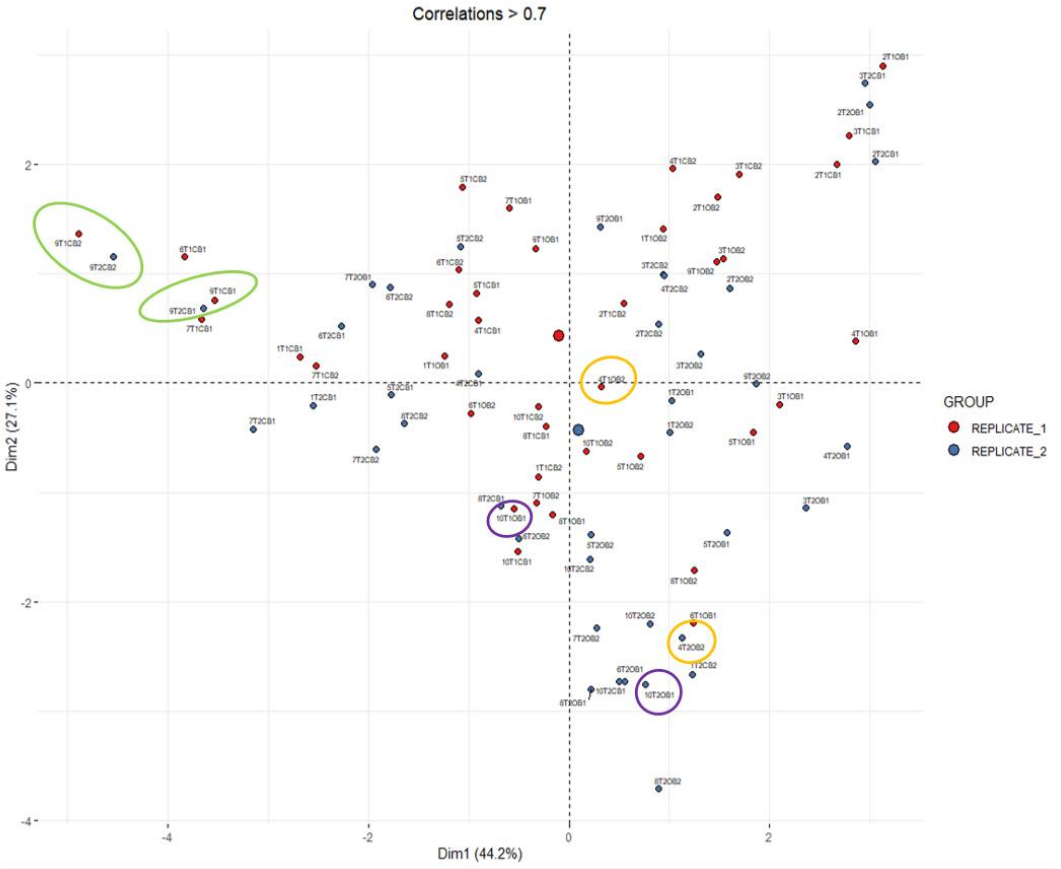


Appendix-Figure 3.1 Printed samples in the nitrocellulose membranes. Each group of samples in the red square (1,2,3, ...12,..18) contains all the 80 potato samples extracted with CDTA and NaOH. The microarray robot additionally created printing replicates (PR1 and PR2).

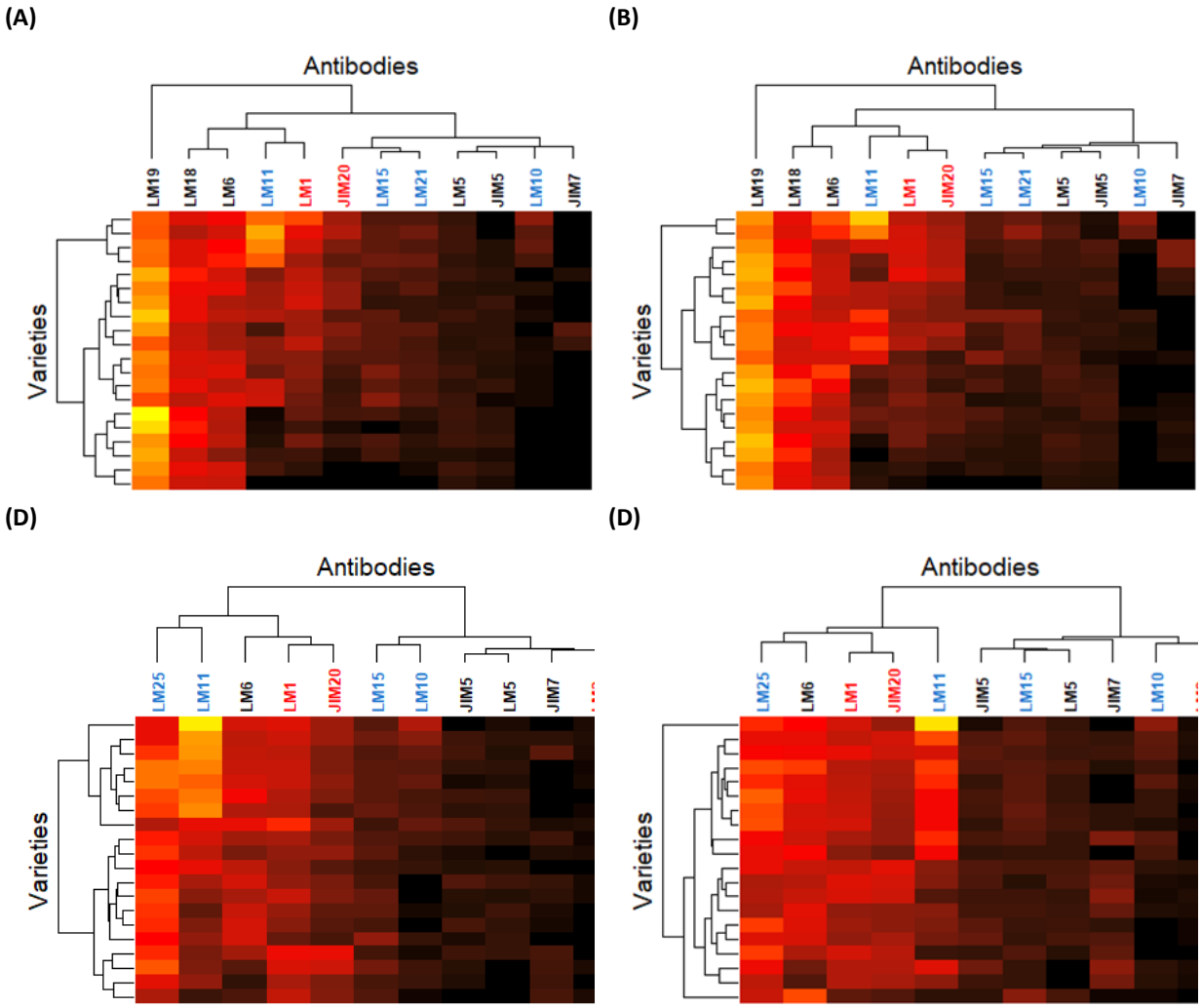
(A)


Correlations > 0.6
Dim2 (23.4%)
Dim1 (38.1%)
2.5
0.0
-2.5
-4
-2
0
2
GROUP
REPLICATE_1
REPLICATE_2

(B)



Appendix-Figure 3.2 PCA plot of the total potato samples using antibodies whose correlation values between technical replicates 1 and 2 are more than 0.6. In (A) the data include antibodies with correlation values more than 0.6. In green, purple, and orange circles, the figure shows that individuals from different replicates are closer as in Figure 4.8-B (correlation more than 0.5). In (B) the data include antibodies with correlation values more than 0.7. Here, some points remain close (green circles), but other points that were close in Figure 4.8-B are now distant (purple and orange circles).



Appendix-Figure 3.3 Cluster of heatmap of the data come from antibodies with corr. > 0.5 in the conventional system and separated by technical replicates. On the right side of each heatmap are the name of each variety in code. In red area the tolerant, in blue are the susceptible, and in green are the intermediate varieties. At the top of each heatmap are the name of the antibodies, in black are the antibodies for pectin, in blue for hemicellulose, and in red for glycoproteins.

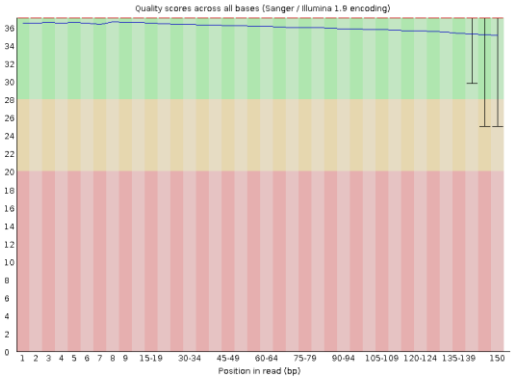
Appendix - Chapter 4

Appendix-Protocol 4. 1 Protocol for RNA extraction with Trizol

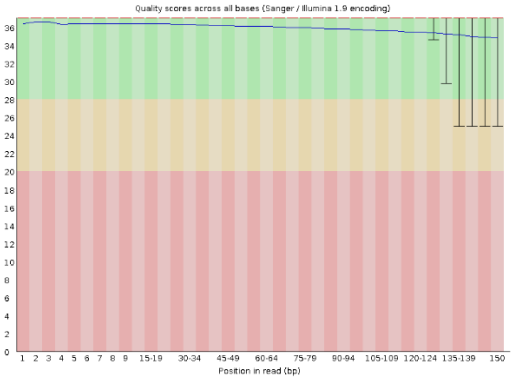
Made under the hood. Use ice to put or move the sample from one location to another.

- Homogenise the sample in liquid N_2.
- Add 1 ml of TRIZOL per 100 mg of leaf.
- Incubate the sample for 5 min at RT.
- Transfer the sample in a new tube.
- Spin at 12000 g for 10 min at 2-8°C
- Transfer the supernatant in a new tube.
- Add 0.2 ml of chloroform per ml of TRIZOL used.
- Shake vigorously the sample by hand for 15 sec.
- Incubate the sample at RT for 2-3 min.
- Spin at 12000 g for 15 min at 2-8°C
- Following centrifugation three phases should be present: a lower dark-red, an intermediate layer of phenol-chloroform and the upper aqueous phase (with RNA, around 60% of the original TRIZOL volume used)
- Transfer the aqueous supernatant in a new tube.
- Precipitate the RNA adding 0.5 ml of isopropanol per ml of TRIZOL used. (In case of difficult samples add 0.25 ml of isopropanol and 0.25 ml of high salt precipitation solution (0.8 M sodium citrate e 1,2 M NaCl) instead)
- Incubate the sample 10 min at RT.
- Spin at 12000 g for 10 min 2-8°C
- Remove the supernatant with a pipet.
- Wash the pellet with 1m of ethanol 75% per ml of TRIZOL used.
- Vortex the sample
- Spin at 7500 g for 5 min at 2-8°C
- Remove the supernatant.
- Dry the pellet with air (do not dry too much!)
- Resuspend the RNA with H20 RNase-free (50uL)

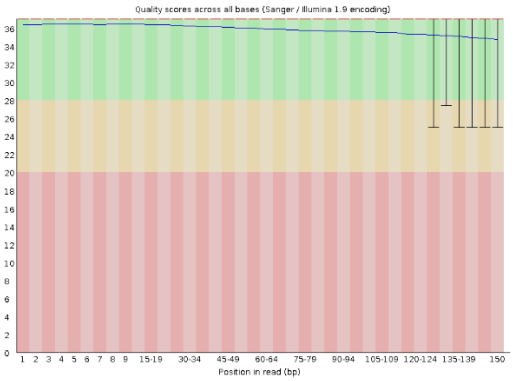
A) R1-1



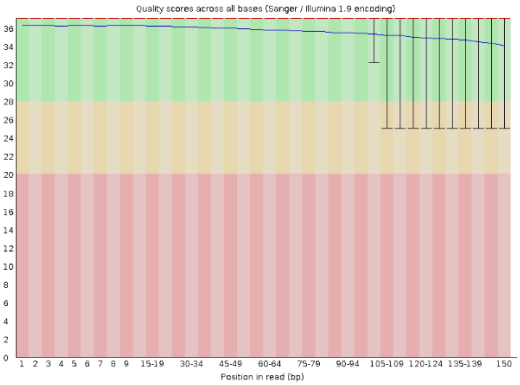
B) R1-2



C) R2-1



D) R2-2



Appendix-Figure 4.1 Sequence quality of the of the pair-ends libraries. Each graphs shows the per base quality of each library. The X- axis indicates ach base position into the reads, from 1 to 150, where 150 was the length of the reads. The y-axis indicates the base quality in one position of all the reads. Red and blue lines represent the median and the mean quality in each position, respectively, and the lower and upper whiskers are representative of the 10 and 90% points, respectively. In red, yellow, and green colours are the range for a poor, intermediate, and good base quality. These 4 graphs are representative of the 36 libraries generated in this study. R1 and R2 are the libraries for Sarpo Mira in the control sample, for the biological replicate 1 and replicate 2 respectively. a) and c) are the quality for read-1, while b) and d) are for read-2 of the pair end libraries of each biological replicate sample.

Appendix-Table 4.1 Number of reads after cleaning and trimming steps.

Sample name	Library name	Replicate	Variety	Treatment	Initial		After trimming				Uniquely mapped reads	
					Number of reads_1	Number of reads_2	Number of reads_1	%	Number of reads_2	%	Number of reads	%
SMira-C1	R1	1	SMira	Control	30,850,929	30,850,929	30,850,929	100%	30,850,929	100%	28499276	92.45%
SMira-C2	R2	2	SMira	Control	30,544,808	30,544,808	30,544,808	100%	30,544,808	100%	27829813	91.24%
SMira-C3	R3	3	SMira	Control	33,032,532	33,032,532	33,032,532	100%	33,032,532	100%	30402156	92.12%
SMira-OI1	R4	1	SMira	Out of Infected area	31,063,113	31,063,113	31,063,113	100%	31,063,113	100%	28295992	91.20%
SMira-OI2	R5	2	SMira	Out of Infected area	29,432,873	29,432,873	29,432,873	100%	29,432,873	100%	26938002	91.62%
SMira-OI3	R6	3	SMira	Out of Infected area	33,154,971	33,154,971	33,154,971	100%	33,154,971	100%	30354678	91.61%
Duke-C1	R7	1	Duke	Control	30,804,484	30,804,484	30,804,484	100%	30,804,484	100%	28144994	91.43%
Duke-C2	R8	2	Duke	Control	30,383,676	30,383,676	30,383,676	100%	30,383,676	100%	27746832	91.40%
Duke-C3	R9	3	Duke	Control	29,919,957	29,919,957	29,919,957	100%	29,919,957	100%	27333958	91.44%
Duke-OI1	R10	1	Duke	Out of Infected area	32,783,687	32,783,687	32,783,687	100%	32,783,687	100%	29968352	91.47%
Duke-OI2	R11	2	Duke	Out of Infected area	32,474,491	32,474,491	32,474,491	100%	32,474,491	100%	29808485	91.86%
Duke-OI3	R12	3	Duke	Out of Infected area	30,972,635	30,972,635	30,972,635	100%	30,972,635	100%	28134925	90.95%
SShona_C1	R13	1	SShona	Control	33,447,662	33,447,662	33,447,662	100%	33,447,662	100%	28327530	84.76%
SShona_C2	R14	2	SShona	Control	29,573,506	29,573,506	29,573,506	100%	29,573,506	100%	26994790	91.36%
SShona_C3	R15	3	SShona	Control	32,177,726	32,177,726	32,177,726	100%	32,177,726	100%	29460697	91.63%
SShona_OI1	R16	1	SShona	Out of Infected area	30,098,860	30,098,860	30,098,860	100%	30,098,860	100%	27577209	91.71%
SShona_OI2	R17	2	SShona	Out of Infected area	32,030,503	32,030,503	32,030,503	100%	32,030,503	100%	29300440	91.56%
SShona_OI3	R18	3	SShona	Out of Infected area	30,373,173	30,373,173	30,373,173	100%	30,373,173	100%	27917693	91.98%
SMira_I48h1	A19	1	SMira	Infected area	34,434,741	34,434,741	34,434,741	100%	34,434,741	100%	31283960	90.93%
SMira_I48h2	A20	2	SMira	Infected area	35,755,231	35,755,231	35,755,231	100%	35,755,231	100%	32052671	89.72%

SMira_I48h3	A21	3	SMira	Infected area	34,351,956	34,351,956	34,351,956	100%	34,351,956	100%	30321359	88.35%
Duke_I48h1	A22	1	Duke	Infected area	31,880,470	31,880,470	31,880,470	100%	31,880,470	100%	28940541	90.86%
Duke_I48h2	A23	2	Duke	Infected area	34,385,963	34,385,963	34,385,963	100%	34,385,963	100%	30762790	89.55%
Duke_I48h3	A24	3	Duke	Infected area	32,871,617	32,871,617	32,871,617	100%	32,871,617	100%	29656888	90.29%
Sshona_I48h1	A25	1	SShona	Infected area	38,071,986	38,071,986	38,071,986	100%	38,071,986	100%	34057598	89.51%
Sshona_I48h2	A26	2	SShona	Infected area	36,034,503	36,034,503	36,034,503	100%	36,034,503	100%	32664601	90.71%
Sshona_I48h3	A27	3	SShona	Infected area	39,082,489	39,082,489	39,082,489	100%	39,082,489	100%	34778796	89.06%
SMira_M48h1	A28	1	SMira	Mock	33,517,825	33,517,825	33,517,825	100%	33,517,825	100%	30749635	91.81%
SMira_M48h2	A29	2	SMira	Mock	32,838,115	32,838,115	32,838,115	100%	32,838,115	100%	29940090	91.25%
SMira_M48h3	A30	3	SMira	Mock	35,652,343	35,652,343	35,652,343	100%	35,652,343	100%	32454449	91.11%
Duke_M48h1	A31	1	Duke	Mock	33,552,619	33,552,619	33,552,619	100%	33,552,619	100%	30553575	91.13%
Duke_M48h2	A32	2	Duke	Mock	39,285,665	39,285,665	39,285,665	100%	39,285,665	100%	35842453	91.29%
Duke_M48h3	A33	3	Duke	Mock	34,189,991	34,189,991	34,189,991	100%	34,189,991	100%	31183824	91.27%
SShona_M48h1	A34	1	SShona	Mock	35,157,843	35,157,843	35,157,843	100%	35,157,843	100%	32306274	91.96%
SShona_M48h2	A35	2	SShona	Mock	31,541,066	31,541,066	31,541,066	100%	31,541,066	100%	28914387	91.77%
SShona_M48h3	A36	3	SShona	Mock	46,744,821	46,744,821	46,744,821	100%	46,744,821	100%	42886827	91.82%

Appendix-Table 4.2 Enriched GO terms after 48 hours post infection with *P. infestans* found inside the infected regions of potato leaves. The DEGs for this enrichment analysis come from the comparison of Inf.48 vs Mock48. Output from gProfiler2.

(A)Sarpo Mira

source	term_id	term_name	term_size	intersection_size_up-regulated	intersection_size_down-regulated	p_value_up-regulated	p_value_down-regulated
GO_BP	GO:0046185	aldehyde_catabolic_process	8	NA	3	NA	9.4e-04
GO_BP	GO:0043447	alkane_biosynthetic_process	14	NA	3	NA	6.0e-03
GO_MF	GO:0071771	aldehyde_decarbonylase_activity	8	NA	3	NA	6.8e-04
GO_MF	GO:1990465	aldehyde_oxygenase_(deformylating)_activity	11	NA	3	NA	2.0e-03
GO_MF	GO:0009924	octadecanal_decarbonylase_activity	11	NA	3	NA	2.0e-03
GO_MF	GO:0000254	C-4_methylsterol_oxidase_activity	15	NA	3	NA	5.4e-03
GO_BP	GO:0009416	response_to_light_stimulus	310	25	NA	1.4e-09	NA
GO_BP	GO:0009768	photosynthesis,_light_harvesting_in_photosystem_I	36	10	NA	2.4e-08	NA
GO_BP	GO:0009644	response_to_high_light_intensity	75	9	NA	5.1e-04	NA
GO_BP	GO:0048544	recognition_of_pollen	82	9	NA	1.1e-03	NA
GO_BP	GO:0009699	phenylpropanoid_biosynthetic_process	66	8	NA	1.9e-03	NA
GO_BP	GO:0019253	reductive_pentose-phosphate_cycle	20	5	NA	3.2e-03	NA
GO_BP	GO:0032922	circadian_regulation_of_gene_expression	23	5	NA	6.7e-03	NA
GO_BP	GO:0007623	circadian_rhythm	144	10	NA	1.8e-02	NA
GO_BP	GO:0009809	lignin_biosynthetic_process	181	11	NA	2.6e-02	NA
GO_CC	GO:0009535	chloroplast_thylakoid_membrane	448	36	NA	7.1e-15	NA
GO_CC	GO:0009579	thylakoid	288	24	NA	5.1e-10	NA
GO_CC	GO:0009534	chloroplast_thylakoid	322	24	NA	5.3e-09	NA
GO_CC	GO:0010287	plastoglobule	130	16	NA	5.7e-09	NA
GO_CC	GO:0009941	chloroplast_envelope	741	34	NA	2.8e-07	NA
GO_CC	GO:0048046	apoplast	434	23	NA	8.5e-06	NA
GO_CC	GO:0009507	chloroplast	6294	134	NA	3.2e-05	NA
GO_CC	GO:0009522	photosystem_I	4	3	NA	9.7e-04	NA
GO_CC	GO:0005576	extracellular_region	3372	75	NA	6.6e-03	NA
GO_CC	GO:0042651	thylakoid_membrane	32	5	NA	7.3e-03	NA
GO_CC	GO:0009517	PSII_associated_light-harvesting_complex_II	10	3	NA	2.7e-02	NA
GO_CC	GO:0009570	chloroplast_stroma	832	25	NA	4.0e-02	NA
GO_MF	GO:0047100	glyceraldehyde-3-phosphate_dehydrogenase_(NADP+)_(phosphorylating)_activity	5	4	NA	5.5e-05	NA
GO_MF	GO:0020037	heme_binding	814	32	NA	8.5e-05	NA
GO_MF	GO:0005506	iron_ion_binding	676	27	NA	5.5e-04	NA
GO_MF	GO:0009055	electron_transfer_activity	184	13	NA	7.7e-04	NA
GO_MF	GO:0016168	chlorophyll_binding	47	7	NA	1.2e-03	NA
GO_MF	GO:0004365	glyceraldehyde-3-phosphate_dehydrogenase_(NAD+)_(phosphorylating)_activity	11	4	NA	3.4e-03	NA
GO_MF	GO:0010277	chlorophyllide_a_oxygenase_[overall]_activity	7	3	NA	2.6e-02	NA
GO_MF	GO:0005516	calmodulin_binding	307	14	NA	4.3e-02	NA

(B)Sarpo Shona

source	term_id	term_name	term_size	intersection_size_up-regulated	intersection_size_down-regulated	p_value_up-regulated	p_value_down-regulated
GO_CC	GO:0005576	extracellular_region	3372	NA	58	NA	2.0e-03
GO_MF	GO:0008810	cellulase_activity	31	NA	4	NA	4.3e-02
GO_BP	GO:0009699	phenylpropanoid_biosynthetic_process	66	12	NA	2.2e-07	NA
GO_BP	GO:0009809	lignin_biosynthetic_process	181	16	NA	1.5e-05	NA
GO_BP	GO:0080163	regulation_of_protein_serine/threonine_phosphatase_activity	21	6	NA	3.0e-04	NA
GO_BP	GO:0009805	coumarin_biosynthetic_process	35	7	NA	4.8e-04	NA
GO_BP	GO:0071456	cellular_response_to_hypoxia	431	21	NA	2.8e-03	NA
GO_BP	GO:0006355	regulation_of_transcription,_DNA-templated	1717	51	NA	4.9e-03	NA
GO_BP	GO:0009873	ethylene-activated_signaling_pathway	75	8	NA	1.1e-02	NA
GO_BP	GO:0006952	defense_response	462	20	NA	2.5e-02	NA
GO_BP	GO:0080051	cutin_transport	15	4	NA	3.3e-02	NA
GO_MF	GO:0046409	p-coumarate_3-hydroxylase_activity	18	7	NA	1.7e-06	NA
GO_MF	GO:0003700	DNA-binding_transcription_factor_activity	1932	62	NA	1.4e-05	NA
GO_MF	GO:0020037	heme_binding	814	35	NA	2.3e-05	NA
GO_MF	GO:0004497	monooxygenase_activity	184	15	NA	6.6e-05	NA
GO_MF	GO:0005506	iron_ion_binding	676	30	NA	1.0e-04	NA
GO_MF	GO:0016709	oxidoreductase_activity,_acting_on_paired_donors,_with_incorporation_or_reduction_of_molecular_oxygen,_NAD(P)H_as_one_donor,_and_incorporation_of_one_atom_of_oxyge	227	16	NA	1.9e-04	NA
GO_MF	GO:0010427	abscisic_acid_binding	25	6	NA	5.6e-04	NA
GO_MF	GO:0004864	protein_phosphatase_inhibitor_activity	28	6	NA	1.1e-03	NA
GO_MF	GO:0038023	signaling_receptor_activity	61	7	NA	1.3e-02	NA
GO_MF	GO:0015245	fatty_acid_transmembrane_transporter_activity	14	4	NA	1.5e-02	NA
GO_MF	GO:0000976	transcription_cis-regulatory_region_binding	588	23	NA	1.8e-02	NA

(C)Duke of York

source	term_id	term_name	term_size	intersection_size_up-regulated	intersection_size_down-regulated	p_value_up-regulated	p_value_down-regulated
GO_BP	GO:0009737	response_to_abscisic_acid	686	NA	39	NA	7.2e-06
GO_BP	GO:0010200	response_to_chitin	226	NA	20	NA	3.0e-05
GO_BP	GO:0031347	regulation_of_defense_response	73	NA	11	NA	1.6e-04
GO_BP	GO:0009738	abscisic_acid-activated_signaling_pathway	168	NA	16	NA	2.4e-04
GO_BP	GO:0071230	cellular_response_to_amino_acid_stimulus	35	NA	8	NA	2.4e-04
GO_BP	GO:0071456	cellular_response_to_hypoxia	431	NA	26	NA	6.7e-04
GO_BP	GO:2000022	regulation_of_jasmonic_acid_mediated_signaling_pathway	53	NA	9	NA	6.7e-04
GO_BP	GO:0009611	response_to_wounding	474	NA	27	NA	1.2e-03
GO_BP	GO:0006874	cellular_calcium_ion_homeostasis	51	NA	8	NA	4.9e-03
GO_BP	GO:0046274	lignin_catabolic_process	15	NA	5	NA	5.0e-03
GO_BP	GO:0006816	calcium_ion_transport	52	NA	8	NA	5.7e-03
GO_BP	GO:0019722	calcium-mediated_signaling	70	NA	9	NA	7.3e-03
GO_BP	GO:0048480	stigma_development	9	NA	4	NA	1.1e-02
GO_BP	GO:0009800	cinnamic_acid_biosynthetic_process	20	NA	5	NA	2.4e-02
GO_BP	GO:0006591	ornithine_metabolic_process	11	NA	4	NA	2.9e-02
GO_BP	GO:0009414	response_to_water_deprivation	534	NA	26	NA	2.9e-02
GO_CC	GO:0048046	apoplast	434	NA	24	NA	9.1e-04
GO_MF	GO:0038023	signaling_receptor_activity	61	NA	11	NA	1.4e-05
GO_MF	GO:0005217	intracellular_ligand-gated_ion_channel_activity	31	NA	8	NA	5.3e-05
GO_MF	GO:0008066	glutamate_receptor_activity	31	NA	8	NA	5.3e-05
GO_MF	GO:0015276	ligand-gated_ion_channel_activity	31	NA	8	NA	5.3e-05
GO_MF	GO:0005262	calcium_channel_activity	46	NA	8	NA	1.3e-03
GO_MF	GO:0052694	jasmonoyl-isoleucine-12-hydroxylase_activity	8	NA	4	NA	3.7e-03
GO_MF	GO:0004332	fructose-bisphosphate_aldolase_activity	8	NA	4	NA	3.7e-03
GO_MF	GO:0016301	kinase_activity	745	38	34	5.0e-07	5.5e-03
GO_MF	GO:0003700	DNA-binding_transcription_factor_activity	1932	NA	67	NA	7.5e-03
GO_MF	GO:0016841	ammonia-lyase_activity	18	NA	5	NA	8.1e-03
GO_MF	GO:0045548	phenylalanine_ammonia-lyase_activity	18	NA	5	NA	8.1e-03
GO_MF	GO:0005516	calmodulin_binding	307	NA	19	NA	8.1e-03
GO_MF	GO:0008080	N-acetyltransferase_activity	51	NA	7	NA	2.6e-02
GO_BP	GO:0006468	protein_phosphorylation	1459	58	NA	2.0e-07	NA
GO_BP	GO:0009755	hormone-mediated_signaling_pathway	131	13	NA	1.3e-04	NA
GO_BP	GO:0010411	xyloglucan_metabolic_process	53	8	NA	1.2e-03	NA
GO_BP	GO:0071918	urea_transmembrane_transport	3	3	NA	2.2e-03	NA
GO_BP	GO:0009809	lignin_biosynthetic_process	181	13	NA	5.1e-03	NA
GO_BP	GO:0042546	cell_wall_biogenesis	65	8	NA	5.5e-03	NA
GO_BP	GO:0090357	regulation_of_tryptophan_metabolic_process	4	3	NA	8.7e-03	NA
GO_BP	GO:0080163	regulation_of_protein_serine/threonine_phosphatase_activity	21	5	NA	9.6e-03	NA
GO_BP	GO:0006995	cellular_response_to_nitrogen_starvation	26	5	NA	2.9e-02	NA
GO_BP	GO:0002215	defense_response_to_nematode	28	5	NA	4.2e-02	NA
GO_BP	GO:0000160	phosphorelay_signal_transduction_system	28	5	NA	4.2e-02	NA
GO_BP	GO:0010338	leaf_formation	6	3	NA	4.2e-02	NA
GO_BP	GO:0062211	root_regeneration	6	3	NA	4.2e-02	NA
GO_BP	GO:0010183	pollen_tube_guidance	87	8	NA	4.7e-02	NA
GO_CC	GO:0005886	plasma_membrane	5162	137	NA	5.1e-07	NA
GO_CC	GO:0005576	extracellular_region	3372	100	NA	5.3e-07	NA
GO_CC	GO:0009505	plant-type_cell_wall	393	18	NA	1.0e-02	NA
GO_CC	GO:0005615	extracellular_space	137	9	NA	4.0e-02	NA
GO_MF	GO:0106310	protein_serine_kinase_activity	1028	43	NA	1.4e-05	NA
GO_MF	GO:0106311	protein_threonine_kinase_activity	1028	43	NA	1.4e-05	NA
GO_MF	GO:0016762	xyloglucan:xyloglucosyl_transferase_activity	41	8	NA	1.0e-04	NA
GO_MF	GO:0010427	abscisic_acid_binding	25	6	NA	8.5e-04	NA
GO_MF	GO:0015370	solute:sodium_symporter_activity	3	3	NA	1.3e-03	NA
GO_MF	GO:0004864	protein_phosphatase_inhibitor_activity	28	6	NA	1.7e-03	NA

Appendix-Table 4.3 Enriched GO terms after 48 hours post infection with *P. infestans* found outside the infected regions of potato leaves. The DEGs for this enrichment analysis come from the comparison of OutInf.48 vs Mock48. Output from gProfiler2.

(A)Sarpo Mira

source	term_id	term_name	term_size	intersection_size_up-regulated	intersection_size_down-regulated	p_value_up-regulated	p_value_down-regulated
GO_BP	GO:0018171	peptidyl-cysteine_oxidation	4	NA	2	NA	4.7e-03
GO_BP	GO:0070483	detection_of_hypoxia	7	NA	2	NA	1.7e-02
GO_MF	GO:0017172	cysteine_dioxygenase_activity	7	NA	2	NA	1.1e-02
GO_MF	GO:0016799	hydrolase_activity,_hydrolyzing_N-glycosyl_compounds	13	NA	2	NA	4.0e-02
GO_BP	GO:0009768	photosynthesis,_light_harvesting_in_photosystem_I	36	16	NA	3.0e-18	NA
GO_BP	GO:0009416	response_to_light_stimulus	310	28	NA	1.9e-12	NA
GO_BP	GO:0015979	photosynthesis	85	12	NA	9.2e-07	NA
GO_BP	GO:0009644	response_to_high_light_intensity	75	11	NA	2.9e-06	NA
GO_BP	GO:0007623	circadian_rhythm	144	14	NA	6.0e-06	NA
GO_BP	GO:0009645	response_to_low_light_intensity_stimulus	11	5	NA	9.2e-05	NA
GO_BP	GO:0019253	reductive_pentose-phosphate_cycle	20	6	NA	9.6e-05	NA
GO_BP	GO:0006833	water_transport	46	7	NA	1.3e-03	NA
GO_BP	GO:0080167	response_to_karrikin	229	14	NA	1.8e-03	NA
GO_BP	GO:0009773	photosynthetic_electron_transport_in_photosystem_I	20	5	NA	2.8e-03	NA
GO_BP	GO:0010207	photosystem_II_assembly	34	6	NA	2.8e-03	NA
GO_BP	GO:0071483	cellular_response_to_blue_light	4	3	NA	4.5e-03	NA
GO_BP	GO:0009767	photosynthetic_electron_transport_chain	22	5	NA	4.6e-03	NA
GO_BP	GO:0032922	circadian_regulation_of_gene_expression	23	5	NA	5.9e-03	NA
GO_BP	GO:0048544	recognition_of_pollen	82	8	NA	7.8e-03	NA
GO_BP	GO:0010196	nonphotochemical_quenching	16	4	NA	2.5e-02	NA
GO_CC	GO:0009535	chloroplast_thylakoid_membrane	448	50	NA	1.4e-28	NA
GO_CC	GO:0009579	thylakoid	288	37	NA	5.1e-23	NA
GO_CC	GO:0009534	chloroplast_thylakoid	322	36	NA	2.8e-20	NA
GO_CC	GO:0009507	chloroplast	6294	167	NA	2.6e-18	NA
GO_CC	GO:0010287	plastoglobule	130	22	NA	5.4e-16	NA
GO_CC	GO:0009941	chloroplast_envelope	741	43	NA	1.5e-13	NA
GO_CC	GO:0009570	chloroplast_stroma	832	35	NA	4.5e-07	NA
GO_CC	GO:0042651	thylakoid_membrane	32	6	NA	3.6e-04	NA
GO_CC	GO:0009517	PSII_associated_light-harvesting_complex_II	10	4	NA	5.7e-04	NA
GO_CC	GO:0009522	photosystem_I	4	3	NA	8.5e-04	NA
GO_CC	GO:0048046	apoplast	434	18	NA	3.0e-03	NA
GO_CC	GO:0010319	stromule	55	6	NA	8.8e-03	NA
GO_CC	GO:0009512	cytochrome_b6f_complex	8	3	NA	1.1e-02	NA
GO_CC	GO:0031969	chloroplast_membrane	21	4	NA	1.5e-02	NA
GO_CC	GO:0009543	chloroplast_thylakoid_lumen	45	5	NA	3.1e-02	NA
GO_CC	GO:0009523	photosystem_II	11	3	NA	3.3e-02	NA
GO_CC	GO:0009783	photosystem_II_antenna_complex	3	2	NA	4.7e-02	NA
GO_MF	GO:0016168	chlorophyll_binding	47	12	NA	3.8e-10	NA
GO_MF	GO:0047100	glyceraldehyde-3-phosphate_dehydrogenase_(NADP+)_(phosphorylating)_activity	5	4	NA	4.8e-05	NA
GO_MF	GO:0015250	water_channel_activity	52	7	NA	1.9e-03	NA
GO_MF	GO:0004365	glyceraldehyde-3-phosphate_dehydrogenase_(NAD+)_(phosphorylating)_activity	11	4	NA	3.0e-03	NA
GO_MF	GO:0003729	mRNA_binding	1308	38	NA	3.9e-03	NA
GO_MF	GO:0010277	chlorophyllide_a_oxygenase_[overall]_activity	7	3	NA	2.4e-02	NA

(B)Sarpo Shona

source	term_id	term_name	term_size	intersection_size_up-regulated	intersection_size_down-regulated	p_value_up-regulated	p_value_down-regulated
GO_BP	GO:0009451	RNA_modification	130	NA	5	NA	3.2e-03
GO_BP	GO:0010200	response_to_chitin	226	17	NA	3.6e-07	NA
GO_BP	GO:0006355	regulation_of_transcription,_DNA-templated	1717	49	NA	6.5e-07	NA
GO_BP	GO:0071456	cellular_response_to_hypoxia	431	20	NA	4.5e-05	NA
GO_BP	GO:0009644	response_to_high_light_intensity	75	8	NA	8.0e-04	NA
GO_BP	GO:0080167	response_to_karrikin	229	13	NA	8.7e-04	NA
GO_BP	GO:0007623	circadian_rhythm	144	10	NA	2.2e-03	NA
GO_BP	GO:0010117	photoprotection	24	5	NA	2.4e-03	NA
GO_BP	GO:0006833	water_transport	46	6	NA	4.8e-03	NA
GO_BP	GO:0080051	cutin_transport	15	4	NA	7.5e-03	NA
GO_BP	GO:0015908	fatty_acid_transport	17	4	NA	1.3e-02	NA
GO_BP	GO:0010222	stem_vascular_tissue_pattern_formation	20	4	NA	2.5e-02	NA
GO_BP	GO:0009611	response_to_wounding	474	16	NA	4.2e-02	NA
GO_BP	GO:0032922	circadian_regulation_of_gene_expression	23	4	NA	4.5e-02	NA
GO_BP	GO:0042754	negative_regulation_of_circadian_rhythm	2	2	NA	4.8e-02	NA
GO_CC	GO:0042651	thylakoid_membrane	32	4	NA	2.9e-02	NA
GO_CC	GO:0009897	external_side_of_plasma_membrane	35	4	NA	4.0e-02	NA
GO_MF	GO:0003700	DNA-binding_transcription_factor_activity	1932	61	NA	2.1e-11	NA
GO_MF	GO:0000976	transcription_cis-regulatory_region_binding	588	22	NA	1.9e-04	NA
GO_MF	GO:0015250	water_channel_activity	52	7	NA	3.6e-04	NA
GO_MF	GO:0015245	fatty_acid_transmembrane_transporter_activity	14	4	NA	2.8e-03	NA
GO_MF	GO:0010277	chlorophyllide_a_oxygenase_[overall]_activity	7	3	NA	9.4e-03	NA

(B)Duke of York

source	term_id	term_name	term_size	intersection_size_up-regulated	intersection_size_down-regulated	p_value_up-regulated	p_value_down-regulated
GO_BP	GO:0071230	cellular_response_to_amino_acid_stimulus	35	NA	8	NA	9.3e-08
GO_BP	GO:0019722	calcium-mediated_signaling	70	NA	9	NA	1.6e-06
GO_BP	GO:0006874	cellular_calcium_ion_homeostasis	51	NA	8	NA	2.3e-06
GO_BP	GO:0006816	calcium_ion_transport	52	NA	8	NA	2.6e-06
GO_BP	GO:0031347	regulation_of_defense_response	73	NA	7	NA	6.3e-04
GO_BP	GO:0010200	response_to_chitin	226	9	11	2.8e-02	7.6e-04
GO_BP	GO:0009611	response_to_wounding	474	NA	15	NA	2.5e-03
GO_BP	GO:0071456	cellular_response_to_hypoxia	431	NA	14	NA	3.7e-03
GO_BP	GO:0048480	stigma_development	9	NA	3	NA	1.2e-02
GO_BP	GO:0006468	protein_phosphorylation	1459	NA	27	NA	1.7e-02
GO_BP	GO:0006591	ornithine_metabolic_process	11	NA	3	NA	2.4e-02
GO_MF	GO:0005217	intracellular_ligand-gated_ion_channel_activity	31	NA	8	NA	4.2e-08
GO_MF	GO:0015276	ligand-gated_ion_channel_activity	31	NA	8	NA	4.2e-08
GO_MF	GO:0008066	glutamate_receptor_activity	31	NA	8	NA	4.2e-08
GO_MF	GO:0038023	signaling_receptor_activity	61	NA	9	NA	6.4e-07
GO_MF	GO:0005262	calcium_channel_activity	46	NA	8	NA	1.3e-06
GO_MF	GO:0004620	phospholipase_activity	57	NA	6	NA	2.1e-03
GO_MF	GO:0016984	ribulose-bisphosphate_carboxylase_activity	6	NA	3	NA	2.6e-03
GO_MF	GO:0052694	jasmonoyl-isoleucine-12-hydroxylase_activity	8	NA	3	NA	7.1e-03
GO_MF	GO:0008080	N-acetyltransferase_activity	51	NA	5	NA	1.7e-02
GO_MF	GO:0005516	calmodulin_binding	307	NA	11	NA	1.9e-02
GO_MF	GO:0016301	kinase_activity	745	18	18	8.4e-03	2.4e-02
GO_MF	GO:0033741	adenylyl-sulfate_reductase_(glutathione)_activity	3	NA	2	NA	4.4e-02
GO_MF	GO:0009973	adenylyl-sulfate_reductase_activity	3	NA	2	NA	4.4e-02
GO_BP	GO:0010411	xyloglucan_metabolic_process	53	7	NA	6.5e-05	NA
GO_BP	GO:0071918	urea_transmembrane_transport	3	3	NA	1.5e-04	NA
GO_BP	GO:0042546	cell_wall_biogenesis	65	7	NA	2.7e-04	NA
GO_BP	GO:0090357	regulation_of_tryptophan_metabolic_process	4	3	NA	6.1e-04	NA
GO_BP	GO:0062211	root_regeneration	6	3	NA	3.0e-03	NA
GO_BP	GO:0006995	cellular_response_to_nitrogen_starvation	26	4	NA	1.6e-02	NA
GO_CC	GO:0005576	extracellular_region	3372	47	NA	2.0e-02	NA
GO_MF	GO:0016762	xyloglucan:xyloglucosyl_transferase_activity	41	7	NA	7.8e-06	NA
GO_MF	GO:0015370	solute:sodium_symporter_activity	3	3	NA	1.1e-04	NA
GO_MF	GO:0015204	urea_transmembrane_transporter_activity	11	4	NA	2.8e-04	NA
GO_MF	GO:0004553	hydrolase_activity,_hydrolyzing_O-glycosyl_compounds	88	7	NA	1.6e-03	NA

Appendix - Chapter 5

Appendix-Table 5.1 Transcription factors differentially expressed in only the tolerant varieties in response to a specific stress (abiotic or biotic).

Potato gene ID v6	Gene Function	Drought						P. infestans			*A. thaliana* geneID
		Tolerant			Susceptible			SM	SS	D	
		T1	T2	T3	T1	T2	T3	Inf 48 vs Mock 48			
Soltu.DM.06G010320	DRE-binding protein 2A	2.07	NA	NA	NA	NA	NA	NA	NA	NA	AT2G40340
Soltu.DM.05G022450	DRE-binding protein 2A	2.02	NA	NA	NA	NA	NA	NA	NA	NA	AT5G05410
Soltu.DM.12G005300	bifunctional nuclease in basal defense response	2.28	2.50	NA	NA	NA	NA	NA	NA	NA	AT1G75380
Soltu.DM.03G037200	GRAS family transcription factor	1.85	1.94	NA	NA	NA	NA	NA	NA	NA	AT3G13840
Soltu.DM.10G024900	growth-regulating factor	-1.99	-1.63	-1.97	NA	NA	NA	NA	NA	NA	AT3G52910
Soltu.DM.08G028420	Integrase-type DNA-binding superfamily protein	NA	1.29	1.36	NA	NA	NA	NA	NA	NA	AT1G68550
Soltu.DM.03G005790	DREB and EAR motif protein	NA	1.16	1.38	NA	NA	NA	NA	NA	NA	AT3G50260
Soltu.DM.02G009190	plastid transcription factor	NA	1.41	1.54	NA	NA	NA	NA	NA	NA	AT3G02150
Soltu.DM.04G035890	BEL1-like homeodomain	NA	1.18	1.94	NA	NA	NA	NA	NA	NA	AT1G75410
Soltu.DM.10G028350	CCCH-type zinc finger protein with ARM repeat domain	NA	1.15	1.66	NA	NA	NA	NA	NA	NA	AT2G41900
Soltu.DM.09G011140	WRKY DNA-binding protein	NA	2.43	2.68	NA	NA	NA	NA	NA	NA	AT3G56400
Soltu.DM.12G012040	Calmodulin binding protein-like	NA	2.34	2.73	NA	NA	NA	NA	NA	NA	AT1G73805
Soltu.DM.05G020000	WRKY family transcription factor	NA	1.92	2.34	NA	NA	NA	NA	NA	NA	AT4G11070
Soltu.DM.05G009650	AGAMOUS-like	NA	3.04	2.98	NA	NA	NA	NA	NA	NA	AT4G02235
Soltu.DM.10G020670	GRAS family transcription factor family protein	NA	2.30	3.28	NA	NA	NA	NA	NA	NA	AT2G01570
Soltu.DM.08G015910	WRKY DNA-binding protein	NA	4.19	4.16	NA	NA	NA	NA	NA	NA	AT1G80840
Soltu.DM.08G015040	Integrase-type DNA-binding superfamily protein	NA	-1.80	-1.72	NA	NA	NA	NA	NA	NA	AT5G25810
Soltu.DM.01G030510	WRKY family transcription factor	NA	-1.00	-1.23	NA	NA	NA	NA	NA	NA	AT2G44745
Soltu.DM.02G020630	PHD finger family protein / bromo-adjacent homology (BAH) domain-containing protein	NA	-0.77	-1.08	NA	NA	NA	NA	NA	NA	AT4G22140
Soltu.DM.08G023770	ureidoglycine aminohydrolase	NA	-0.91	-1.07	NA	NA	NA	NA	NA	NA	AT4G17050
Soltu.DM.06G011930	response regulator	NA	-2.17	-3.89	NA	NA	NA	NA	NA	NA	AT3G56380
Soltu.DM.03G021700	heat shock protein	NA	4.67	NA	NA	NA	NA	NA	NA	NA	AT4G27670
Soltu.DM.10G002230	NAC domain containing protein	NA	4.61	NA	NA	NA	NA	NA	NA	NA	AT3G15510
Soltu.DM.01G020840	ethylene-responsive element binding protein	NA	1.03	NA	NA	NA	NA	NA	NA	NA	AT3G16770
Soltu.DM.10G015560	ENHANCED DOWNY MILDEW	NA	1.03	NA	NA	NA	NA	NA	NA	NA	AT5G55390
Soltu.DM.11G018220	NIN like protein	NA	1.01	NA	NA	NA	NA	NA	NA	NA	AT1G76350
Soltu.DM.06G032890	cytokinin response factor	NA	1.12	NA	NA	NA	NA	NA	NA	NA	AT4G27950
Soltu.DM.08G019590	Basic-leucine zipper (bZIP) transcription factor family	NA	1.12	NA	NA	NA	NA	NA	NA	NA	AT2G40950

	protein										
Soltu.DM.01G047500	GATA transcription factor	NA	1.26	NA	NA	NA	NA	NA	NA	NA	AT4G17570
Soltu.DM.02G028870	heat shock factor	NA	1.21	NA	NA	NA	NA	NA	NA	NA	AT4G36990
Soltu.DM.02G015370	WRKY DNA-binding protein	NA	1.37	NA	NA	NA	NA	NA	NA	NA	AT1G29280
Soltu.DM.06G024390	Integrase-type DNA-binding superfamily protein	NA	1.73	NA	NA	NA	NA	NA	NA	NA	AT1G16060
Soltu.DM.05G003320	C2H2 and C2HC zinc fingers superfamily protein	NA	1.69	NA	NA	NA	NA	NA	NA	NA	AT1G68360
Soltu.DM.06G025800	NAC domain containing protein	NA	1.83	NA	NA	NA	NA	NA	NA	NA	AT5G61430
Soltu.DM.10G024830	Duplicated homeodomain-like superfamily protein	NA	2.41	NA	NA	NA	NA	NA	NA	NA	AT3G10000
Soltu.DM.05G005150	myb domain protein	NA	2.39	NA	NA	NA	NA	NA	NA	NA	AT1G69560
Soltu.DM.03G013150	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	NA	-1.41	NA	NA	NA	NA	NA	NA	NA	AT2G40200
Soltu.DM.01G031000	ethylene-responsive element binding factor	NA	-1.60	NA	NA	NA	NA	NA	NA	NA	AT2G44840
Soltu.DM.12G006040	Zinc-finger domain of monoamine-oxidase A repressor R1	NA	-2.10	NA	NA	NA	NA	NA	NA	NA	AT2G23530
Soltu.DM.12G004640	ARID/BRIGHT DNA-binding domain-containing protein	NA	-2.41	NA	NA	NA	NA	NA	NA	NA	AT1G76510
Soltu.DM.07G021060	ovate family protein	NA	NA	1.62	NA	NA	NA	NA	NA	NA	AT5G01840
Soltu.DM.03G013350	WRKY DNA-binding protein	NA	NA	1.57	NA	NA	NA	NA	NA	NA	AT3G56400
Soltu.DM.09G008990	GRAS family transcription factor	NA	NA	1.39	NA	NA	NA	NA	NA	NA	AT4G08250
Soltu.DM.10G015570	ENHANCED DOWNY MILDEW	NA	NA	1.33	NA	NA	NA	NA	NA	NA	AT5G55390
Soltu.DM.07G011520	GATA type zinc finger transcription factor family protein	NA	NA	1.87	NA	NA	NA	NA	NA	NA	AT5G56860
Soltu.DM.08G010780	Transducin family protein / WD-40 repeat family protein	NA	NA	1.04	NA	NA	NA	NA	NA	NA	AT1G80490
Soltu.DM.06G029490	transcription factor-related	NA	NA	1.03	NA	NA	NA	NA	NA	NA	AT2G27230
Soltu.DM.01G019980	calmodulin binding;transcription regulators	NA	NA	1.12	NA	NA	NA	NA	NA	NA	AT3G16940
Soltu.DM.02G009240	ABI five binding protein	NA	NA	5.27	NA	NA	NA	NA	NA	NA	AT3G29575
Soltu.DM.12G026290	Integrase-type DNA-binding superfamily protein	NA	NA	5.10	NA	NA	NA	NA	NA	NA	AT1G19210
Soltu.DM.06G012920	Integrase-type DNA-binding superfamily protein	NA	NA	6.16	NA	NA	NA	NA	NA	NA	AT5G52020
Soltu.DM.08G000070	dehydration response element B1A	NA	NA	3.21	NA	NA	NA	NA	NA	NA	AT1G12610
Soltu.DM.04G034270	myb domain protein	NA	NA	3.19	NA	NA	NA	NA	NA	NA	AT3G50060
Soltu.DM.12G006180	salt tolerance zinc finger	NA	NA	3.68	NA	NA	NA	NA	NA	NA	AT1G27730
Soltu.DM.02G025020	late embryogenesis abundant domain-containing protein / LEA domain-containing protein	NA	NA	-1.89	NA	NA	NA	NA	NA	NA	AT1G75250
Soltu.DM.12G007690	Duplicated homeodomain-like superfamily protein	NA	NA	-1.39	NA	NA	NA	NA	NA	NA	AT1G76890
Soltu.DM.03G031590	Transducin family protein / WD-40 repeat family protein	NA	NA	-1.00	NA	NA	NA	NA	NA	NA	AT1G15750
Soltu.DM.02G000060	BREAST CANCER 2 like 2A	NA	NA	-1.02	NA	NA	NA	NA	NA	NA	AT5G01630
Soltu.DM.03G031620	hypothetical protein	NA	NA	-1.13	NA	NA	NA	NA	NA	NA	AT1G15750
Soltu.DM.03G000450	AUX/IAA transcriptional regulator family protein	NA	NA	-1.20	NA	NA	NA	NA	NA	NA	AT1G04240
Soltu.DM.04G008290	Integrase-type DNA-binding superfamily protein	NA	NA	NA	NA	NA	NA	1.75	2.14	NA	AT5G64750
Soltu.DM.02G027750	BTB and TAZ domain protein	NA	NA	NA	NA	NA	NA	1.75	1.72	NA	AT5G67480
Soltu.DM.05G005300	NAC-like, activated by AP3/PI	NA	NA	NA	NA	NA	NA	1.28	1.14	NA	AT1G69490
Soltu.DM.10G011330	phytochrome A	NA	NA	NA	NA	NA	NA	-1.39	-1.09	NA	AT1G09570

Appendix-Table 5.2 W-box domains within the 1,000 bp upstream region of genes putatively regulated by *Soltu.DM.03G030960* (AtWRKY40). Shown are the locations of the *cis* elements in the genes differentially expressed in only the tolerant varieties in response to drought and *P. infestans* infection.

Factor or Site Name	Loc.(Str.)	Signal Sequence
***Cis*-elements in: *Soltu.DM.02G020560*-chitin elicitor receptor kinase**		
WRKY71OS	4 (-)	TGAC
WRKY71OS	15 (+)	TGAC
WRKY71OS	53 (+)	TGAC
WRKY71OS	168 (-)	TGAC
WRKY71OS	253 (+)	TGAC
WRKY71OS	348 (-)	TGAC
WRKY71OS	426 (-)	TGAC
WRKY71OS	614 (+)	TGAC
WRKY71OS	720 (+)	TGAC
WRKY71OS	903 (-)	TGAC
WBOXNTERF3	3 (-)	TGACY
WBOXNTERF3	15 (+)	TGACY
WBOXNTERF3	253 (+)	TGACY
WBOXNTERF3	347 (-)	TGACY
WBOXNTERF3	614 (+)	TGACY
WBOXNTERF3	720 (+)	TGACY
WBOXNTERF3	902 (-)	TGACY
WBOXNTCHN48	719 (+)	CTGACY
WBOXHVISO1	253 (+)	TGACT
WBOXHVISO1	347 (-)	TGACT
WBOXHVISO1	614 (+)	TGACT
WBOXHVISO1	720 (+)	TGACT
WBOXATNPR1	4 (-)	TTGAC
WBOXATNPR1	348 (-)	TTGAC
WBOXATNPR1	426 (-)	TTGAC
WBOXATNPR1	903 (-)	TTGAC
***Cis*-elements in: *Soltu.DM.12G006670*-cysteine-rich RLK (RECEPTOR-like protein kinase)**		

WRKY71OS	125 (-)	TGAC
WRKY71OS	206 (-)	TGAC
WRKY71OS	353 (+)	TGAC
WRKY71OS	360 (+)	TGAC
WRKY71OS	576 (-)	TGAC
WRKY71OS	949 (-)	TGAC
WBOXNTERF3	353 (+)	TGACY
WBOXNTERF3	360 (+)	TGACY
WBOXATNPR1	206 (-)	TTGAC
WBOXATNPR1	576 (-)	TTGAC
WBOXATNPR1	949 (-)	TTGAC
***Cis*-elements in: *Soltu.DM.04G010950*-heat shock transcription factor B3**		
WRKY71OS	28 (-)	TGAC
WRKY71OS	120 (-)	TGAC
WRKY71OS	156 (-)	TGAC
WRKY71OS	591 (-)	TGAC
WRKY71OS	977 (+)	TGAC
WBOXNTERF3	27 (-)	TGACY
WBOXNTERF3	119 (-)	TGACY
WBOXNTERF3	155 (-)	TGACY
WBOXNTERF3	977 (+)	TGACY
WBOXHVISO1	27 (-)	TGACT
WBOXHVISO1	119 (-)	TGACT
WBOXHVISO1	977 (+)	TGACT
WBOXATNPR1	28 (-)	TTGAC
WBOXATNPR1	120 (-)	TTGAC
WBOXATNPR1	591 (-)	TTGAC
WBBOXPCWRKY1	27 (-)	TTTGACY
WBBOXPCWRKY1	119 (-)	TTTGACY
***Cis*-elements in: *Soltu.DM.10G003320*-conserved hypothetical protein**		
WRKY71OS	145 (-)	TGAC
WRKY71OS	173 (-)	TGAC
WRKY71OS	200 (-)	TGAC

WRKY71OS	272 (+)	TGAC
WRKY71OS	321 (-)	TGAC
WRKY71OS	438 (+)	TGAC
WRKY71OS	523 (+)	TGAC
WRKY71OS	701 (-)	TGAC
WRKY71OS	810 (+)	TGAC
WRKY71OS	841 (+)	TGAC
WRKY71OS	872 (+)	TGAC
WRKY71OS	903 (+)	TGAC
WRKY71OS	932 (+)	TGAC
WRKY71OS	993 (+)	TGAC
WBOXNTERF3	144 (-)	TGACY
WBOXNTERF3	172 (-)	TGACY
WBOXNTERF3	199 (-)	TGACY
WBOXNTERF3	841 (+)	TGACY
WBOXNTERF3	872 (+)	TGACY
WBOXNTERF3	903 (+)	TGACY
WBOXNTERF3	932 (+)	TGACY
WBOXNTERF3	993 (+)	TGACY
WBOXHVISO1	172 (-)	TGACT
WBOXHVISO1	199 (-)	TGACT
WBOXHVISO1	841 (+)	TGACT
WBOXHVISO1	872 (+)	TGACT
WBOXHVISO1	903 (+)	TGACT
WBOXHVISO1	932 (+)	TGACT
WBOXATNPR1	271 (+)	TTGAC
WBOXATNPR1	321 (-)	TTGAC
WBOXATNPR1	522 (+)	TTGAC
WBOXATNPR1	701 (-)	TTGAC

Appendix - Linkage disequilibrium in autotetraploids

A. Introduction

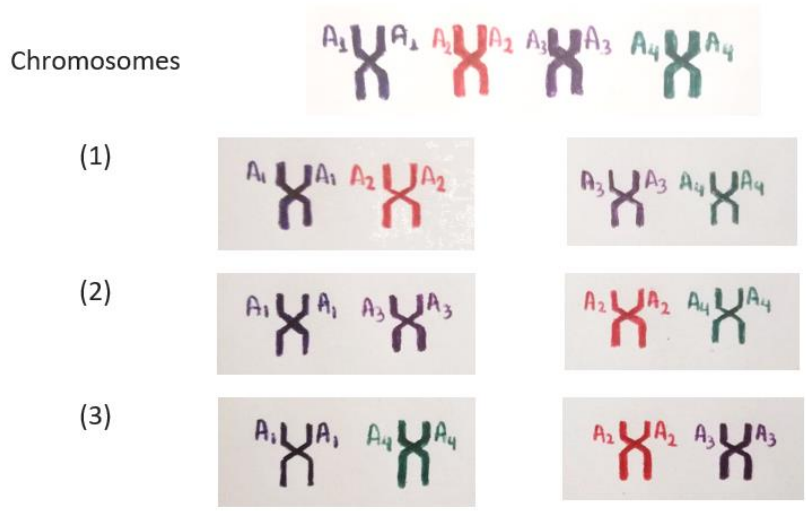
In nature, many economically important crops are autotetraploid, including blueberry, rye, oat, and potato. Autotetraploids are organisms with four sets of chromosomes originating from a single species, usually by fusion of unreduced gametes (Parisod, Holderegger, and Brochman, 2010). During meiosis, there is no preferential pairing between homologous chromosomes, and different structures can be observed such as univalent, bivalents, trivalents, and quadrivalents (Choudhary *et al*., 2020).

A.1. Mechanics of meiosis in autotetraploids

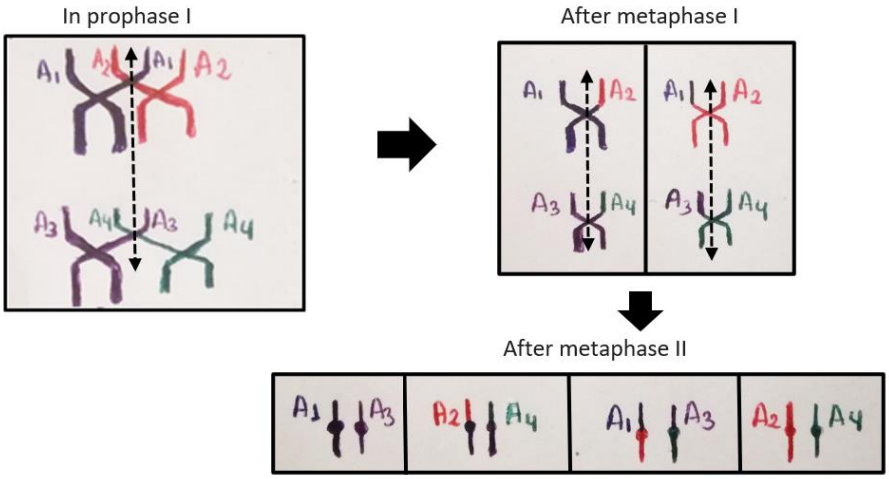
Meiosis in autotetraploids is more complex than in diploids because with four copies per chromosome, different pairing structures can form, including bivalents, trivalent, quadrivalents, or a mixture (Choudhary *et al*., 2020) leading to complex segregation patterns.

A.1.1. Bivalent formation

In tetraploids (2n=4X), when bivalents are formed, there are three possible pairings between the four chromosome copies in metaphase I (**Appendix-Figure 6.1**). Then, paired chromosomes are separated and each one migrates to a different pole in anaphase I. After meiosis I, the number of chromosomes is reduced by half, similar to diploids, but in this case n=2X. After meiosis II, sister chromatids are separated and, and each gamete will contain two sister chromatids, each one from different chromosomes (**Appendix-Figure 6.2**). If the segregation of gene A with four different alleles (heterozygote: $A_1A_2A_3A_4$) is analyzed, after meiosis I, each newly formed cell will have two chromosomes with four different alleles, because of a recombination event. Then, after meiosis II, each gamete will contain two chromosomes, each one with one sister chromatid (**Appendix-Figure 6.2**). Also, each gamete will contain two different alleles with 6 possibilities: A_1A_2, A_1A_3, A_1A_4, A_2A_3, A_2A_4 or A_3A_4.



Appendix-Figure 6.1 Possible bivalents generated in tetraploids. There are three possible combinations of homologous chromosomes pairings in metaphase I. A_1-A_4 represent alleles carried on the same homologous chromosome.



Appendix-Figure 6.2 Meiosis in tetraploids when bivalents are generated. Meiosis in a tetraploid with the first combination of homologous chromosomes pairings shown in **Appendix-Figure 6.1**. *A_1-A_4* represent alleles carried on the same homologous chromosome and each pair of homologous chromosomes has a single crossover during prophase I. After metaphase I, diploid cells with chromosomes consisting of two sister chromatids are formed (*A_1A_2* and *A_3A_4* for one cell, and *A_1A_2* and *A_3A_4* in the other one). After metaphase II, chromatids separate and pairs of single chromatids (now called chromosomes) will form a diploid gamete, with 4 possible combinations of alleles *A_1A_3, A_2A_4, A_1A_3, and A_2A_4*.

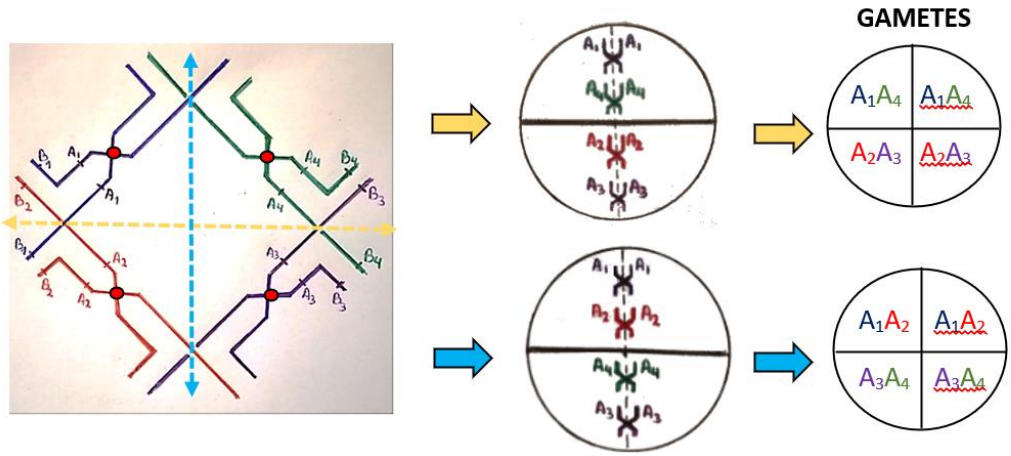
A.1.2. Quadrivalent formation

When a quadrivalent is formed, balanced diploid gametes may be generated, but the allelic segregation pattern can be different from what it is obtained with bivalents. This is because identical

alleles on sister chromatids, from the same chromosome, can migrate to the same pole and unexpected gametes and allelic frequencies are produced. To visualize this phenomenon, called double reduction, it is necessary to show how the segregation pattern of a gene occurs, depending on the occurrence of recombination between the gene and the centromere of homologous chromosomes.

A.1.2.1. Quadrivalents with no recombination between a gene and the centromere.

In quadrivalents, the frequency of recombination between the centromere and the nearest genes may be very low, and recombination will rarely occur. As an example, the same case of the parental tetraploid cell with four different alleles for gene A (heterozygote: $A_1A_2A_3A_4$) will be taken to analyze its segregation pattern. In meiosis I, there are two possible axes by which quadrivalents can be separated (blue and yellow in **Appendix-Figure 6.3**). However, with both axes after meiosis II, each gamete will contain two different alleles: A_1A_2, A_1A_3, A_1A_4, A_2A_3, A_2A_4 *or* A_3A_4, similar to the case of bivalent formation. Each allele in the gamete will come from different sister chromatids (**Appendix-Figure 6.3**).



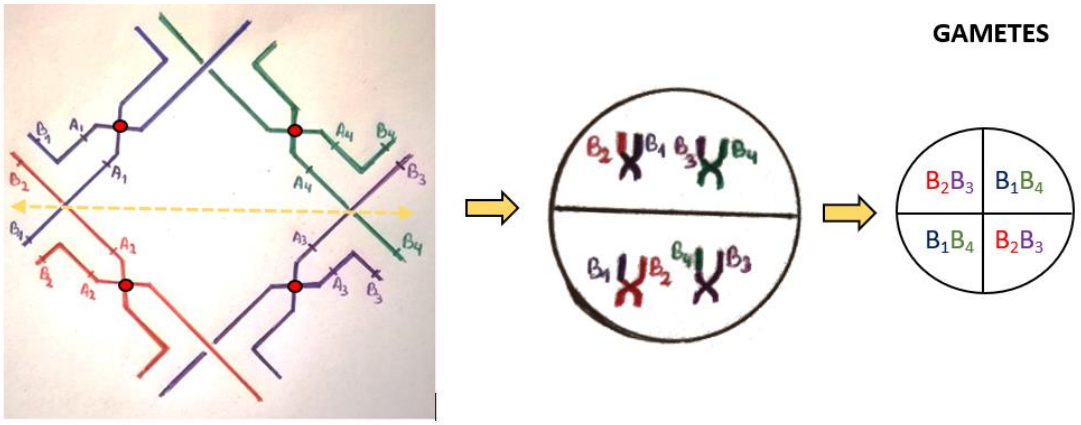
Appendix-Figure 6.3 Meiosis in autotetraploid when a quadrivalent structure is formed and no recombination occurs between gene A and the centromere. The figure in the left side represents a quadrivalent, where each red dot represents a centromere. A_1-A_4 and B_1-B_4 represent the alleles of a gene *A* and gene *B*, respectively, with *A* closer to the centromere than *B*. Dotted yellow and light blue lines represent the two possible divisions that could occur after metaphase I. In this example, there is no recombination between the centromere and gene *A*, so chromosomes carrying only one type of allele will be formed after

metaphase I. After metaphase II, alleles from different sister chromatids will migrate into different gametes. Adapted from Wu *et al*., 2001.

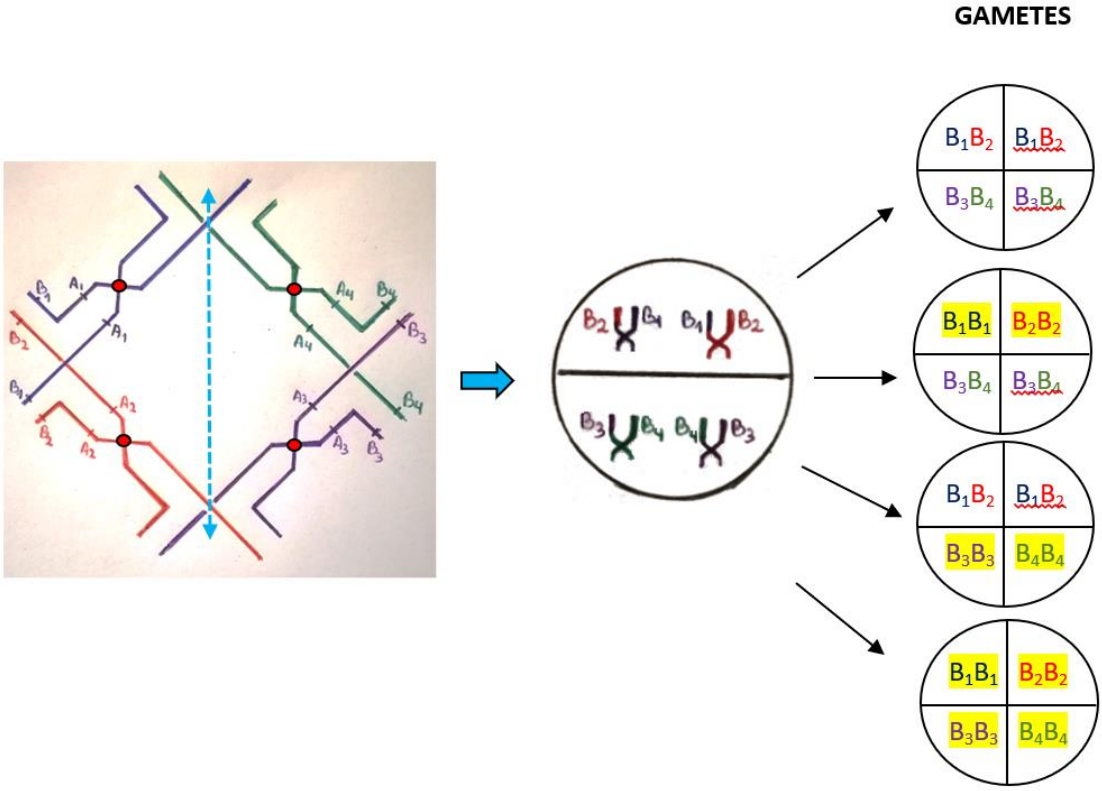
A.1.2.2. Quadrivalents with recombination between a gene and the centromere.

In this case, depending on which of the two axes can separate the quadrivalent, new combination of alleles in the gametes can be observed (**Appendix-Figure 6.4**). As an example, the same case of the parental tetraploid cell with four different alleles for a gene *B* (heterozygote: $B_1B_2B_3B_4$) will be taken to analyze its segregation pattern. After anaphase I, if the chromosomes that are recombined migrate to opposite poles (**Appendix-Figure 6.4**-**A**), after anaphase II, each gamete will contain two different alleles: B_1B_2, B_1B_3, B_1B_4, B_2B_3, B_2B_4 or B_3B_4, each one coming from different sister chromatids, similar to the case of bivalent and quadrivalent with no recombination. (**Appendix-Figure 6.4**-**A**). However, if in anaphase I the chromosomes which are recombined are not segregated to the same pole (**Appendix-Figure 6.4**-**B**), after anaphase II, alleles from the same sister chromatids can migrate to the same pole and new allele combinations can appeared in the gamete: B_1B_1, B_2B_2, B_3B_3, B_4B_4. (**Appendix-Figure 6.4**-**B**). This phenomenon is called “double reduction.”

(A)



(B)



Appendix-Figure 6.4 Meiosis in a tetraploid when a quadrivalent is formed and recombination occurs between gene B and the centromere. Depending on how the quadrivalent is separated in anaphase I, double reduction will occur. If the recombined chromosomes segregate to opposite poles (A), no double reduction will occur. However, if the recombined chromosomes segregate to the same pole (B) double reduction occurs and gametes will contain alleles coming from the same sister chromatids (highlighted in yellow). Adapted from Wu *et al*., 2001.

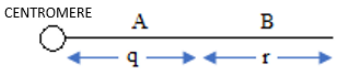
In summary, in a tetraploid individual, a gene A with four different alleles ($A_1A_2A_3A_4$), without the occurrence of double reduction, can generate 6 possible gametes A_1A_2, A_1A_3, A_1A_4, A_2A_3, A_2A_4, A_3A_4, but with the occurrence of double reduction, homozygous genotypes can be also observed in the gametes: A_1A_1, A_2A_2, A_3A_3, A_4A_4.

A.2. Focus on Double reduction

Double reduction is a phenomenon produced in autopolyploids, where homologous segments of two sister chromatids segregate to the same gamete (Fisher, 1947; Rehmsmeier, 2013) (**Appendix-Figure 6.4**), creating new genotypes and segregation ratios that differ from those assumed under independent assortment (Butruille and Boiteux, 2000). Double reduction affects the distribution of gene frequencies in an autopolyploid population, generating segregation distortion, and because it leads to an excess of homozygotes, the recessive alleles may be at a higher frequency than in diploids (Welch, 1962). Double reduction is also a position-dependent phenomenon. The probability of generating double reduction is greater when a locus is more distal from the centromere (Welch, 1962). Since double reduction is a function of the cross-over distance between a locus and the centromere, the gene segregation pattern in tetraploids would vary from gene to gene.

Theoretically, the maximum value of the probability or coefficient of double reduction (α) has been calculated as a function of the recombination frequency. If α and β are the coefficient of double reduction of genes A and B, respectively, and both of them are in the same chromosome in the following order: the centromere, locus *A*, and locus *B*, with *r* being the recombinant frequency between the gene *A* and *B* (**Appendix-Figure 6.5**), then the coefficient of double reduction, according to Luo *et al*. will be:

$$\beta = \frac{1}{9}\left[\alpha(3-4r)^2 + 2r(3-2r)\right]$$ … Luo, Zhang, and Kersey 2004.



Appendix-Figure 6.5 Two linked loci in a chromosome arm, where their positions are: centromere, locus *A*, and locus *B*. The recombination frequency between the centromere and the locus A is q, and between locus A and B is r.

In tetraploids, the maximum value of recombination frequency is equal to 0.75, instead of 0.5 like in diploids (Luo *et al*., 2006). Because double reduction depends on the recombination frequency between an allele and a centromere, its maximum value will depend on the maximum value of the recombination frequency*.* Then, replacing r by 0.75 in the formula of Luo *et al*, 2004, the maximum value of β, the coefficient of double reduction, will be equal to 1/4 (Luo *et al*., 2006).

Double reduction influences the segregation pattern of gametic genotypes, so it is not only dependent on recombination as in diploids or when only bivalents are formed during meiosis in autotetraploids (Luo *et al*.,2001) (**Appendix-Table 6.1**). The dependency between the probability of gametic formation, recombination frequency and double reduction of autotetraploids was previously described by Luo, Zhang, and Kersey (2004) (**Appendix-Table 6.2**).

Appendix-Table 6.1 Probability of gametic genotypes for two loci A and B, in a random mixed population when bivalent formation occurs. It shows the probability distribution for two linked loci, A and B, each one with a maximum of 4 possible alleles, where i, j, k, l represent different allele for each locus A or B. The frequency is for the number of genotypes observed for each represented gamete mode. The recombination events are the number of recombinations observed between loci for a specific gametic mode. In the gamete probabilities r is the recombination frequency between A and B. Reproduced from Luo *et al*., 2001.

Gametes	Frequency	Recombination events	Gamete Probabilities
A_iB_i/AjBj	6	1	$(1-r)^2/6$
A_iB_j/AjBk	24	1	$(1-r)r/12$
A_iB_j/AkBl	12	2	$r^2/12$

Appendix-Table 6.2 Probability distribution of the 11 diploid gamete mode formation when quadrivalents occur in autetraploids. It shows the probability distribution for two linked loci, A and B, each one with a maximum of 4 possible alleles, where i, j, k, l represent different allele for each locus A or B. The frequency is for the number of genotypes observed for each represented gamete mode. i.e., for a parent with 4 alleles in each locus A and B ($A_1B_1/A_2B_2/A_3B_3/A_4B_4$) there would be 4 possible gametes of the mode A_iB_i/A_iB_i, (A_1B_1/A_1B_1, A_2B_2/A_2B_2, A_3B_3/A_3B_3, A_4B_4/A_4B_4), and this mode will have a probability of $27\alpha(1-r)^2/108$. This mode of formation would be observed when double reduction occurs in A and B locus, but without any recombination events between A and B. α is the coefficient of double reduction for the locus A, and r is the frequency of recombination between A and B. Reproduced from Luo, Zhang, and Kersey, 2004.

Gametes	Frequency	Double reduction (Recombination) events	Gamete Probability
A_iB_i/A_iB_i	4	A y B (0)	$27\alpha(1-r)^2/108$
A_iB_j/A_iB_j	12	A y B (2)	$3\alpha r^2/108$
A_iB_i/A_iB_j	12	A (1)	$18\alpha r(1-r)/108$
A_iB_j/A_iB_k	12	A (2)	$6\alpha r^2/108$
A_iB_i/A_jB_i	12	B (1)	$6(1-\alpha)r(1-r)/108$
A_iB_j/A_kB_i	12	B (2)	$2(1-\alpha)r^2/108$
A_iB_i/A_jB_j	6	– (0)	$18(1-\alpha)(1-r)^2/108$
A_iB_i/A_jB_k	24	– (1)	$6(1-\alpha)r(1-r)/108$
A_iB_j/A_jB_i	6	– (2)	$2(1-\alpha)r^2/108$
A_iB_j/A_jB_k	24	– (2)	$2(1-\alpha)r^2/108$
A_iB_j/A_kB_l	12	– (2)	$2(1-\alpha)r^2/108$

The majority of agronomic traits are quantitative, which depend on the expression of several genes called quantitative trait loci (QTLs). For autotetraploids, methodologies have been implemented to detect genetic markers linked to QTLs employing a biparental population where double reduction and recombination frequency were included in the analysis (Leach *et al.*, 2010; Chen, *et al*., 2021). However, there is a preference to use a naturally existing populations because they have experienced many more recombination events that increase the probability of finding an association between a marker and a quantitative trait in high resolution (Bernardo, 2010). This methodology receives the name of genome-wide association study (GWAS) and it makes use of the concept of linkage disequilibrium (*D*) to detect genetic markers linked to QTLs.

A.3. Linkage disequilibrium

To refine population genetic analyses in autotetraploid species, the key concept of linkage disequilibrium (*D*) needs to be fully described. In general, *D* describes a non-random association

between alleles at two different loci within a non-related naturally existing population, and it is calculated by the deviation (*D*) from the linkage equilibrium. *D* is measured by the observed frequency of a gamete (observed genotypic or haplotype frequency) in a population minus the product of the frequencies of the corresponding alleles (expected genotypic frequency when the loci are independent).

Considering two loci A and B, each one with their possible alleles A_i and B_j, then: $D = P(A_iB_j) - P(A_i)P(B_j)$ (Thomson and Klitz, 1987), where: $P(A_iB_j)$ is the observed frequency of gamete/genotype A_iB_j, $P(A_i)$ is the frequency of allele A_i and $P(B_j)$ is the frequency of allele B_j. Considering these two loci are bi-allelic, with the alleles for locus *A* as A_1 and A_2, and the alleles for locus *B* as B_1 and B_2, with frequencies denoted as *p*, then the expected probability of each gametic genotype or haplotype will be as shown in **Appendix-Table 6.3**.

Appendix-Table 6.3 Probability of gametic genotypes for two loci with two alleles in diploids. The actual probability of a genotype A_1B_1 is equal to P_{A1A1}, with the expected probability equal to $P_{A1}P_{B1}$. The deviation between the actual and the expected probability is called linkage disequilibrium (*D*). Therefore, P_{A1B1} will be equal to $P_{A1}P_{B1} + D$. Under equilibrium, $D = 0$. Reproduced from Thomson and Klitz, 1987.

Alleles		Locus B	
		B_1	B_2
Locus A	A_1	Actual: p_{A1B1} Expected: $p_{A1}\,p_{B1}$	Actual: p_{A1B2} Expected: $p_{A1}\,p_{B2}$
	A_2	Actual: p_{A2B1} Expected: $p_{A2}\,p_{B1}$	Actual: p_{A2B1} Expected: $p_{A2}\,p_{B2}$
Including D:			
Locus A	A_1	$p_{A1B1} = p_{A1}\,p_{B1} + D$	$p_{A1B2} = p_{A1}\,p_{B2} - D$
	A_2	$p_{A2B1} = p_{A2}\,p_{B1} - D$	$p_{A2B2} = p_{A2}\,p_{B1} + D$

Then, if any allele of the locus A is in linkage equilibrium with any allele of the locus B, then $D=0$ (independent segregation), but if they are in linkage disequilibrium, then $D\neq0$. The value of LD (*D*) can

be positive or negative depending on the configuration of their alleles. When two alleles from different loci are frequently associated on the same chromosome (haplotype), it said that they are in coupling, and their *D* value will be positive. On the other hand, when these two alleles are more frequently located on different copies of the homologous chromosome, it said they are in repulsion and their D value will be negative (Gallais, 2003). However, because *D* is a measure of association between two loci, its magnitude is more important than its sign.

D decays with each generation after random mating. If the recombination frequency between two loci is r, then the disequilibrium after t generations of recombination is $(1 - r)^t$ of the initial disequilibrium: $D = (1-r)^t D_0$, where D_0 is the initial linkage disequilibrium (Falconer, 1981, Bernardo, 2010). With a weak linkage (r close to 0.5), *D* will decay faster and the two loci will approach equilibrium faster too, while with a strong linkage, *D* can persist for many generations. Generally, loci that are physically close exhibit stronger *D* than loci that are farther apart on a chromosome, though D may not always arise due to physical linkage.

A.3.1. Linkage disequilibrium in autotetraploids

In the case of autotetraploids, gametes are not haploid; instead, they contain two copies of each chromosome, for which two the same or different alleles are observed for each locus. Also, in the gamete of an autotetraploid species, two loci can be observed in coupling or repulsion phase. In coupling phase, the two parental alleles are linked in the same haplotype (*AB/ab*), while in repulsion phase the two parental alleles are separated (*Ab/aB*). As a consequence, it can generate more than one type of disequilibrium. The first one is commonly called linkage disequilibrium, also observed in diploids and referring to a correlation in the segregation of two alleles, each one from different loci but on the same chromosome. The second one is called chromosomal gametic disequilibrium, which not only involve two loci, instead it refers to a non-random association of the two complete

chromosomes copies in the diploid gamete. It means that the segregation of these two chromosomes is not always independent (Gallais 2003, Griswold 2017).

In autotetraploids, previous works have evaluated the expected value of segregation of the alleles of two loci (*A, B*) without disequilibrium, that is, at equilibrium, which occurs in a population under random matting experiencing an infinitive number of generations (Geiringer 1949a, 1949b; Bennet 1954; Crow 1954). The probability of a genotype at two loci is the multiplication of their independent allelic genotypes, as in diploids (Bennet, 1954; Crow, 1954). Therefore, at equilibrium, the frequency of a two locus genotype is the product of the separate loci, as in diploids.

$$\lim_{n\to\infty} P_n(AB) = p(A)p(B)$$

This result was one of the first steps to decipher linkage disequilibrium and its decay in autotetraploids. Once it is known what is occurring at equilibrium, the next step is to calculate how linkage disequilibrium in autotetraploids occurs and how it is affected by double reduction and the recombination frequency, which could be different to what is observed in diploids. Due to the importance of the analysis of *D* for population genetics and its influence in the identification of QTLs in GWAS, it is important to analyze the *D* in autotetraploid species, analytically and theoretically. Linkage disequilibrium was previously evaluated in autotetraploid species, including potato, using this definition of deviation from the equilibrium (Stich *et al*., 2013; Vos *et al*., 2017; Sharma *et al.*, 2018). However, *D* has not been fully theoretically defined in terms of its dependent variables, which in autotetraploids are recombination frequency (*r*) and double reduction (*α*), both influencing the probability of the gametic genotype. For this reason, this chapter aims to develop the population genetic model of *D* for autotetraploids, where the basic definition and measure of *D* from diploids to autotetraploids will be extended, by first analyzing *D* analytically, then algebraically.

B. Material and methods

To determine the influence of double reduction in the analysis of linkage disequilibrium in a random mating population, the analysis was carried out analytically and theoretically. The analytical part was an exploratory analysis that simulated gamete formation and calculated the frequency of each one with the aim of determining the linkage disequilibrium after several generations and the influence of double reduction. In the theoretical part, the symmetry of *D*, its dependence on double reduction, and its decay after generations, was derived.

B.1. Analytical study of *D* in autotetraploids

The analysis was carried out for a population in which two linked loci (A and B) have two alleles each (A and a, and B and b, respectively). The assumption is that the position of each locus on the chromosome is centromere, locus A, and locus B. All the possible zygotic genotypes were identified, and from each possible zygotic genotype, the probability of each of its descendant gametes was calculated, assuming only full quadrivalent formation. For simplicity, in the first generation (generation 0), the parental genotype was assumed to be AB/ab/ab/ab.

The probability of each possible generated gamete was calculated based on the probability distribution described by Luo, Zhang, and Kersey, 2004, which included the 11 modes of gamete formation described by Fisher (Fisher 1947, Luo, Zhang, and Kersey, 2004). Luo et al., described the probability of each 11 gamete types in terms of α (double reduction) and r(recombination frequency) (**Appendix-Table 6.2**).

Then, in the following descendants, which became new parental individuals, each one with a zygotic genotype, the probability of each possible gamete was also calculated. This analysis was continued for 4 generations. The analysis included different values of recombination frequency (r) and double reduction (α), as follows:

(1st) The probability of each gamete was calculated in a population at equilibrium when r_{max} occurs (r_{max} = 0.75) (Luo *et al.*, 2006) and no double reduction is observed (α = 0).

(2nd) The probability of each gamete was calculated when the population is not at equilibrium under different r (0.25 and 0.5) and α (0.1 and 0.2) values.

With these two sets of probabilities, *D* was calculated as the difference between the probability at equilibrium and the probability not under equilibrium. Therefore, *D* was calculated under different recombination frequencies and double reduction coefficients, across 4 generations. In the simulation, it is assumed that r and α values were constant in the population.

For these analyses, custom algorithms were constructed in RStudio v 1.2.1335 and Microsoft Excel.

B.2. Theoretical study of *D* in autotetraploids

For the theoretical analysis, to determine linkage disequilibrium in autotetraploids, the probability of two parental loci (*A, B*), each with two alleles (*A, a,* and *B, b*), inherited over generations was analyzed. The analysis considered the 11 gamete formation modes described by Fisher (1947) and the probability of each mode, defined in terms of the recombination frequency (*r*) and the coefficient of double reduction (*α*) by Luo, Zhang, and Kersey, 2004. For the meiotic pairing model, full bivalent, full quadrivalent, and a mixture of pairings was analyzed.

Bennett (1954) defined the probability of the genotype *AB* in a generation *n+1* ($P_{n+1}AB$) in a random mating population as:

$$P_{n+1}AB = P_nAB + \frac{r}{6}[4P_AP_B - 4P_nAB + P_n(Ab/aB) - P_n(AB/ab)]$$

Where $P_n(Ab/aB)$ and $P_n(AB/ab)$ are the gametic probabilities of the respective genotype in generation *n*. The model of gamete formation, its probabilities and the equation described by

Bennett, were applied to the definition of *D*: $D = P_{AB} - P_A.P_B$. Theoretically, this part evaluated the symmetry of *D*, its dependence on double reduction, and its decay over generations.

C. Results

C.1. Analytical study of *D* in autotetraploids

Here, *D* was evaluated over four generations, where the initial population has two linked loci (*A* and *B*) each with two alleles (*A* and a, and *B* and *b*, respectively). Different values of recombination frequency and double reduction were assumed to calculate the gametic frequency of each possible genotype. In an autotetraploid population, if two linked loci (*A* and *B*) each have two possible alleles, 35 phased zygotic genotypes are possible (**Appendix-Table 6.4**).

Appendix-Table 6.4 Possible phased zygotic genotypes for two loci (*A* and *B*) each with two alleles (*A* and *a* for locus *A*; *B* and b for locus *B*).

One allele at both loci	**Two alleles for each of two loci**
1 *AB/AB/AB/AB*	17 *AB/Ab/aB/ab*
2 *Ab/Ab/Ab/Ab*	18 *AB/AB/AB/ab*
3 *aB/aB/aB/aB*	19 *AB/ab/ab/ab*
4 *ab/ab/ab/ab*	20 *Ab/Ab/Ab/aB*
One allele at one of the two loci	21 *Ab/aB/aB/aB*
5 *AB/AB/AB/Ab*	22 *Ab/ab/ab/aB*
6 *AB/AB/AB/aB*	23 *AB/Ab/Ab/ab*
7 *AB/AB/aB/aB*	24 *AB/aB/aB/ab*
8 *AB/aB/aB/aB*	25 *AB/AB/Ab/aB*
9 *AB/Ab/Ab/Ab*	26 *AB/ab/ab/Ab*
10 *Ab/Ab/Ab/ab*	27 *Ab/Ab/aB/ab*
11 *Ab/Ab/ab/ab*	28 *AB/AB/aB/ab*
12 *Ab/ab/ab/ab*	29 *AB/aB/aB/Ab*
13 *aB/aB/aB/ab*	30 *AB/ab/ab/aB*
14 *aB/aB/ab/ab*	31 *AB/Ab/Ab/aB*
15 *aB/ab/ab/ab*	32 *AB/AB/Ab/ab*
16 *AB/AB/Ab/Ab*	33 *Ab/aB/aB/ab*
	34 *AB/AB/ab/ab*
	35 *Ab/Ab/aB/aB*

From each possible zygotic genotype, from the 35 observed in **Appendix-Table 6.4**, the probability of each of the 11 possible gametes was calculated based on **Appendix-Table 6.2**. The analysis began with a parental genotype AB/ab/ab/ab, then its descendant was evaluated over 4 generations.

C.1.1. Generation 0 - Case *AB/ab/ab/ab*

For two loci *A* and *B*, each with 2 alleles, 10 gametic genotypes can be formed: *AB/AB, AB/Ab, AB/aB, AB/ab, Ab/Ab, Ab/aB, Ab/ab, aB/aB, aB/ab, and ab/ab* (**Appendix-Table 6.5**), which can be grouped into the 11 gamete modes. In generation 0, for simplicity, it was assumed that the parental zygotic genotype was *AB/ab/ab/ab*. From this parental genotype, the probability of the 10 gametic genotypes (classified into the 11 gametes mode according to **Appendix-Table 6.2**) was calculated, first at equilibrium (*r* = 0.75) and without double reduction (**Appendix-Table 6.5**).

Appendix-Table 6.5 Probability of each gamete genotype from a parental genotype *AB/ab/ab/ab* when *A* and *B* are at equilibrium (*r* = 0.75) and without any double reduction. The table shows the probability of each possible gamete and the probability of each of the 11 gametic mode documented by Fisher.

			Possible gametes inside each mode and its probabilities.									
			1	2	3	4	5	6	7	8	9	10
Number	**Mode**	**Probability**	**AB/AB**	**AB/Ab**	**AB/aB**	**AB/ab**	**Ab/Ab**	**Ab/aB**	**ab/Ab**	**aB/aB**	**aB/ab**	**ab/ab**
1	AiBi/AiBi	0.000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	AiBj/AiBj	0.000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	AiBi/AiBj	0.000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	AiBj/AiBk	0.000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	AiBi/AjBi	**0.125**	0.0000	0.0000	**0.0313**	0.0000	0.0000	0.0000	**0.0313**	0.0000	0.0000	**0.0625**
6	AiBj/AkBi	**0.125**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0625**	**0.0313**	0.0000	**0.0313**
7	AiBi/AjBj	**0.063**	0.0000	0.0000	0.0000	**0.0313**	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0313**
8	AiBi/AjBk	**0.250**	0.0000	0.0000	0.0000	**0.0625**	0.0000	0.0000	**0.0625**	0.0000	**0.0625**	**0.0625**
9	AiBj/AjBi	**0.063**	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0313**	0.0000	0.0000	0.0000	**0.0313**
10	AiBj/AjBk	**0.250**	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0625**	**0.0625**	0.0000	**0.0625**	**0.0625**
11	AiBj/AkBl	**0.125**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0625**	0.0000	**0.0625**	0.0000
		Total	0.00000	0.0000	**0.0313**	**0.0938**	0.0000	**0.0938**	**0.2813**	**0.0313**	**0.1875**	**0.2813**
		Grand total	1									

Then, the probability of each haploid genotype was calculated with the probability of each gametic genotype, as follows:

- $P(AB) = P(AB/AB) + \frac{P(AB/Ab)}{2} + \frac{P(AB/aB)}{2} + \frac{P(AB/ab)}{2}$
- $P(Ab) = P(Ab/Ab) + \frac{P(AB/Ab)}{2} + \frac{P(Ab/aB)}{2} + \frac{P(AB/ab)}{2}$
- $P(aB) = P(aB/aB) + \frac{P(AB/aB)}{2} + \frac{P(Ab/aB)}{2} + \frac{P(aB/ab)}{2}$
- $P(ab) = P(ab/ab) + \frac{P(AB/ab)}{2} + \frac{P(Ab/ab)}{2} + \frac{P(aB/ab)}{2}$

As a result, the probability of each haploid genotype was (**Appendix-Table 6.6**):

Appendix-Table 6.6 Probability of each haploid genotype from a parental genotype *AB/ab/ab/ab* when *A* and *B* are at equilibrium (*r* = 0.75) and without any double reduction.

Haploid genotype	Probability
AB	0.0625
Ab	0.1875
aB	0.1875

Then, the same methodology was applied to calculate the haploid genotypes when $r = 0.25$ and 0.5 and $\alpha = 0.1$ and 0.2 (**Appendix-Table 6.7**):

Appendix-Table 6.7 Linkage disequilibrium at G0. This table shows the probability of each haploid genotype and linkage disequilibrium values under different values of *r* (0.25, and 0.50) and *α* (0.1 and 0.2).

r and α values	Haploid genotypes			
	AB	***Ab***	***aB***	***ab***
r = 0.75 and *α* = 0 At equilibrium	0.0625	0.1875	0.1875	0.5625
r = 0.25 and *α* = 0.1 Linkage disequilibrium	0.1875 0.125	0.0625 -0.125	0.0625 -0.125	0.6875 0.125
r = 0.25 and *α* = 0.2 Linkage disequilibrium	0.1875 0.125	0.0625 -0.125	0.0625 -0.125	0.6875 0.125
r = 0.5 and *α* = 0.1 Linkage disequilibrium	0.125 0.0625	0.125 -0.0625	0.125 -0.0625	0.625 0.0625
r = 0.5 and *α* = 0.2 Linkage disequilibrium	0.125 0.0625	0.125 -0.0625	0.125 -0.0625	0.625 0.0625

C.1.2. Generation 1 (G1)

Assuming random mating, in the following generation, the probability of each zygotic gamete will depend on the combination and the probability of gametic genotype. As an example:

- $P(AB/AB/AB/AB) = P^2(AB/AB)$
- $P(AB/ab/ab/ab) = 2P(AB/ab)P(ab/ab)$
- $P(AB/Ab/ab/ab) = 2P(AB/Ab)P(ab/ab) + 2P(AB/ab)P(Ab/ab)$
- $P(AB/AB/ab/ab) = 2P(AB/AB)P(ab/ab) + P^2(AB/ab)$

According to G0, the probability of the 10 gametic genotypes at equilibrium was shown in **Appendix-Table 6.5**. With these probabilities, the probability of each zygotic genotype in G1 and the probability of their respective gametic genotypes that each one is formed was calculated. The probability of each gametic genotype was calculated as its probability to be formed in the gamete per the probability of the zygotic (parental) genotype. With the probability of each gametic genotype, the probability of each haplotype was calculated in the population, as previously. At equilibrium and without double reduction, the haplotype frequencies were the same as in G0 (**Appendix-Table 6.8**).

Appendix-Table 6.8 Probabilities in the generation 1. This table shows the zygotic, gametic, and haplotype probabilities calculated in G1 at equilibrium and when there is no double reduction in G0.

Generation 1 (G1)			Gamete probability in G_1										Haplotype Probability			
	Zygote genotypes	Zygote probability	*AB/AB*	*AB/Ab*	*AB/aB*	*AB/ab*	*Ab/Ab*	*Ab/aB*	*ab/Ab*	*aB/aB*	*aB/ab*	*ab/ab*	*AB*	*Ab*	*aB*	*ab*
1	*AB/AB/AB/AB*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	*Ab/Ab/Ab/Ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	*aB/aB/aB/aB*	0.0010	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000
4	*ab/ab/ab/ab*	0.0791	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0791	0.0000	0.0000	0.0000	0.0791
5	*AB/AB/AB/Ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
6	*AB/AB/AB/aB*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7	*AB/AB/aB/aB*	0.0010	0.0002	0.0000	0.0007	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0005	0.0000	0.0005	0.0000
8	*AB/aB/aB/aB*	0.0020	0.0000	0.0000	0.0010	0.0000	0.0000	0.0000	0.0000	0.0010	0.0000	0.0000	0.0005	0.0000	0.0015	0.0000
9	*AB/Ab/Ab/Ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
10	*Ab/Ab/Ab/ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
11	*Ab/Ab/ab/ab*	0.0791	0.0000	0.0000	0.0000	0.0000	0.0132	0.0000	0.0527	0.0000	0.0000	0.0132	0.0000	0.0396	0.0000	0.0396
12	*Ab/ab/ab/ab*	0.1582	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0791	0.0000	0.0000	0.0791	0.0000	0.0396	0.0000	0.1187
13	*aB/aB/aB/ab*	0.0117	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0066	0.0044	0.0007	0.0000	0.0000	0.0088	0.0029
14	*aB/aB/ab/ab*	0.0527	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0132	0.0264	0.0132	0.0000	0.0000	0.0264	0.0264
15	*aB/ab/ab/ab*	0.1055	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0066	0.0396	0.0593	0.0000	0.0000	0.0264	0.0791
16	*AB/AB/Ab/Ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
17	*AB/Ab/aB/ab*	0.0352	0.0015	0.0029	0.0059	0.0059	0.0015	0.0059	0.0059	0.0015	0.0029	0.0015	0.0088	0.0088	0.0088	0.0088
18	*AB/AB/AB/ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
19	*AB/ab/ab/ab*	0.0527	0.0000	0.0000	0.0016	0.0049	0.0000	0.0049	0.0148	0.0016	0.0099	0.0148	0.0033	0.0099	0.0099	0.0297
20	*Ab/Ab/Ab/aB*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
21	*Ab/aB/aB/aB*	0.0059	0.0000	0.0000	0.0016	0.0005	0.0000	0.0005	0.0002	0.0016	0.0011	0.0002	0.0011	0.0004	0.0033	0.0011

22	*Ab/ab/ab/aB*	0.1582	0.0000	0.0000	0.0049	0.0148	0.0000	0.0148	0.0445	0.0049	0.0297	0.0445	0.0099	0.0297	0.0297	0.0890
23	*AB/Ab/Ab/ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
24	*AB/aB/aB/ab*	0.0176	0.0000	0.0000	0.0049	0.0016	0.0000	0.0016	0.0005	0.0049	0.0033	0.0005	0.0033	0.0011	0.0099	0.0033
25	*AB/AB/Ab/aB*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
26	*AB/ab/ab/Ab*	0.0527	0.0005	0.0033	0.0022	0.0066	0.0049	0.0066	0.0198	0.0005	0.0033	0.0049	0.0066	0.0198	0.0066	0.0198
27	*Ab/Ab/aB/ab*	0.0527	0.0005	0.0033	0.0022	0.0066	0.0049	0.0066	0.0198	0.0005	0.0033	0.0049	0.0066	0.0198	0.0066	0.0198
28	*AB/AB/aB/ab*	0.0059	0.0005	0.0004	0.0022	0.0007	0.0001	0.0007	0.0002	0.0005	0.0004	0.0001	0.0022	0.0007	0.0022	0.0007
29	*AB/aB/aB/Ab*	0.0059	0.0005	0.0004	0.0022	0.0007	0.0001	0.0007	0.0002	0.0005	0.0004	0.0001	0.0022	0.0007	0.0022	0.0007
30	*AB/ab/ab/aB*	0.0527	0.0000	0.0000	0.0066	0.0066	0.0000	0.0066	0.0066	0.0066	0.0132	0.0066	0.0066	0.0066	0.0198	0.0198
31	*AB/Ab/Ab/aB*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
32	*AB/AB/Ab/ab*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
33	*Ab/aB/aB/ab*	0.0527	0.0000	0.0000	0.0066	0.0066	0.0000	0.0066	0.0066	0.0066	0.0132	0.0066	0.0066	0.0066	0.0198	0.0198
34	*AB/AB/ab/ab*	0.0088	0.0004	0.0007	0.0015	0.0015	0.0004	0.0015	0.0015	0.0004	0.0007	0.0004	0.0022	0.0022	0.0022	0.0022
35	*Ab/Ab/aB/aB*	0.0088	0.0004	0.0007	0.0015	0.0015	0.0004	0.0015	0.0015	0.0004	0.0007	0.0004	0.0022	0.0022	0.0022	0.0022
Total		1	0.0046	0.0117	0.0456	0.0586	0.0254	0.0586	0.2539	0.0592	0.1523	0.3301	0.0625	0.1875	0.1875	0.5625

With the same procedure, the probability of each haplotype and linkage disequilibrium were calculated for different *r* (0.1, 0.25, and 0.50) and *α* (0.1 and 0.2) values (**Appendix-Table 6.9**).

Appendix-Table 6.9 Linkage disequilibrium at G1. This table shows the probability of each haploid genotype and the linkage disequilibrium values under different values of *r* (0.1, 0.25, and 0.50) and *α* (0.1 and 0.2).

r and α values	Haploid genotypes			
	AB	***Ab***	***aB***	***ab***
r = 0.75 and *α* = 0 (Indep./Equil.)	0.0625	0.1875	0.1875	0.5625
r = 0.10 and *α* = 0.1	0.2077	0.0423	0.0423	0.7077
Disequilibrium at generation 1	0.1452	-0.1452	-0.1452	0.1452
r = 0.10 and *α* = 0.2	0.2084	0.0416	0.0416	0.7084
Disequilibrium at generation 1	0.1459	-0.1459	-0.1459	0.1459
r = 0.25 and *α* = 0.1	0.1542	0.0958	0.0958	0.6542
Disequilibrium at generation 1	0.0917	-0.0917	-0.0917	0.0917
r = 0.25 and *α* = 0.2	0.1556	0.0944	0.0944	0.6556
Disequilibrium at generation 1	0.0931	-0.0931	-0.0931	0.0931
r = 0.50 and *α* = 0.1	0.0917	0.1583	0.1583	0.5917
Disequilibrium at generation 1	0.0292	-0.0292	-0.0292	0.0292
r = 0.50 and *α* = 0.2	0.0931	0.1569	0.1569	0.5931
Disequilibrium at generation 1	0.0306	-0.0306	-0.0306	0.0306

C.1.3. Linkage disequilibrium across different generations.

Linkage disequilibrium was evaluated until generation 4 (**Appendix-Table 6.10**). Over these generations, the result showed that linkage disequilibrium was influenced by the recombination frequency and double reduction and in every case *D* was symmetric ($D_{AB} = - D_{Ab} = D_{ab} = - D_{aB}$), similar to what occurs in diploids.

Appendix-Table 6.10 *D* under different *r* and *α* values, over 4 generations.

	AB	***Ab***	***aB***	***ab***
r = 0.10 and *α* = 0.1				
G0	0.1625	-0.1625	-0.1625	0.1625
G1	0.1452	-0.1452	-0.1452	0.1452
G2	0.1310	-0.1310	-0.1310	0.1310
G3	0.1186	-0.1186	-0.1186	0.1186
G4	0.1074	-0.1074	-0.1074	0.1074
r = 0.10 and *α* = 0.2				
G0	0.1625	-0.1625	-0.1625	0.1625
G1	0.1459	-0.1459	-0.1459	0.1459
G2	0.1323	-0.1323	-0.1323	0.1323
G3	0.1204	-0.1204	-0.1204	0.1204
G4	0.1096	-0.1096	-0.1096	0.1096
r = 0.25 and *α* = 0.1				
G0	0.1250	-0.1250	-0.1250	0.1250
G1	0.0917	-0.0917	-0.0917	0.0917
G2	0.0697	-0.0697	-0.0697	0.0697
G3	0.0537	-0.0537	-0.0537	0.0537
G4	0.0416	-0.0416	-0.0416	0.0416
r = 0.25 and *α* = 0.2				
G0	0.1250	-0.1250	-0.1250	0.1250
G1	0.0931	-0.0931	-0.0931	0.0931
G2	0.0719	-0.0719	-0.0719	0.0719
G3	0.0561	-0.0561	-0.0561	0.0561
G4	0.0440	-0.0440	-0.0440	0.0440
r = 0.50 and *α* = 0.1				
G0	0.0625	-0.0625	-0.0625	0.0625
G1	0.0292	-0.0292	-0.0292	0.0292
G2	0.0161	-0.0161	-0.0161	0.0161
G3	0.0094	-0.0094	-0.0094	0.0094
G4	0.0056	-0.0056	-0.0056	0.0056
r = 0.50 and *α* = 0.2				
G0	0.0625	-0.0625	-0.0625	0.0625
G1	0.0306	-0.0306	-0.0306	0.0306
G2	0.0175	-0.0175	-0.0175	0.0175
G3	0.0105	-0.0105	-0.0105	0.0105
G4	0.0064	-0.0064	-0.0064	0.0064

C.2. Theoretical analysis of *D* in autotetraploids

D was evaluated algebraically, by taking into account Bennet's formulas (Bennet, 1954) and including the probabilities of the 11 modes of gamete formation in autotetraploids (Fisher 1947, Luo, Zhang,

and Kersey, 2004). This analysis included evaluation of the symmetry of *D*, its relationship with double reduction, and its decay over generations.

C.2.1. Symmetry of *D*

According to Bennet and Crow for autotetraploids, in a population at equilibrium in panmixia, the probability for two alleles from different loci (A and B) is equal to the probability that each allele segregates independently, as in diploids: $P_{AB}\ at\ equilibrium = P_A P_B$(Bennet, 1954; Crow, 1954). This analysis begins with the following equation from Bennet, 1954:

$$\boldsymbol{P_{n+1}AB = P_nAB + \frac{r}{6}[4P_AP_B - 4P_nAB + P_n(Ab/aB) - P_n(AB/ab)]} \textbf{ .... (1)}$$

Where $P_{n+1}AB$ is the probability of the haplotype AB in generation $n+1$.

Let $P_n(Ab/aB) - P_n(AB/ab) = m_n$. In a population, for two loci (A and B) each with two alleles (A, a, B, b), we can have 4 different haplotypes (*AB*, *ab*, *Ab,* and *aB*). If equation (1) continues to be analysed for the other 3 haplotypes, we have:

- $P_{n+1}ab = P_nab + \frac{r}{6}[4P_aP_b - 4P_nab + P_n(aB/Ab) - P_n(ab/AB)]$ .... **(2)**
- $P_{n+1}Ab = P_nAb + \frac{r}{6}[4P_AP_b - 4P_nAb + P_n(AB/ab) - P_n(Ab/aB)]$ .... **(3)**
- $P_{n+1}aB = P_naB + \frac{r}{6}[4P_aP_B - 4P_naB + P_n(ab/AB) - P_n(aB/Ab)]$ .... **(4)**

And:

- $For\ the\ haplotype\ ab$: $P_n(aB/Ab) - P_n(ab/AB) = m_n$
- $For\ the\ haplotype\ Ab$: $P_n(AB/ab) - P_n(Ab/aB) = -m_n$
- $For\ the\ haplotype\ aB$: $P_n(ab/AB) - P_n(aB/Ab) = -m_n$

Then, the algebraic calculation of linkage disequilibrium between the alleles of the respective haplotypes (*AB*, *ab*, *Ab,* and *aB*) will be:

- For haplotype AB, *D* at generation *n+1* will be:

$$\boldsymbol{D_{n+1}AB = P_{n+1}AB\ (observed) - P_{n+1}AB\ (expected, at\ equilibrium)}$$

Replacing $P_{n+1}AB$ from equation **(1)** and the value of $P_{n+1}AB$ at equilibrium:

$$D_{n+1}AB = P_nAB + \frac{r}{6}[4P_AP_B - 4P_nAB + P_n(Ab/aB) - P_n(AB/ab)] - P_AP_B$$

$$D_{n+1}AB = P_nAB\left(\frac{3-2r}{3}\right) - P_AP_B\left(\frac{3-2r}{3}\right) + m_n \times \frac{r}{6}$$

- For haplotype *ab*, *D* at generation *n+1* will be:

$$\boldsymbol{D_{n+1}ab = P_{n+1}ab\,(observed) - P_{n+1}ab\,(expected, at\ equilibrium)}$$

Replacing $P_{n+1}ab$ from equation **(2)** and the value of $P_{n+1}ab$ at equilibrium:

$$D_{n+1}ab = P_nab + \frac{r}{6}[4P_aP_b - 4P_nab + P_n(aB/Ab) - P_n(ab/AB)] - P_aP_b$$

$$D_{n+1}ab = P_nab\left(\frac{3-2r}{3}\right) - P_aP_b\left(\frac{3-2r}{3}\right) + m_n \times \frac{r}{6}$$

$$D_{n+1}ab = (1 - P_nAB - P_nAb - P_naB)\left(\frac{3-2r}{3}\right) - (1-P_A)(1-P_B)\left(\frac{3-2r}{3}\right) + m_n \times \frac{r}{6}$$

$$D_{n+1}ab = \left(\frac{3-2r}{3}\right)(1 - P_nAB - P_nAb - P_naB + P_A + P_B) - P_AP_B\left(\frac{3-2r}{3}\right) + m_n \times \frac{r}{6}$$

$$D_{n+1}ab = \left(\frac{3-2r}{3}\right)(1 - P_nAB - P_nAb - P_naB + P_nAB + P_nAb + P_nAB + P_naB) - P_AP_B\left(\frac{3-2r}{3}\right) + m_n \times \frac{r}{6}$$

$$D_{n+1}ab = P_nAB\left(\frac{3-2r}{3}\right) - P_AP_B\left(\frac{3-2r}{3}\right) + m_n \times \frac{r}{6}$$

- For haplotype *Ab*, *D* at generation *n+1* will be:

$$\boldsymbol{D_{n+1}Ab = P_{n+1}Ab\,(observed) - P_{n+1}Ab\,(expected, at\ equilibrium)}$$

Replacing $P_{n+1}Ab$ from equation **(3)** and the value of $P_{n+1}Ab$ at equilibrium:

$$D_{n+1}Ab = P_nAb + \frac{r}{6}[4P_AP_b - 4P_nAb + P_n(AB/ab) - P_n(Ab/aB)] - P_AP_b$$

$$D_{n+1}Ab = P_nAb\left(\frac{3-2r}{3}\right) - P_AP_b\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}Ab = P_nAb\left(\frac{3-2r}{3}\right) - (P_A)(1-P_B)\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}Ab = \left(\frac{3-2r}{3}\right)(P_nAb - P_A) + P_AP_B\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}Ab = \left(\frac{3-2r}{3}\right)(P_nAb - P_nAB - P_nAb) + P_AP_B\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}Ab = -P_nAB\left(\frac{3-2r}{3}\right) + P_AP_B\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

- For haplotype *aB*, *D* at generation *n+1* will be:

$$\boldsymbol{D_{n+1}aB = P_{n+1}aB\,(observed) - P_{n+1}aB\,(expected, at\ equilibrium)}$$

Replacing $P_{n+1}aB$ from equation **(4)** and the value of $P_{n+1}aB$ at equilibrium:

$$D_{n+1}aB = P_naB + \frac{r}{6}[4P_aP_B - 4P_naB + P_n(ab/AB) - P_n(aB/Ab)] - P_aB$$

$$D_{n+1}aB = P_naB\left(\frac{3-2r}{3}\right) - P_aP_B\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}aB = P_naB\left(\frac{3-2r}{3}\right) - (1 - P_A)(P_B)\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}aB = \left(\frac{3-2r}{3}\right)(P_nAb - P_B) + P_AP_B\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}aB = \left(\frac{3-2r}{3}\right)(P_nAb - P_nAB - P_naB) + P_AP_B\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

$$D_{n+1}aB = -P_nAB\left(\frac{3-2r}{3}\right) + P_AP_B\left(\frac{3-2r}{3}\right) - m_n \times \frac{r}{6}$$

Therefore, if we compare the probability of linkage disequilibrium of the 4 haplotypes, we obtain the following:

$$\boldsymbol{D_{n+1}AB = D_{n+1}ab = -D_{n+1}Ab = -D_{n+1}aB =}$$

$$\boldsymbol{P_nAB\left(\frac{3-2r}{3}\right) - P_AP_B\left(\frac{3-2r}{3}\right) + m_n \times \frac{r}{6}} \quad \textbf{.... (5)}$$

This result demonstrates algebraically that linkage disequilibrium is symmetric in autotetraploids, as in diploids, as it was observed analytically. In equation (5), *D* depends not only on *r*, but also on *m,* which is equal to the subtraction of $P_n(Ab/aB) - P_n(AB/ab)$ in the population.

C.2.2. *D* and its relationship with double reduction

From equation (**5**), *D* can continue to be analysed by calculating algebraically $\boldsymbol{m_n}$ in the population. For two loci each with two alleles, there are 35 phased zygotes genotypes. For these 35 zygotes, the probability $P_n(Ab/aB)$ and $P_n(AB/ab)$ was calculated, in terms of *α* and *r* (**Appendix-Table 6.11**) by employing the probabilities of gamete formation described by Luo *et al*. (Luo *et al*., 2001, Luo, Zhang, and Kersey, 2004) (**Appendix-Table 6.11**).

Appendix-Table 6.11 Probability of $\boldsymbol{P_n(Ab/aB) - P_n(AB/ab)}$ in a population, when bivalent, quadrivalent formation or a mix of them occurs during meiosis. These probabilities were calculated by employing the gamete probabilities described in Luo *et al*., 2001 and 2004. The probability under a mix of bivalent and quadrivalent chromosomal paring in meiosis (column 5) assumes that the proportion of bivalent:quadrivalent is 1:1.

	Phased parental genotype	$\boldsymbol{m_n = P_n(Ab/aB) - P_n(AB/ab)}$		
		Under bivalent formation	**Under quadrivalent formation**	**Under mix of bivalent and quadrivalent (1:1)**
1	AB/AB/AB/AB	0	0	0
2	Ab/Ab/Ab/Ab	0	0	0
3	aB/aB/aB/aB	0	0	0
4	ab/ab/ab/ab	0	0	0
5	AB/AB/AB/Ab	0	0	0
6	AB/AB/AB/aB	0	0	0
7	AB/AB/aB/aB	0	0	0
8	AB/aB/aB/aB	0	0	0
9	AB/Ab/Ab/Ab	0	0	0
10	Ab/Ab/Ab/ab	0	0	0
11	Ab/Ab/ab/ab	0	0	0
12	Ab/ab/ab/ab	0	0	0
13	aB/aB/aB/ab	0	0	0
14	aB/aB/ab/ab	0	0	0
15	aB/ab/ab/ab	0	0	0
16	AB/AB/Ab/Ab	0	0	0
17	AB/Ab/aB/ab	0	0	0
18	AB/AB/AB/ab	-(1-r)/2	$18(1-\propto)(4r-3)/108$	$-3[\frac{(1-\mathrm{r})}{6}-\frac{(1-\propto)(4r-3)}{18}]$

19	AB/ab/ab/ab	-(1-r)/2	$18(1-\propto)(4r-3)/108$	$-3[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
20	Ab/Ab/Ab/aB	(1-r)/2	$-18(1-\propto)(4r-3)/108$	$3[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
21	Ab/aB/aB/aB	(1-r)/2	$-18(1-\propto)(4r-3)/108$	$3[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
22	Ab/ab/ab/aB	(1-r)/6	$-6(1-\propto)(4r-3)/108$	$[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
23	AB/Ab/Ab/ab	-(1-r)/6	$6(1-\propto)(4r-3)/108$	$-[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
24	AB/aB/aB/ab	-(1-r)/6	$6(1-\propto)(4r-3)/108$	$-[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
25	AB/AB/Ab/aB	(1-r)/6	$-6(1-\propto)(4r-3)/108$	$[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
26	AB/ab/ab/Ab	-(1-r)/3	$12(1-\propto)(4r-3)/108$	$-2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
27	Ab/Ab/aB/ab	(1-r)/3	$-12(1-\propto)(4r-3)/108$	$2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
28	AB/AB/aB/ab	-(1-r)/3	$12(1-\propto)(4r-3)/108$	$-2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
29	AB/aB/aB/Ab	(1-r)/3	$-12(1-\propto)(4r-3)/108$	$2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
30	AB/ab/ab/aB	-(1-r)/3	$12(1-\propto)(4r-3)/108$	$-2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
31	AB/Ab/Ab/aB	(1-r)/3	$-12(1-\propto)(4r-3)/108$	$2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
32	AB/AB/Ab/ab	-(1-r)/3	$12(1-\propto)(4r-3)/108$	$-2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
33	Ab/aB/aB/ab	(1-r)/3	$-12(1-\propto)(4r-3)/108$	$2[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
34	AB/AB/ab/ab	-2(1-r)/3	$24(1-\propto)(4r-3)/108$	$-4[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$
35	Ab/Ab/aB/aB	2(1-r)/3	$-24(1-\propto)(4r-3)/108$	$4[\frac{(1-r)}{6}-\frac{(1-\propto)(4r-3)}{18}]$

C.2.2.1. Linkage disequilibrium when only bivalent formation occurs in meiosis.

According to **Appendix-Table 6.11**, when only the formation of bivalents occurs during meiosis, the $P_n(Ab/aB) - P_n(AB/ab)$ in the population will be:

$$P_n(Ab/aB) - P_n(AB/ab) = \sum_{i=1}^{i=35} K_i(1-r)/6$$

Where $K_i = 0, \text{or a multiple of } \pm 1, \pm 2, \pm 3 \text{ or } \pm 4$, because many individuals from the same genotype can be present (or absent) in the population.

If: $\sum_{i=1}^{i=35} K_i = K$, then, $P_n(Ab/aB) - P_n(AB/ab) = K(1-r)/6$, and given equation (5) and considering that $m_n = P_n(Ab/aB) - P_n(AB/ab)$, then:

$D_{n+1}AB = P_nAB\left(\frac{3-2r}{3}\right) - P_AP_B\left(\frac{3-2r}{3}\right) + \frac{K(1-r)}{6} \times \frac{r}{6}$, where *K* is a constant.

As it can be observed, when only bivalents are formed during meiosis, *D* is only affected by recombination frequency, which appears in the factor (3-2*r*)/3.

C.2.2.2. Linkage disequilibrium when only quadrivalent formation occurs in meiosis.

According to **Appendix-Table 6.11**, $P_n(Ab/aB) - P_n(AB/ab)$ in the population will be as follows:

$$P_n(Ab/aB) - P_n(AB/ab) = \sum_{i=1}^{i=35} K'_i(1-\propto)(4r-3)/18$$

Where $K'_i = 0, \text{or a multiple of } \pm 1, \pm 2, \pm 3 \text{ or } \pm 4$, because many individuals from the same genotype can be present (or absent) in the population.

If: $\sum_{i=1}^{i=35} K'_i = K'$, then $P_n(Ab/aB) - P_n(AB/ab) = K'(1-\propto)(4r-3)/18$, and given the equation (5) and considering that $m_n = P_n(Ab/aB) - P_n(AB/ab)$, then:

$$D_{n+1}AB = P_nAB\left(\frac{3-2r}{3}\right) - P_AP_B\left(\frac{3-2r}{3}\right) + \frac{K'(1-\propto)(4r-3)}{18} \times \frac{r}{6}$$

Where $K'_i = 0, \text{or a multiple of } \pm 1, \pm 2, \pm 3 \text{ or } \pm 4$, because many individuals from the same genotype can be present (or absent) in the population.

Here, we observe that *D* depends on the recombination frequency (*r*) and double reduction coefficient (∝).

C.2.2.3. Linkage disequilibrium when mix of bivalent and quadrivalent formation occurs in meiosis.

From equation (2), again, the probability of $m_n = P_n(Ab/aB) - P_n(AB/ab)$ was calculated for each zygotic/parental genotype, but in this case, when a mix of bivalents and quadrivalents are formed during meiosis in an individual, in a proportion of 1:1. According to **Appendix-Table 6.11**, $P_n(Ab/aB) - P_n(AB/ab)$ in the population will be as follows:

$$P_n(Ab/aB) - P_n(AB/ab) = \sum_{i=1}^{i=35} K''_i[\frac{(1-\mathrm{r})}{6} - \frac{(1-\propto)(4r-3)}{18}]$$

Where $K''_i = 0, \text{or multiple of } \pm 1, \pm 2, \pm 3 \text{ or } \pm 4$, because many individuals from the same genotype can be present (or absent) in the population.

If: $\sum_{i=1}^{i=35} K'_i = K'$, then $P_n(Ab/aB) - P_n(AB/ab) = K''[\frac{(1-\mathrm{r})}{6} - \frac{(1-\propto)(4r-3)}{18}]$, and given the equation (5) and considering that $m_n = P_n(Ab/aB) - P_n(AB/ab)$, then:

$$D_{n+1}AB = P_nAB\left(\frac{3-2r}{3}\right) - P_AP_B\left(\frac{3-2r}{3}\right) + K''[\frac{(1-\mathrm{r})}{6} - \frac{(1-\propto)(4r-3)}{18}] \times \frac{r}{6}$$

Here, it is demonstrating again that linkage disequilibrium depends on the recombination frequency (*r*) and double reduction coefficient (∝).

C.2.3. Linkage disequilibrium decay

To calculate *D* decay in autotetraploids, the analysis began again from Bennet (equation **(1)**), from which the following equations can be developed:

$$P_{n+1}AB = P_nAB + \frac{4r}{6}P_AP_B - \frac{4r}{6}P_nAB + (P_n(Ab/aB) - P_n(AB/ab)) \times \frac{r}{6}$$

$$P_{n+1}AB - P_AP_B = P_nAB + \frac{4r}{6}P_AP_B - P_AP_B - \frac{4r}{6}P_nAB + (P_n(Ab/aB) - P_n(AB/ab)) \times \frac{r}{6}$$

$$P_{n+1}AB - P_AP_B = P_nAB(1 - \frac{4r}{6}) - P_AP_B(1 - \frac{4r}{6}) + (P_n(Ab/aB) - P_n(AB/ab)) \times \frac{r}{6}$$

$$D_{n+1}AB = (P_nAB - P_AP_B)(1 - \frac{4r}{6}) + (P_n(Ab/aB) - P_n(AB/ab)) \times \frac{r}{6}$$

This last equation can be described also as:

$$D_{n+1} = D_n(1 - \frac{2r}{3}) + (P_n(Ab/aB) - P_n(AB/ab)) \times \frac{r}{6}$$

that can be also written as:

$$D_{n+1} = D_n\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_n$$

Where: $m_n = P_n(Ab/aB) - P_n(AB/ab)$, and m_n depends on the probability of each parental genotype in the population and may not be constant from one generation to another.

Then, if we evaluate linkage disequilibrium in each generation, we observe that:

- $D_1 = D_0\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_0$

- $D_2 = D_1\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_1 = [D_0\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_0]\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_1$

  $D_2 = D_0\left(1 - \frac{2r}{3}\right)^2 + \frac{r}{6}\left(1 - \frac{2r}{3}\right)m_0 + \frac{r}{6} m_1$

- $D_3 = D_2\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_2$

  $D_3 = [D_0\left(1 - \frac{2r}{3}\right)^2 + \frac{r}{6}\left(1 - \frac{2r}{3}\right)m_0 + \frac{r}{6} m_1]\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_2$

  $D_3 = D_0\left(1 - \frac{2r}{3}\right)^3 + \frac{r}{6}\left(1 - \frac{2r}{3}\right)^2 m_0 + \frac{r}{6}\left(1 - \frac{2r}{3}\right) m_1 + \frac{r}{6} m_2$

- $D_4 = D_3\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_3$

  $D_4 = [D_0\left(1 - \frac{2r}{3}\right)^3 + \frac{r}{6}\left(1 - \frac{2r}{3}\right)^2 m_0 + \frac{r}{6}\left(1 - \frac{2r}{3}\right) m_1 + \frac{r}{6} m_2]\left(1 - \frac{2r}{3}\right) + \frac{r}{6} m_3$

  $D_4 = D_0\left(1 - \frac{2r}{3}\right)^4 + \frac{r}{6}\left(1 - \frac{2r}{3}\right)^3 m_0 + \frac{r}{6}\left(1 - \frac{2r}{3}\right)^2 m_1 + \frac{r}{6}\left(1 - \frac{2r}{3}\right) m_2 + \frac{r}{6} m_3$

Therefore, the following equation for linkage decay results:

$$\boldsymbol{D_n = D_0\left(1-\frac{2r}{3}\right)^n + \frac{r}{6}\left(1-\frac{2r}{3}\right)^{n-1} m_0 + \frac{r}{6}\left(1-\frac{2r}{3}\right)^{n-2} m_1 + \frac{r}{6}\left(1-\frac{2r}{3}\right)^{n-3} m_2 + \ldots + \frac{r}{6}\left(1-\frac{2r}{3}\right)^{0} m_{n-2} + \frac{r}{6} m_{n-1}}$$

Where: $m_n = P_n(Ab/aB) - P_n(AB/ab)$

From this result, it can be observed that LD decay is different from diploids, which only depends on the recombination frequency of the initial population and the generation time: $\boldsymbol{D_n = (1-r)^n D_0}$.

In the case of autetraploids, *D* decays over generations by a factor of $\left(1-\frac{2r}{3}\right)$, which contains the recombination frequency, and *D* decay also depends on double reduction, which is included in the factors $m_0, m_1, m_2, etc.$

D. Discussion

The analysis of linkage disequilibrium is an important component in GWAS studies because linkage disequilibrium determines if a genetic marker is close to the gene responsible for the evaluated phenotype. Moreover, *D* has many other applications, such as determining segments of the genome that are strongly associated, possibly due to selective pressure, in which case *D* will be maintained after many generations (Sved and Hill, 2018). In practice, *D* is also important to determine the number of markers required for the study in a particular species to obtain an adequate mapping resolution (Vos *et al*., 2017).

Although general formulas are applied in the analysis of the LD parameter (Griswold and Williamson., 2017), there is not a mathematical framework that establishes the theory of LD in autotetraploids. In this work, it was formally demonstrated how LD is influenced by the rate of chromosomal recombination and double reduction events. Here, the symmetry of LD was also demonstrated in autotetraploids, which until now was only assumed. Although it was previously described that double reduction may decrease the rate of LD decay in autotetraploids (Griswold and Williamson, 2017), here the relationship with double reduction was fully described for the first time. LD decay was described in terms of double reduction, showing that it is not only dependent on generation time and recombination frequency as in diploids. Further work will be required to validate whether double reduction actually reduces the rate of decay. In addition, in autotetraploids, here we can observe that the recombination affects LD over generations with a factor of $\left(1-\frac{2r}{3}\right)$, different to what occurs in diploids; the same was also found by Gallais (2003), though he did not include both double reduction and recombination frequency in the formulation. The availability of detailed models such as the one developed here specific for quantitative genetic analysis of autotetraploids, will facilitate more accurate population genetic analysis in such species that can incorporate information on the frequency of quadrivalent pairing and hence double reduction events.

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