DOCTORAL (PhD) DISSERTATION

JENY JOSE

Gödöllő

2024

Analysing disease susceptibility genes to generate potato plants resistant to bacterial wilt and late blight

DOI: 10.54598/007080

JENY JOSE

Gödöllő

2024

Doctoral School: Doctoral School of Plant Science

Name: Jeny Jose

Discipline: Plant Science

Head: Lajos Helyes, DSc
Professor, Doctor of the Hungarian Academy of Sciences,
Director General of Gödöllő Campus, MATE

Supervisor(s): Csaba Éva, PhD
Senior research associate
Agricultural Institute, HUN-REN Centre for Agricultural Research

Zsófia Bánfalvi, DSc
Group leader, Doctor of the Hungarian Academy of Sciences
MATE, Institute of Genetics and Biotechnology

....

Approval of the Head of Doctoral School Approval of the Supervisor

……..

Approval of the Supervisor

TABLE OF CONTENTS

ABBREVIATIONS

AAO3: Abscisic Aldehyde Oxidase 3
ABA: Abscisic Acid
AGO: Argonaute
AP1: Antimicrobial Peptide 1
ARLPK: Atypical Receptor-like Pseudokinase
BR: Balatoni Rózsa
Bt: Botond
BSL: BRI1-Suppressor1-Like
CC-NB-LRR: Coiled-Coil-Nucleotide-Binding Leucine-Rich-Repeat
CDPKs: Ca^{2+}-dependent protein kinases
CERK1: Chitin Elicitor Receptor Kinase 1
CG: 'Calalo Gaspar'
CIM: Callus Induction Medium
CR: 'Cruza 148'
CRISPR/Cas: Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-Associated Protein
CsLOB1: Lateral Organ Boundaries 1
CTR: Control
DCL1: Dicer-Like 1
DEG: Differentially Expressed Genes
DES: 'Désirée'
DMR6: Downy Mildew Resistance 6
DND1: Defence, No Death 1
DPI: Days Post-Inoculation
DSBs: Double Strand Breaks
EBD: Enteric B-Defensin
EBEs: Effector Binding Elements
EDR1: Enhanced Disease Resistance 1
EFR: Elongation Factor-Tu Receptor
EF-Tu: Elongation Factor Thermo unstable
EPS: Extracellular Polymeric Substances
ET: Ethylene
ETI: Effector-Triggered Immunity
FDR: False Discovery rate
FLS2: Flagellin Sensitive 2
FW: Fresh weight
GA: Gibberellic acid
GFP: Green Fluorescent Protein
GO: Gene Ontology
GRF: Growth-Regulating Factor
HPLC: High-Performance Liquid Chromatography
HR: Hypersensitive Response
HRc: Homologous Recombination
IAA: Indole-Acetic Acid
INF: Infected
JA: Jasmonic Acid
KEGG: Kyoto Encyclopedia of Genes and Genomes
LRR: Leucine-Rich-Repeat
LysM: Lysin-Motif
MAP: Mitogen-Activated Protein
MAPK: Mitogen-Activated Protein Kinase
MAPK9: MAPK 9
MEKK1: MAPK Kinase Kinase 1
miRNAs: MicroRNAs
MS: Murashige and Skoog
mRNA: messenger RNA
NBS: Nucleotide Binding Site
NHEJ: Non-Homologous End-Joining
NLR: Nucleotide-binding domain and Leucine-rich Repeats
NPR1: Nonexpressor of PR-1
OD: Optical Density
PAL: Phenylalanine Ammonia-Lyase
PAM: Protospacer Adjacent Motif
PAMPs: Pathogen-Associated Molecular Patterns
PCA: Principal Component Analysis
PCD: Programmed Cell Death
PEN1: Penetration 1
PHA: Dihydrophaseic Acid
Pi: *Phytophthora infestans*
PM: Powdery Mildew
POD: Peroxidase
PPO: Polyphenol Oxidase
PR: Pathogenesis-Related
pri-miRNAs: Primary miRNAs
PRRs: Pattern Recognition Receptors
PTI: PAMP-Triggered Immunity
PVA: Potato Virus A
R genes: Resistance genes
RDR6: RNA Dependent RNA polymerase 6
RIN: RNA Integrity Numbers
RIPs: Ralstonia-Injected Proteins
RLK: Receptor-Like Kinase
RNAi: RNA Interference
ROI: Region of Interest
ROS: Reactive Oxygen Species
Rs: *Ralstonia solanacearum*
RRS1: Resistance to Rs 1
Rssc: Ralstonia solanacearum species complex
S genes: Susceptibility genes
SA: Salicylic Acid
SAR: Systemic Acquired Resistance
sgRNA: Single Guide RNA
siRNAs: small interfering RNAs
SIM: Shoot Induction Medium
SSNs: Site-Specific Nucleases
STAS: Sulphate Transporter and Anti-Sigma factor antagonist
StDMR1: Solanum tuberosum DOWNY MILDEW RESISTANCE 1
StDMR6: Solanum tuberosum DOWNY MILDEW RESISTANCE 6
StDND1: Solanum tuberosum Defense, No Death 1
StPM1: Solanum tuberosum Plasma Membrane protein 1
StSYR1: Solanum tuberosum Syntaxin-Related1
SULTR: Sulphate Transporter
T2SS: Type II Secretion System
T3SS: Type III Secretion System
TAL: Transcription Activator-Like
TALEN: Transcription Activators Like Effector Nuclease
UPLC-MS: Ultra-Performance Liquid Chromatography-Mass Spectrometry
Xcc: *Xanthomonas citri* subsp. citri
Xoo: *Xanthomonas oryzae* pv. oryzae
ZFN: Zinc Finger Nuclease

1. INTRODUCTION

The intricate interplay between plants and pathogens is a perpetual struggle, with far-reaching consequences, particularly when it comes to crops of paramount significance. One such crop, the potato (*Solanum tuberosum* L.), ranks as the fourth most vital global crop, renowned for its nutritional value, notably its high carbohydrate production. However, the limited genetic diversity within potato cultivars presents a formidable challenge when it comes to developing resistance against devastating pathogens. Two diseases that have left a historical and economic impact are bacterial wilt (BW), caused by the biotrophic pathogen *Ralstonia solanacearum* (*Rs*), and late blight, driven by the hemibiotrophic oomycete *Phytophthora infestans* (*Pi*). Despite sustained efforts in managing these pathogens since their identification in the 19th century, achieving ultimate control has remained elusive, as underscored by historical crises like the Irish famine.

The persistent consumption of potatoes highlights the pressing need for effective remedial measures to mitigate potential crises. Efforts to induce resistance through traditional breeding methods, particularly by incorporating genetic diversity from wild varieties, have encountered successes but also significant challenges. Environmental variations, pathogen heterogeneity, ploidy, and the intricacies of resistance induction have made it a formidable task.

Pathogens, following their recognition of a suitable host, navigate through the plant's constitutive defence barriers, infiltrate tissues and cell walls, and are then met with a barrage of pathogen-induced defences. Most pathogens, especially biotrophs, require host cooperation to establish compatible interactions, allowing them to form feeding structures such as haustoria within host cells. The genes that facilitate pathogen infection and host compatibility are referred to as susceptibility (S) genes, and their mutation or loss can curtail pathogen success. The emergence of CRISPR/Cas genome editing technology has opened new avenues for controlling plant diseases by targeting these susceptibility genes at the genomic level.

A comprehensive understanding of plant defence mechanisms is pivotal in the identification of susceptibility gene candidates for disease resistance. The remarkable adaptability of pathogens like *Rs* and *Pi*, characterised by their wide host range and resilience, necessitates a comprehensive investigation into their infection strategies, the role of injected effectors, shifts in plant transcriptome and metabolome, and structural modifications. These interactions give rise to notable changes in biological and molecular functions, encompassing activities like cell wall modification, binding, and catalytic activity. Our first research aim was to understand the resistance mechanisms displayed by known resistant potato varieties to *Rs*, such as ‘Calalo Gaspar’ and ‘Cruza 148’, by comparing their responses with that of a commercially grown susceptible

variety like ‘Désirée’. By leveraging insights from the existing literature and transcriptomic data from *Rs*-resistant lines, a list of susceptibility gene candidates could be systematically compiled for both *Rs* and *Pi*.

Polyphenol oxidases (PPOs) are a class of enzymes responsible for catalysing the oxidation of monophenols and o-phenols, and their role in defence against a range of pathogens and herbivores is well-documented. However, the precise role of PPOs in response to *Rs* and *Pi* infections has remained elusive. As part of our research, our second aim was to generate PPO mutants and rigorously analyse their responses when subjected to *Rs* and *Pi* infections.

MiR396, a non-coding RNA molecule ranging from 20-24 nucleotides, is a central regulator in plant biology. It plays a pivotal role in regulating plant growth, development, and responses to environmental stressors and diseases. Notably, miR396's involvement in disease resistance has been observed through its reduced levels in *Rs*-resistant peanut plants and its capacity to suppress hypersensitive responses. In the context of potatoes, miR396 is predicted to target genes associated with disease resistance, including multicystain proteins and NBS-LRR disease resistance proteins (Zhang *et al*., 2013). It had also been known to influence *Pi* colonisation. Given its significance in both *Rs* and *Pi* resistance responses, a key research aim involved generating miR396 mutants and systematically evaluating their resistance responses.

Plant sulphate transporters (SULTR) are central to the absorption and distribution of sulphur, an essential element for plant growth. Recent evidence suggests that plants can strategically withhold sulphur from pathogens as part of their immune response (Criollo-Arteaga *et al.*, 2021). Within this framework, the research sets its sights on analysing *Arabidopsis* SULTR mutants, offering an opportunity to scrutinise their responses to *Rs* in light of the literature that supports the potential of sulphate transporters as susceptibility genes (Cernadas *et al.*, 2014).

This integrated approach aims to contribute to a deeper understanding of plant-pathogen interactions, resistance mechanisms, and the potential for innovative strategies to combat these devastating diseases, ultimately benefiting both producers and consumers contributing to the ultimate aim of this study.

2. OBJECTIVES TO ACHIEVE

1. Analysing resistance mechanisms of *Rs*-resistant potato lines
2. Studying the effect of PPO knockout in tetraploid potato on resistance to *Rs* and *Pi*
3. Studying the effect of miR396 knockout in tetraploid potato on resistance to *Rs* and *Pi*
4. Studying the effect of sulphate transporters in resistance response to *Rs* in *Arabidopsis thaliana*

3. LITERATURE REVIEW

Climate change is expected to elevate the frequency of extreme weather events, presenting a formidable challenge to crop production in agriculture (Fedoroff *et al.*, 2010). The primary impediments to crop yield and the distribution of wild plant species arise from both biotic and abiotic stresses. A myriad of pathogens, including bacteria, fungi, oomycetes, viruses, and nematodes, infect plants displaying varying lifestyles ranging from biotrophs (which derive nutrients from living cells), hemibiotrophs (which derive nutrients with initial biotrophic-like phase and a latter necrotrophic phase) to necrotrophs (which derive nutrients from dead cells) (Hane *et al.*, 2020). These pathogens employ diverse infection strategies, such as intracellular or extracellular methods, targeting different plant tissues such as the xylem, phloem, roots, or leaves. The escalating incidence and severity of plant disease outbreaks pose significant threats with consequences that extend beyond immediate agricultural concerns to encompass substantial risks to global food security and environmental sustainability on a worldwide scale. (Fones *et al.*, 2020; Ristaino *et al.*, 2021).

Potato (*Solanum tuberosum* L. (2n=4x=48)) stands as the world's fourth most important food crop, following rice, wheat, and maize (FAO, 2006; He *et al.*, 2012), with an annual global yield of approximately 330 million tons (Tang *et al.*, 2018). Belonging to the Solanaceae family, it holds a prominent position among vegetable crops in terms of both production quantity and human consumption, playing a vital role in food security (Birch *et al.*, 2012; Devaux *et al.*, 2014). Potatoes are unrivalled in their efficiency in generating food energy, making them indispensable (Karim *et al.*, 2018; Tolessa, 2018).

Despite its significance, the potato faces numerous threats from various pathogens. Many plant viruses, nematodes, bacteria, and fungi are documented as severe pests of the potato crop. Among bacterial diseases, brown rot, bacterial wilt, and blackleg stand out as particularly destructive (Rupp and Jacobsen, 2017; Charkowski *et al*., 2020), while fungal diseases such as early blight, late blight, and powdery mildew pose significant challenges (Rupp and Jacobsen, 2017; Adolf *et al.*, 2019). Additionally, viral pathogens like PVY and PLRV, which cause potato mosaic and leaf roll, can result in complete crop loss (Kreuze *et al.*, 2019).

Plant pathogens significantly contribute to substantial decreases in crop yields. Estimates indicate that approximately 13% to 16% of global crop production is directly lost each year encompassing factors such as yield and quality due to the impact of pathogens (Oerke, 2006; Vurro *et al.*, 2010). Plant resistance becomes even more challenging when dealing with complex and damaging bacterial pathogens like *Ralstonia solanacearum* or persistant oomycetes like *Phytophthora infestans* capable of damaging an entire field.

3.1. *Ralstonia solanacearum* and resistance breeding of plants

Ralstonia solanacearum (*Rs*), a versatile biotrophic phytopathogen, induces the destructive bacterial wilt disease and is found in around 200 host species across over 50 botanical families (Buddenhagen and Kelman, 1964; Hayward, 1991; Patil *et al.*, 2012). Its impact extends beyond banana, eggplant, peanut, pepper, tobacco, and tomato, with potatoes (*Solanum tuberosum* L.) being a significant global target (Elphinstone, 2005; Álvarez *et al.*, 2010). The economic repercussions of *Rs* infections amount to approximately 1 billion USD annually, leading to regional potato crop losses ranging from 10% to 80% (Kinyua *et al.*, 2014; Kurabachew and Ayana, 2017; Karim *et al.*, 2018; Savary *et al.*, 2019). Recognised as the second most impactful plant pathogenic bacterium globally, *Rs* earns this distinction due to its lethal effects, causing wilting in host plants, and its challenging eradication due to prolonged survival and environmental persistence (Van Elsas *et al.*, 2000; Mansfield *et al.*, 2012; Kong *et al.*, 2014)

Since the early 1990s, *Rs* has been classified as a quarantine pest in Europe, and its rapid spread across the continent is linked to the global import of numerous host plants carrying latent infections (Janse *et al.*, 1998, 2004). The distinct classifications of *Rs* can be seen in **Figure 1**, where it is classified into races based on the host range and into biovars on the basis of its ability to metabolise sugar alcohols (mannitol, sorbitol, dulcitol) and disaccharides (sucrose, maltose, lactose) and three hexose alcohols (Denny, 2001). They were further classified into phylotypes based on variations of the internal transcribed spacer region (Fegan and Prior, 2005). The strains of *Rs* responsible for causing brown rot disease in European potatoes fall into race 3 and biovar 2 (R3B2; **Figure 1 A and B**; Buddenhagen *et al.*, 1962; Hayward, 1964), as well as phylotype IIB and sequevar 1 (PIIB-1; Fegan and Prior, 2005). The pathogenicity of *Rs* is contingent upon factors such as extracellular polymeric substances (EPS), flagellin, antioxidant enzymes, type II secretion system (T2SS) secreted proteins (e.g., endoglucanase), and notably, the type III secretion system (T3SS) secreted proteins (Genin and Denny, 2012). Each RSSC strain typically harbours 50-70 T3SS proteins, also known as *Ralstonia*-injected proteins (Rips), which are injected into the host to enable the bacterium to suppress plant defence responses (Block *et al.*, 2008; Remigi *et al.*, 2011; Deslandes and Rivas, 2012). Crucial elements in impeding the dissemination of *Rs* within vascular tissues include physical barriers like the reinforcement of the cell wall and the production of tylose (Grimault and Prior, 1993; Grimault *et al.*, 1994; Nakaho *et al.*, 2004; Caldwell *et al.*, 2017). Substantiating this, Ferreira *et al.* (2017) observed that when comparing various potato clones with different rootstocks for their resistance to *Rs*, the tolerant clones exhibited a lower bacterial load in the stems compared to the susceptible ones. This observation underscores the root-restriction basis of resistance in potatoes.

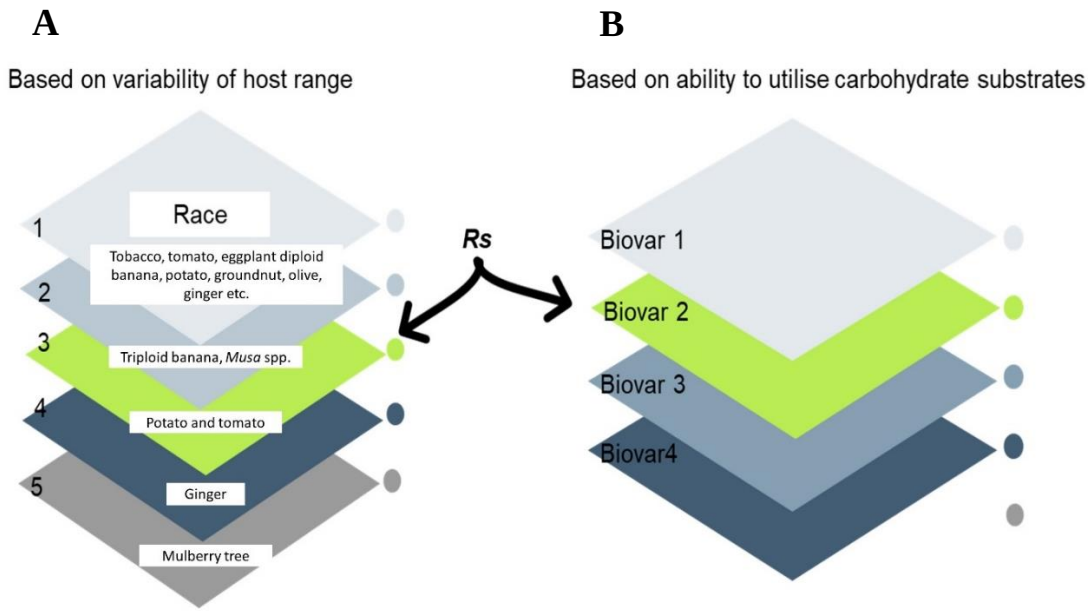


Figure 1. Classification of *Ralstonia solanacearum* spp. complex. Based on variability of host range (A). Based on ability to utilise carbohydrate substrates including glucose, trehalose, mannitol, sorbitol, dulcitol and to oxidise lactose, maltose, and cellobiose (B).

Despite over 2 million years of co-evolution between potato plants (Särkinen *et al.*, 2013; Aversano *et al.*, 2015) and the *Rs* bacterium (Marin *et al.*, 2017), natural resistance or immunity within cultivated *S. tuberosum* genotypes has been exceedingly rare (Nielsen and Haynes, 1960; Jaworski *et al.*, 1980). The pathogenic success of *Rs* is attributed to the coordinated action of various virulence factors and effectors, many of which are delivered into their host(s) through the type II and type III secretion systems (Genin and Denny, 2012; Coll and Valls, 2013; Peeters *et al.*, 2013).

Previously the locus position of two genes responsible for resistance to *Rs* (race 1, biovar 3) was presumed to be on chromosomes 2 and 9 of *S. chacoense*, respectively (Chen *et al.*, 2013). Then, Habe *et al*. (2019) mapped five QTLs associated with resistance in diploid resistant clone induced from 'Saikai 35'. It was hypothesised that *S. phureja* possesses three primary resistance genes (Sequeira and Rowe, 1969), while the resistance in *S. tuberosum* is governed by a recessive resistance gene (Katayama and Kimura, 1987). However, ongoing observations have revealed that the resistance response is significantly influenced by factors such as the bacterial strain, potato genotype, and environmental conditions (Tung *et al.*, 1990b; Watanabe *et al.*, 1999) making it complex. Numerous breeding initiatives were launched in the 1960s to incorporate resistance to *Rs* from wild relatives like *S. phureja* (Thurston and Lozano, 1968; Sequeira and Rowe, 1969), *S. microdontum* (Tyagi *et al.*, 1980), and *S. commersonii* (Carputo *et al.*, 2009; Siri *et al.*, 2009; Andino *et al.*, 2022). Somatic hybridisation was explored due to genetic distance and sexual incompatibility with resistant wild species, aiming to transfer *Rs* resistance from various sources, including *S. phureja, S. stenotomum, S. chacoense, S. commersonii*, and even eggplant (*S. melongena*; Laferriere *et al.*, 1999; Fock *et al.*, 2000, 2001; Kim-Lee *et al.*, 2005; Chen *et al.*,

2013; Yu *et al.*, 2013; Liu *et al.*, 2016; Cao *et al.*, 2020). However, along with successes, these attempts faced challenges such as linkage drag of unfavourable traits, like high-temperature sensitivity in the case of *S. phureja*, resulting in primarily *Rs*-resistant breeding lines rather than commercially successful cultivars (French *et al.*,1998; Patil *et al.*, 2012; Huet, 2014; Park *et al.*, 2016; Muthoni *et al.*, 2020).

The bacterium is drawn to plant root injuries, where it senses the presence of amino and organic acids. Using specialised pili, *Rs* attaches itself to root surfaces and gain entry through wounded or secondary roots, colonise the xylem, forming biofilms that can lead to the partial or complete wilting of the host plant by impeding water transport (Grimault and Prior, 1993; Caldwell *et al.*, 2017). Remarkably, these bacteria can remain viable for extended periods in both water and soil environments. Furthermore, when plants exhibit wilting symptoms, *Rs* can migrate from the roots back into the surrounding soil (reviewed by Wang *et al.*, 2023). In mounting a defence against *Rs*, changes occur in various gene expression, accompanied by a nuanced interplay of secondary metabolites and phytohormones. Notably, the defence pathways activated during *Rs* infection encompass critical processes such as plant-pathogen interaction, secondary metabolite biosynthesis, MAPK signalling, plant hormone transduction, phenylpropanoid biosynthesis, and glutathione metabolism. This comprehensive orchestration underscores the pivotal role these pathways play in fortifying the plant's defence mechanisms (Zuluaga *et al.*, 2015; Jiang *et al.*, 2019; Cao *et al.*, 2020; Li *et al.*, 2021; Pan *et al.*, 2021; Peng *et al.*, 2021; Alariqi *et al.*, 2022; Chen *et al.*, 2022; Jose *et al.*, 2023; Xiao *et al.*, 2023). The understanding of molecular mechanisms related to *Rs* resistance primarily stems from investigations conducted on the model plant *Arabidopsis thaliana* that constitutes initial defence (Kunze *et al.*, 2004). Pattern recognition receptors (PRRs) are membrane-localised receptor proteins that detect pathogens and start defence response resulting in PAMP-triggered immunity (PTI) (Zipfel, 2014). Elongation Factor-Tu Receptor (EFR) is a PRR receptor that triggers PTI in *A. thaliana* upon perception of the *Rs* effector EF-Tu. Besides, EFR, various receptors have been identified that contribute to *Rs* detection and provide resistance in the host plant. These include RRS1 and RPS4 in the *A. thaliana* ecotype 'ND-1', ERECTA in the *A. thaliana* ecotype 'Columbia', Re-bw from eggplant 'E-31', and AhRRS5 from peanut (Deslandes *et al.*, 2003; Godiard *et al.*, 2003; Xi'ou *et al.*, 2015; Zhang *et al.*, 2017a). Ectopic overexpression of the AhRRS5 receptor demonstrated resistance to *Rs* in tobacco (Zhang *et al.*, 2017a). The expression of antimicrobial peptides, such as Antimicrobial Peptide 1 (AP1) isolated from the resistant potato clone MS42.3, Cecropin B, and Shiva, has been shown to enhance resistance against *Rs* (Jia *et al.*, 1993; Xuping *et al.*, 1996). Potato lacks the PRR Ef-Tu receptors that contribute to resistance in *A. thaliana*, but when these were expressed ectopically, they increased resistance to *Rs* (Boschi *et al.*, 2017). RNA interference (RNAi) PAP2

lines exhibit resistance attributed to increased jasmonic acid (JA) expression and reduced salicylic acid (SA) expression (Chakrabarti *et al*., 2019).

Various cell wall mutants/overexpressing lines have demonstrated enhanced resistance to *Rs* upon pathogen exposure (Bellincampi *et al.*, 2014; Miedes *et al.*, 2014; Kesten *et al.*, 2017). Mutants of *Arabidopsis* genes associated with secondary cell wall synthesis, such as irregular xylem mutants *irx 1,3,5*, or mutants with reduced secondary cell wall thickness like *wat1*, activate defence responses against *Rs* in an abscisic acid (ABA) and SA-dependent manner, respectively (Hernández-Blanco *et al.*, 2007; Denancé *et al.*, 2013). Potatoes expressing the *ENTERIC B-DEFENSIN* (*EBD*) gene, possessing antibacterial activity, exhibit higher resistance towards *Rs* in both *in vitro* and greenhouse conditions (Kumar and Chakrabarti, 2017). A chimeric gene comprising the sequence of a *SYNTHETIC TACHYPLESIN I* gene and barley *HORDOTHIONIN* is reported to inhibit *Erwinia caratovora* subspecies in potatoes (Allefs *et al.*, 1996). All of these results indicate the potential of exploiting the same concept of gene regulation to control pathogens, here *Rs*. Additionally, potatoes expressing antimicrobial proteins, SNAKIN1, and SNAKIN2, demonstrated increased resistance to bacterial wilt (Berrocal-Lobo *et al.*, 2002). *Rs*-resistant potato genotypes show promise for engineering resistance through repurposing intrafamily PRRs and identifying key defence mechanisms (Boschi *et al.*, 2017). Comprehensive molecular analyses of resistant genotypes are vital for uncovering target genes to enhance bacterial wilt resistance in potatoes. Editing candidate susceptibility gene candidates using precise genome editing techniques like CRISPR/Cas is a promising approach towards generating resistance (Tyagi *et al.*, 2021).

3.2. *Phytophthora infestans* and resistance breeding of plants

Phytophthora infestans (*Pi*), an oomycete, exhibits a heterothallic nature and functions as a nearly obligatory hemibiotrophic pathogen in both natural and agricultural settings. The asexual cycle facilitates swift population expansion within susceptible host tissue (Aylor *et al.*, 2001). Studies on gene expression in *Pi* have identified genes associated with sporulation (Ah-Fong and Judelson, 2003; Cvitanich and Judelson, 2003; Kim and Judelson, 2003), zoosporogenesis (Tani *et al.*, 2004), and zoospore behaviour (Latijnhouwers *et al.*, 2004).

Late blight, caused by the oomycete *Pi*, stands out as one of the most destructive potato diseases globally. Guenthner *et al.* (2001) estimated late blight's economic impact in the USA at 287.8 million USD, while Haverkort *et al.* (2009) pegged it in the EU at 900 million EUR. Late blight, caused by *Pi*, devastates global potato production, costing up to 10 billion USD annually. Originating in Central Mexico or South America, it has spread to major potato-producing countries like the USA, Canada, China, and India (Fry *et al.*, 2015; Fry, 2016). Late blight remains the primary threat to global food security, especially in regions heavily reliant on potatoes (Pennisi,

2010), causing annual losses of 3-10 billion USD (Haverkort *et al.*, 2009). In developing nations where chemical control is costly, late blight often leads to over 60% yield loss (Dong and Zhou, 2022). The pathogen disseminates through sporangia, airborne asexual spores, propelled by wind and rain. Infection initiates with the germination of a sporangium on a leaf or the development into a zoosporangium, unleashing zoospores that encyst and germinate (Melhus, 1915; Aylor *et al.*, 2001). Subsequently, the emerging germ tube evolves into an appressorium, breaching the leaf's cuticle and epidermis. The infection further proliferates within the leaf tissue through hyphal growth, manifesting as water-soaked lesions that evolve into blackened areas. Within a few days, the afflicted leaf tissue succumbs to necrosis, entering a phase of sporulation. The rapid devastation of an unprotected potato field can unfold within a mere 10 days, contingent on environmental conditions (Fry, 2008). The exceptional pathogenic capability of *Pi* has prompted numerous endeavours to discover or cultivate resistant hosts. Initiatives were launched in the mid-19th century, leading to the development of 'field resistance,' which achieved partial success (Ingram and Williams, 1991). In the early 20th century, the identification of the first resistance (R) genes in *S. demissum* led to initial optimism and a shift away from field resistance efforts against *Pi*. The discovered R genes provided immunity to specific *Pi* strains, recognising effector proteins and inducing host defences, including the hypersensitive response (HR). However, the rise of "resistance-breaking strains" in the pathogen population, initially undetectable, emerged over time, rendering individual R genes ineffective. As new potato cultivars with novel R genes gained popularity, the chance for strains compatible with the R gene to be selected increased. This recurrent pattern has hindered the long-term efficacy of R genes in practical potato late blight suppression in agriculture (Reddick, 1934; Ferris, 1955; Malcolmson, 1969; Ingram and Williams, 1991; Vleeshouwers *et al.*, 2000; Govers and Gijzen, 2006; Fry, 2008). Over 35 potato R genes providing resistance against late blight have been discovered thus far, with the majority encoding proteins characterised by a nucleotide-binding domain and leucine-rich repeats (NLR) (Vleeshouwers *et al.*, 2011; Rodewald and Trognitz, 2013; Vossen *et al.*, 2016; Armstrong *et al.*, 2019; Elnahal *et al.*, 2020). Although R genes from various *Solanum* species, such as *S. berthaultii* (RPi-ber, located on chromosome 10) (Rauscher *et al.*, 2006) and petunias (Becktell *et al.*, 2006), have been explored, their effectiveness is limited due to pathogen population diversity.

In addition to resistance mediated by R genes, the progression of *Pi* infection can be impeded by bolstering the overall defence capacity of the host. This involves activating defence-related genes, accumulating defensive metabolites, and reinforcing the cell wall (Llorente *et al.*, 2014; Du *et al.*, 2015; Yogendra *et al.*, 2017; Kuźnicki *et al.*, 2019). An alternative method for acquiring disease resistance involves targeting plant susceptibility genes (S genes). This concept has been actively employed in the past decade, contributing to the formulation of innovative

breeding approaches for the management of various crop diseases (Eschen-Lippold *et al.*, 2012; Pessina *et al.*, 2014; Sun *et al.*, 2016a, 2016b). Silencing of S gene orthologs of *A. thaliana* including *StDND1*, *StDMR1*, and *StDMR6*, through RNAi in the late blight-susceptible potato cultivar 'Désirée' resulted in enhanced resistance to *Pi* (Sun *et al.*, 2016a, 2022; Luo *et al.*, 2023). This highlights the potential of disrupting the function of presumed S genes to impede infection and leaf colonisation by *Pi*, ultimately conferring late blight resistance in potatoes. Until now, numerous S genes have been delineated in the interplay between *Pi* and potatoes (He *et al.*, 2020). Notably, members of the BRI1-SUPPRESSOR1-like (BSL) family function as susceptibility factors, actively fostering the virulence of *Pi* (Turnbull *et al.*, 2019). Editing of *S. tuberosum PLASMA MEMBRANE PROTEIN 1* (*StPM1)* in potato also resulted in increased resistance to *Pi* (Bi *et al.*, 2023). It is indeed an interesting and important way of engineering disease resistance. However, while generating edited lines, it is important to consider the versatility of the pathogen. It paves the way for multiple infection assays with varying explants to screen for resistance response and establish a reliable infection system. The **Supplementary Table 1** enlists some of the available literatures that direct the infection methodology and evaluation of *Pi*-potato response to aid research.

3.3. Plant defence responses

Plants face continuous exposure to diverse potentially harmful microorganisms. To ward off infections, plants have developed a sophisticated immune system with multiple layers (Chisholm *et al.*, 2006; Jones and Dangl, 2006). Plant immunity relies on innate immune receptors expressed in each cell, recognising invasion signals to initiate either pathogen-associated molecular patterns (PAMPs) triggered immunity (PTI) or effector-triggered immunity (ETI) (Jones and Dangl, 2006) depending on the molecules being recognised. PTI is activated by a vast array of cell surface-localised pattern recognition receptors (PRRs), including leucine-rich-repeat (LRR) receptor-like kinases (RLKs) like FLS2 and EFR, which identify conserved PAMPs like bacterial flagellin and EF-Tu, respectively, and the lysine-motif (LysM) RLK CERK1, essential for recognising fungal chitin-oligomers and bacterial peptidoglycans (Couto and Zipfel, 2016) as can be seen in **Figure 2.** Upon ligand binding, receptors form complexes with coreceptor/adapter kinases, triggering protein phosphorylation cascades involving RLKs and receptor-like cytoplasmic kinases (Yu *et al.*, 2017). PRR signalling encompasses cytosolic Ca^{2+} and apoplastic reactive oxygen species (ROS) bursts, Ca^{2+} dependent protein kinases (CDPKs), mitogen-activated protein kinase (MAPK) cascades, defence hormone networks, and extensive transcriptional and translational changes, callose deposition and metabolic reprogramming (Jones and Dangl, 2006; Boller and He, 2009; Yu *et al.*, 2017). These responses collectively contribute to PTI, which prevents the infection of most microbes and restricts that of adapted pathogens during basal resistance. While PRR

pathways share similarities in signalling modules, they exhibit divergence in rate-limiting steps, influencing their sensitivity to biotic/abiotic perturbations. Simultaneous engagement of multiple PRR pathways is likely to enhance the robustness of overall PTI signalling against both pathogenic attacks and environmental perturbations (Saijo *et al.*, 2018).

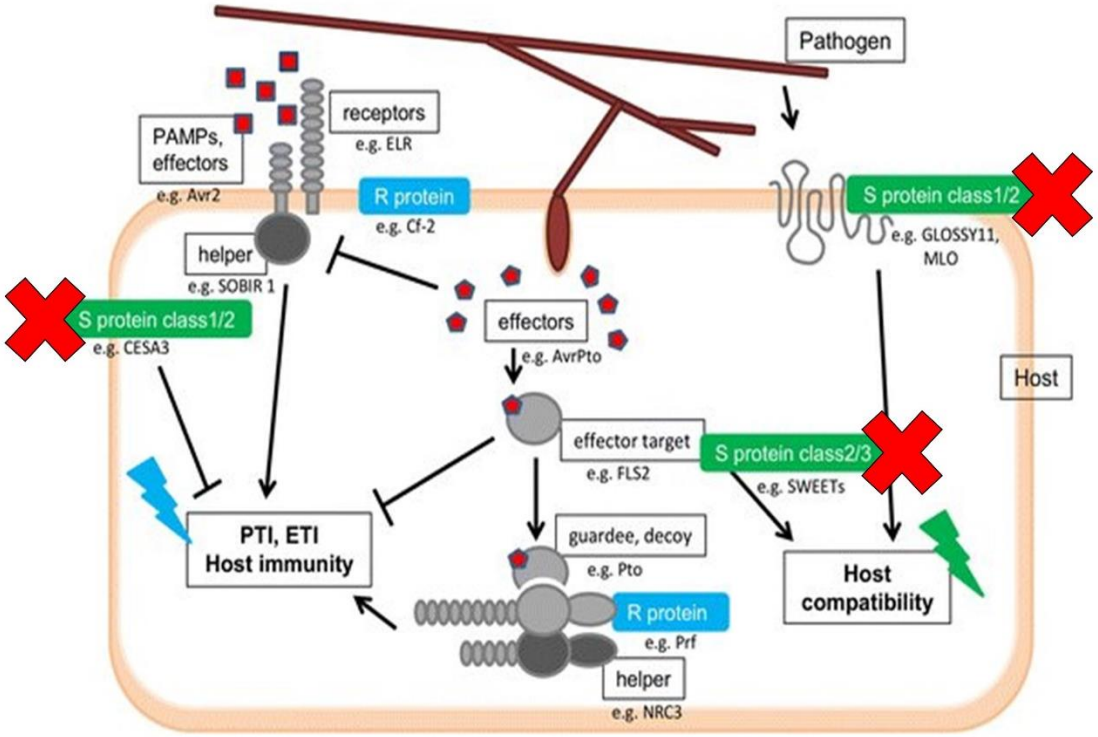


Figure 2. Plant defence mechanisms comprising PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). Receptors located on the plasma membrane recognise apoplastic pathogen-associated molecular patterns (PAMPs/apoplastic effectors) triggering the activation of PAMP-triggered immunity (PTI). Cytoplasmic resistance (R) proteins actively recognise injected intracellular pathogen effectors, or modified effector targets giving rise to effector-triggered immunity (ETI). The co-perception of pathogen-derived components involves helper proteins and guard proteins/decoys. Plant susceptibility genes (S-genes) encode host proteins (S proteins), such as MLO, CESA3, and SWEETs, which play a role in facilitating the entry and growth of pathogens, leading to host compatibility. These S proteins fall into three classes: class 1 genes provide features that aid pathogen entry, class 2 genes enhance innate immunity when disabled, and class 3 genes encode substrates essential for the pathogen. The crosses on the figure taken from Sun *et al.* (2016a) represent the strategy followed to knockout these susceptibility genes to confer resistance.

To evade PTI, pathogenic and mutualistic microbes have developed various effectors that manipulate host immunity and susceptibility components (Toruño *et al.*, 2016; Miwa and Okazaki, 2017). The second level of defence entails plant resistance (R) proteins recognising specific receptors from a pathogen (Avr proteins), resulting in ETI (**Figure 2**). Plants have evolved intracellular receptors, including nucleotide-binding domain and LRR-containing proteins (NLRs, otherwise termed as resistance genes: R-genes), which directly or indirectly via accessory proteins recognise microbial effectors (Dodds and Rathjen, 2010). In the absence of a pathogen-specific receptor, the plant will eventually sense its own damaged components (guard hypothesis) and this will also activate the immune response, nevertheless an early perception of the pathogen could give a plant an advantage and it might decide the outcome of the infection (Dangl and McDowell,

2006). NLR activation leads to an amplified form of defence, often involving localised cell death known as the hypersensitive response (HR). ETI stimulates HRs and triggers programmed cell death (PCD) in infected and surrounding cells (Dangl and McDowell, 2006; Gouveia *et al.*, 2017; Abdul Malik *et al.*, 2020).

ETI and PTI are linked to analogous qualitative changes in transcriptional reprogramming within the host, albeit displaying distinctions in quantity and kinetics (Maleck *et al*., 2000; Tao *et al.*, 2003). Both PTI and ETI signals are often transduced through the MAPK pathway eventually phosphorylating WRKY transcription factors which in turn activate plant effector genes against the pathogen (Ishihama and Yoshioka, 2012). Compared to PTI, ETI is typically more resilient against pathogen-mediated perturbations (Cui *et al.*, 2015; Saijo *et al.*, 2018). Plant immunity also relies on non-cell autonomous signalling, where localised pathogen/damage recognition triggers the rapid generation of long-distance mobile signals transmitted throughout the plant (Hartmann *et al.*, 2018; Toyota *et al.*, 2018). R gene-mediated plant responses can induce systemic acquired resistance (SAR), conferring whole-plant systemic resistance against a broad spectrum of pathogens (Fu and Dong, 2013; Saijo and Loo, 2020). SAR is initiated through local encounters, stimulating resistance in other plant organs to a broad spectrum of pathogens through intraplant communication. SAR is accompanied by the induction or priming of genes encoding pathogenesis-related (PR) proteins, regulated in part through balanced transcriptional control via the SA-binding transcriptional coactivator NPR1 and corepressors NPR3/NPR4 (Ding *et al.*, 2018). To evade ETI, PTI and SAR, pathogen effectors alternately interact with host genes for successful infection. For instance, the transcription activator-like (TAL) effectors of *Xanthomonas* spp. represent an alternative bacterial effector strategy, functioning as transcription factors that induce the expression of specific host genes as seen in **Figure 3**, some of which contribute to symptom development (Kay and Bonas, 2009). Unlike R genes, S genes are essential for the successful infection of pathogens, playing a pivotal role in compatible plant–pathogen interactions. These interactions involve three main molecular mechanisms associated with S genes, as identified by Van Schie and Takken (2014) (i) basic compatibility, aiding in host recognition and penetration; (ii) sustained compatibility, crucial for pathogen proliferation and spread; and (iii) negative regulation of immune signals. Therefore, deploying a strategy to disable S genes is a good approach for plant resistance. A diagrammatic representation of how susceptibility genes work has been entailed in **Figure 3**. The TAL effectors of *Xanthomonas* spp. represent an alternative bacterial effector strategy, functioning as transcription factors that induce the expression of specific host genes, some of which contribute to symptom development (Kay and Bonas, 2009; Boch and Bonas, 2010; Doyle *et al.*, 2013).

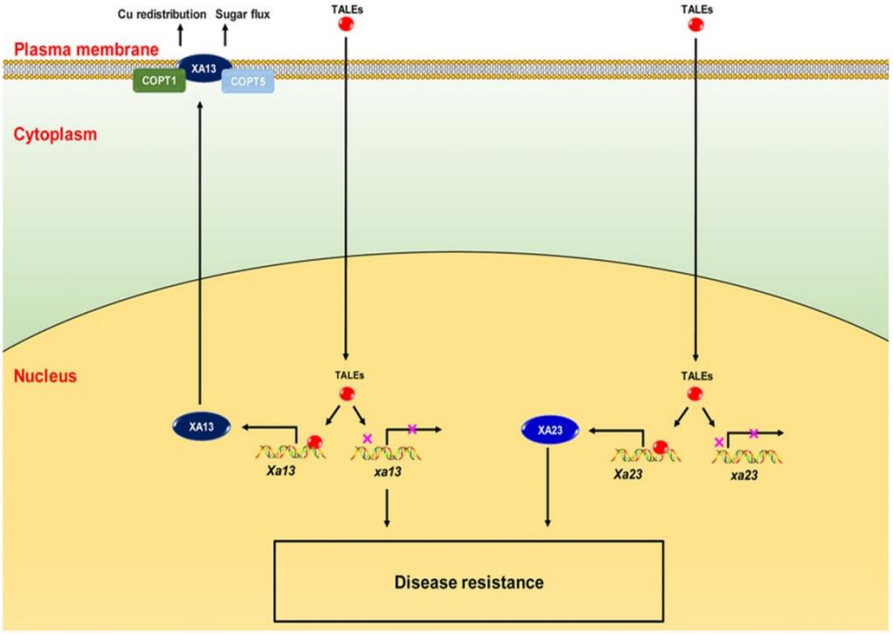


Figure 3. An example of the functioning of S genes. The immune signalling pathways initiated by *Xanthomonas oryzae* involve the SWEET gene *Xa13* and the executor gene *Xa23*. TALEs (Transcription Activator-Like Effectors) are secreted into the cytoplasm of plant cells via the type III secretion system, after which they enter the nucleus, bind to specific promoter elements, and induce the expression of *Xa13*. TALEs exploit XA13 to transport sucrose to the apoplast, providing nutrition to the pathogen. Additionally, TALEs utilise XA13 in conjunction with COPT1 and COPT5 to eliminate toxic Cu from xylem vessels. The resistant allele *xa13*, characterised by mutations in the Effector Binding Elements (EBEs), disrupts the binding of TALEs, resulting in disease resistance. Similarly, akin to SWEET genes, the executor R gene Xa23 undergoes transcriptional activation by TALEs, instigating host defence responses. Figure taken from Jiang *et al.*, (2020).

In rice, the SWEET gene OsSWEET14 is manipulated by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) during bacterial blight infection. *Xoo* utilises endogenous TAL effectors to redirect host sugars, crucial for the pathogen's nutritional needs. Disrupting this interaction, achieved by CRISPR/Cas9, triggers immunity against bacterial blight (Chen *et al.*, 2012a; Zafar *et al.*, 2020).

Over 150 S genes have been documented in *Arabidopsis*, and mounting evidence suggests the existence of orthologs of these S genes in a wide range of crop species (Bai *et al.*, 2008; Van Schie and Takken, 2014; Sun *et al.*, 2016b). The *dnd1* (*Defence, No Death 1*) mutant encoding a cyclic nucleotide-gated cation channel, exhibits resistance against the bacterium *Pseudomonas syringae* pv. *glycinea*, the oomycete *Hyaloperonospora arabidopsidi*s, and fungal pathogens including *Botrytis cinerea* and *Alternaria brassicicola* (Clough *et al.*, 2000; Govrin and Levine, 2000; Jurkowski *et al.*, 2004; Ahn, 2007; Genger *et al.*, 2008; Su'udi *et al.*, 2011). Similarly, mutants of *Arabidopsis* with disrupted *dmr1* (*Downy Mildew Resistance 1*) encoding a homoserine kinase were observed to exhibit resistance against *H. arabidopsidis*, the powdery mildew fungus *Oidium neolycopersici*, and two fusarium pathogens (Van Damme *et al.*, 2008; Huibers *et al.*, 2013; Brewer *et al.*, 2014). In the case of potatoes, reducing expression through RNAi-mediated silencing of the S gene *SYNTAXIN-RELATED1* (*StSYR1*), an *Arabidopsis PEN1* ortholog, led to reduced susceptibility to *Pi* (Eschen-Lippold *et al.*, 2012). Recently, evidence of increased

resistance against *Pi* was reported by CRISPR/Cas editing of *StPM1* in potato (Bi *et al.*, 2023). For over seven decades, the *Mlo* (S gene) mutation has consistently provided resistance to powdery mildew (PM) fungi in the field (Jørgensen, 1992; Freisleben and Lein, 1942; Van Schie and Takken, 2014). *Mlo*, a gene encoding a membrane-associated protein with seven transmembrane domains, is evolutionarily conserved across monocots and dicots and is crucial for PM fungal penetration of host epidermal cells (Acevedo-Garcia *et al.*, 2014).

S genes have also been used to induce immunity against bacterial threats, such as citrus canker caused by *X. citri* subsp. *citri* (Xcc). Mutation of the S gene *LATERAL ORGAN BOUNDARIES 1* (*CsLOB1*) with CRISPR/Cas9 conferred robust resistance against citrus canker, showcasing its critical role in promoting pathogen growth and pustule formation (Peng *et al.*, 2017). Similarly, the S locus *DOWNY MILDEW RESISTANCE 6* (*DMR6*) has been exploited to trigger broad-spectrum resistance against multiple pathogens (Zeilmaker *et al.*, 2015; de Toledo Thomazella *et al.*, 2016). In rice, the SWEET gene *OsSWEET14* is manipulated by *Xoo* during bacterial blight infection. *Xoo* utilises endogenous TAL effectors to redirect host sugars, crucial for the pathogen's nutritional needs. Disrupting this interaction, achieved by CRISPR/Cas9, triggers immunity against bacterial blight (Antony *et al.*, 2010; Chen *et al.*, 2012a).

3.3.1. Genome editing towards generating resistance in plants

Employing genome editing by means of site-specific nucleases (SSNs) to identify particular DNA sequences, is a technique that involves introducing DNA double strand breaks (DSBs) at a specific genomic region or target site, initiating host DNA repair pathways. The presence of DSBs in DNA is extremely harmful, by causing the arrest of DNA-replication. For their repair, higher eukaryotes prefer the error-prone non-homologous end-joining (NHEJ), it is their dominant repair pathway even in the presence of a sister chromosome where perfect repair would also be possible via homologous recombination (HRc) using the sequence information from the sister chromosome (Voytas and Gao, 2014). Explanations include the time requirement of the two methods (~30 mins for NHEJ compared to ~24h for HRc) and the huge genome size of higher eukaryotes, most of which is non-coding so the effect of most mutations would be minimal (Kanaar *et al.*, 1998; Schaefer, 2001; Kass and Jasin, 2010). However we can use this phenomenon to our advantage by mutating coding regions which frequently results in the creation of a dysfunctional allele, thereby modifying the causal trait (Zaidi *et al.*, 2018). When a donor DNA template is available, the HRc pathway is prone to repairing double-stranded DNA breaks (DSBs), leading to accurate alterations at the base level or complete gene replacement. At present, genome editing relies on three primary categories of sequence-specific nucleases: zinc finger nucleases (ZFNs), transcription activators like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) system (Yin and Qiu, 2019).

Genome editing, with its unique advantages, holds the promise to overcome the limitations inherent in conventional resistance breeding methods. Firstly, genome editing allows for the direct modification of target genes in elite varieties, bypassing the time-consuming mating procedures involved in traditional breeding approaches. Secondly, the independence of plant populations with ample genetic variation is not a prerequisite; only the sequence information of the target gene is essential. Thirdly, genome editing ensures alterations are confined to the target sites, mitigating the risk of unintended changes or linkage drag. Fourthly, the application of genome editing in resistance breeding obviates the need for complex genetic crosses and the subsequent selection of segregant progeny, facilitating rapid advancements in crop improvement as reviewed by Yin and Qiu (2019). This strategic application of genome editing holds tremendous potential for revolutionising crop disease management practices. Genome editing provides diverse approaches to enhance plant R genes. Techniques such as base editing enable precise amino acid substitutions in the nucleotide-binding site domain of resistance proteins, resembling naturally resistant wild counterparts (Stirnweis *et al.*, 2014; Kim, 2018). Additionally, CRISPR/Cas9-mediated knock-in offers a method for stacking R genes, providing broad-spectrum resistance (Giannakopoulou *et al.*, 2015; Wolter *et al.*, 2018). Another avenue involves modulating gene expression (Cao *et al.*, 2007; Chintamanani *et al.*, 2008; Chai *et al.*, 2017) through CRISPR activation (CRISPRa) or CRISPR interference (CRISPRi) techniques, enabling refined control over resistance gene expression levels (Yin and Qiu, 2019).

The CRISPR/Cas system, rooted in an adaptive immune mechanism that eliminates foreign plasmids or viral DNA by inducing cleavage within bacteria and archaea, has garnered significant attention (Marraffini and Sontheimer, 2010). This genome editing technology utilises a combination of a single guide RNA (sgRNA) and a Cas protein with nuclease activity. The sgRNA features a scaffold for binding with the Cas protein and a user-defined spacer sequence for precise targeting of genomic sequences. Notably, the simplicity, high efficiency, and user-friendly nature of the CRISPR/Cas system have propelled its applications far beyond those of ZFNs and TALENs in various organisms, including plants (Pennisi, 2013). The CRISPR/Cas system's primary constraint lies in the protospacer adjacent motif (PAM)-dependent cleavage of the target sequence. However, the identification and engineering of numerous CRISPR/Cas systems with diverse PAM specificities, such as CRISPR/saCas9, CRISPR/Cpf1 (Cas12a), and xCas9, have expanded its utility beyond the original CRISPR/Cas9 from *Streptococcus pyogenes* (Wrighton, 2018). Additionally, the CRISPR toolbox has been enriched by RNA-targeting systems, including CRISPR/Cas13a (C2c2), CRISPR/Cas13b, and CRISPR/Cas13d, each offering unique functionalities (Abudayyeh *et al.*, 2016; Smargon *et al.*, 2017; Yan *et al.*, 2018). It is a useful tool for editing plant genomes (Wright *et al.*, 2016) that can be applied across different plant species

to induce resistance (Jiang *et al.*, 2013; Wang *et al.*, 2014; Zaidi *et al.*, 2016; Char *et al.*, 2017). Recently applications of CRISPR/Cas9 technology have successfully mutated *Mlo*, conferring PM resistance in wheat and non-transgenic tomato systems (Shan *et al.*, 2013; Wang *et al.*, 2014; Nekrasov *et al.*, 2017). The susceptibility gene *ENHANCED DISEASE RESISTANCE 1* (*EDR1*), encoding a Raf-like mitogen-activated protein, has also been targeted with CRISPR/Cas9, resulting in a significant reduction of PM in wheat (Zhang *et al.*, 2017b) highlighting the scope of precise genome editing of plant S genes to explore and induce resistance. Successful genome editing necessitates knowledge of host genome sequences and molecular details about the target gene. While an increasing number of plant species have been fully sequenced, extensive genetic and molecular studies have unveiled finer details of plant innate immunity, providing a growing array of targets for pest and disease prevention and control. Negative regulators of plant disease resistance, the host S genes, emerge as promising targets for genome editing, with a particular focus on enhancing disease resistance traits. This is especially pertinent as bacterial diseases pose significant challenges in terms of control, particularly once epidemics have established, owing to the diverse nature, rapid multiplication, and various modes of spread exhibited by bacterial pathogens. Given the potential durability of S genes in field conditions, they have gained popularity as prime candidates for genome editing strategies aimed at developing crops resistant to bacterial diseases. Utilising the functional similarities of S genes across different plant species, genome editing enables the creation of desired mutant S genes in a wide range of plants for breeding, bypassing the need to account for species-specific barriers. The fundamental concept of the disease triangle in plant pathology, which involves a virulent pathogen, a susceptible host, and a conducive environment, highlights the interplay leading to disease manifestation. Genome editing offers a versatile approach by allowing interventions in any of the three factors including S genes, disrupting plant–pathogen interactions and offering a means of disease control.

3.4. Polyphenol oxidases

Polyphenols play a crucial role in defence responses against both biotrophic and hemibiotrophic pathogens, and their resistance-inducing impact is intricately linked to the temporal and spatial dynamics of their accumulation (Nicholson and Hammerschmidt, 1992). Among the key players in this defence network are polyphenol oxidases (PPOs), encompassing tyrosinases (EC 1.14.18.1), o-diphenol: oxygen oxidoreductases, also known as catechol oxidases (EC 1.10.3.1), and laccases (EC 1.10.3.2). The enzymatic browning induced by PPO activity in tubers not only impacts their quality but also raises significant biological and commercial concerns within the realm of potato production. This phenomenon has been a substantial issue, leading to approximately 50% losses in the industrial production of fruits and vegetables (Holderbaum *et al.*, 2010). Specifically in the context of potatoes, during post-harvest, about 20% of the product is

susceptible to compromise due to PPO-mediated browning, significantly affecting consumer acceptance and posing challenges for potato producers (Whitaker and Lee, 1995; Tomás-Barberán and Espín, 2001).

It is noteworthy that, in addition to affecting quality and consumer preference, PPOs have been associated with allergenic effects in certain species (Babu *et al.*, 2017). Moreover, the consequences of food browning extend beyond aesthetic concerns, as it results in the degradation of essential amino acids, reduced digestibility, and the formation of potentially harmful compounds during baking, thereby impacting the nutritional quality of the product (Friedman, 1996). The multifaceted implications of PPO activity highlight the importance of understanding and managing enzymatic browning in crops for both biological and commercial considerations. In the context of potato, genes responsible for *PPO*s exhibit intricate spatial and temporal expression patterns. Notably, *Pot32* (U22921), *Pot33* (U22922), *Nor333* (M95196), and *POT72* (U22923) are reported to be expressed in different parts such as tubers and roots (Thygesen *et al.*, 1995). These copper-containing enzymes, encoded by nuclear genes, primarily operate within plant chloroplasts, with an additional presence in fungi, certain animals, and bacteria (Burke and Cairney, 2002; Mayer, 2006). PPOs catalyse the oxidation of monophenols to o-diphenols and o-phenols to o-quinones, utilising molecular oxygen as a substrate (Van Gelder *et al.*, 1997; Oliveira *et al.*, 2011). The resulting quinones are cytotoxic, capable of cross-linking macromolecules and reducing the nutritional value of alkylated proteins (Felton *et al.*, 1992; Li and Steffens, 2002). Furthermore, these reactive o-quinone products undergo non-enzymatic polymerisation, culminating in the formation of a brown-black coloured melanin-like complex polymer on plant tissues and cell extracts (Golan-Goldhirsh *et al.*, 1984; Duffey and Felton, 1991). PPOs emerge as a dynamic defence system against biotrophic, necrotrophic pathogens, and herbivores, with their expression being induced by SA, JA, and systemin (Nicholson and Hammerschmidt, 1992; Van Gelder *et al.*, 1997; Burke and Cairney, 2002; Li and Steffens, 2002; Mayer, 2006; Oliveira *et al.*, 2011). This defence mechanism involves the formation of melanin complexes that establish a protective barrier, sealing tuber tissues against the entry and spread of pathogens (Johnson *et al.*, 2003). Laccases, a subset of PPOs, demonstrate the ability to oxidise monolignols, producing lignin that reinforces the cell wall, offering an effective protective mechanism against various pathogens (Liu *et al.*, 2020; Bai *et al.*, 2023). Remarkably, studies have shown that tomato plants overexpressing *PPO* exhibit increased resistance to *Pseudomonas syringae* (Li and Steffens, 2002), while the introduction of an antisense *PPO* construct heightens susceptibility (Duffey and Felton, 1991). Additionally, *PPO*-overexpressing strawberries were reported to display enhanced resistance to gray mold (Jia *et al.*, 2016) and Bhonwong *et al.* (2009) observed a decline in the growth rate and nutritional indices of two caterpillar species when reared on tomato plants

overexpressing *PPO*. Further PPO and its involvement with biotic stress has been reviewed by Zhang and Sun, (2021).

While substantial data highlight the protective role of PPOs against pathogens and their multifaceted functions in this defence mechanism, conflicting reports exist. Some studies, such as those by Melo *et al.* (2006) and Richter *et al.* (2012) found no correlation between PPO activity and resistance to fungal pathogens. Conversely, negative relationships were also established in studies like those conducted by Hakimi *et al.* (2002) and Llorente *et al.* (2014). In the context of *Rs*-resistant wild peanuts (*Arachis glabrata*), a significant increase in the expression level of the PPO-targeting miR3508 following *Rs* infection was observed (Zhao *et al.*, 2015). The patent WO2002061101A3 claims that antisense silencing of tuber specific *PPO* lead to enhanced resistance to fungal diseases such as late blight, induced by *Pi* (Hakimi *et al.*, 2002). Notably, the downregulation of *PPO* in potato tubers also led to altered metabolite concentrations, particularly chlorogenate, redirecting phenylpropanoid metabolism and enhancing the accumulation of defensive phenolic compounds, ultimately bolstering pathogenic resistance (Llorente *et al.*, 2014). The enzymatic reaction catalysed by PPOs presents a paradoxical scenario, akin to a double-edged sword. The oxidation resulting from PPO activity implies the loss of chlorogenic acid and other PPO substrates, which are otherwise potent antimicrobial compounds. For instance, chlorogenic acid has been demonstrated to inhibit both *Rs* and *Escherichia coli*, as well as the *Monilia* fungus in peaches (Villarino *et al.*, 2011; Kabir *et al.*, 2014; Yang *et al.*, 2016). Moreover, a myriad of phenolic and polyphenolic compounds have exhibited antibacterial and/or antifungal effects (Coppo and Marchese, 2014; Nakamura *et al.*, 2015; Pagliarulo *et al.*, 2016). This discrepancy in the role of PPOs in pathogen resistance is further complicated by the uncertainty of whether PPOs or the simple phenols/polyphenols (many of which are PPO substrates) represent the more critical and effective defence mechanism. These findings underscore the pivotal role of polyphenols and PPOs in orchestrating a robust defence against diverse pathogens, emphasising their potential for enhancing plant resistance through genetic modification.

Adding to the complexity, certain bacteria and fungi also express *PPO*s, with *Rs* expressing at least three genes exhibiting PPO activity, such as tyrosinases and laccases, contributing to secondary metabolism and defence against phenolic compounds (Hernández-Romero *et al.*, 2005). In the realm of fungal pathogens, melanin, a component of the cell wall, has been shown to protect the fungus from plant immune factors (Pihet *et al.*, 2009). Consequently, the challenge lies in deciphering how to effectively protect plants against *PPO*-expressing pathogens, whether through the activity of PPOs (considering the potential tolerance development by pathogens to PPO products due to their own PPO enzymes) or through the production of simple phenols/polyphenols.

Traditional breeding methods are time-consuming, requiring several years for the development of a new variety (Mullins *et al.*, 2006; Werij *et al.*, 2007). To expedite the process, a more efficient solution involves genetic intervention through genome editing, specifically creating *Pot32* knockout lines in commercial cultivars. This approach aims to produce edited potato lines in a shorter timeframe, simultaneously exploring the relationship between PPO activity and resistance against pathogens such as *Rs* and *Pi* in potatoes. If decreasing PPO activity leads to heightened production of antimicrobial compounds and, consequently, increased resistance to pathogens, this approach offers dual advantages.

3.5 miR396

MicroRNAs (miRNAs) are eukaryotic endogenous, small (20–24 nt) non-coding, single-stranded RNAs serving as vital post-transcriptional regulators in both plant and animal systems. (Finnegan and Matzke, 2003). Primary miRNAs (pri-miRNAs) in plants are generated through transcription by RNA polymerase II from genomic loci dedicated to miRNA (MIR loci, Lee *et al.*, 2004). Following transcription, the extended stem of the looped pri-miRNAs undergoes processing by RNAse III enzymes known as DICER-like 1 (DCL1; Lee *et al.*, 2004; Bologna and Voinnet, 2014). This process involves the formation of hairpin structures (Jones-Rhoades and Bartel, 2004; Gibbings *et al.*, 2009). Following this, mature miRNAs associate with ARGONAUTE (AGO) proteins, forming miRNA/AGO complexes that seek out complementary messenger RNAs (mRNA) for degradation or translational silencing (Tolia and Joshua-Tor, 2007; Mallory *et al.*, 2008; Chen, 2009; Meister, 2013). Interestingly, in animals, approximately 60% of protein-coding genes are regulated by miRNAs but by contrast, only around 1% of plant genes are subject to such a regulation. Still, the effect of miRNA-controlled gene regulation is significant in plants since most targets are transcription factors (Sunkar *et al.*, 2012).

In the plant context, miRNAs play a pivotal role in recognising target mRNAs through highly specific basepairing mechanisms, influencing various processes including development and responses to biotic (bacteria, fungi, oomycetes, etc.) and abiotic stresses (Zhang *et al.*, 2009; Hwang *et al.*, 2011; Li *et al.*, 2017; Chen, 2012; Li *et al.*, 2012; Bologna and Voinnet, 2014; Kumar, 2014; Luan *et al.*, 2015; Chen *et al.*, 2015; Bonar *et al.*, 2018; Natarajan *et al.*, 2018; Jiang *et al.*, 2018; Kondhare *et al.*, 2018; Chen *et al.*, 2019a). Based on multiple studies, it can be concluded that plant microRNAs exert their role on promoting resistance to bacterial pathogens in the following ways: attenuating plant growth and development, promoting early flowering, signalling of IAA (indole-acetic acid), GA (gibberellic acid) and ABA and inhibiting JA but boosting SA-signalling, inducing the synthesis of antimicrobial compounds, and increasing of copper metabolism and hypersensitive response, turning on PTI, ETI and cell wall building (Subramanian *et al.*, 2008; Zhang *et al.*, 2011; Khraiwesh *et al.*, 2012; Pérez-Quintero *et al.*, 2012;

Sunkar *et al.*, 2012; Niu *et al.*, 2016a). The complex and dynamic reprogramming comprising the temporal and intensity of gene regulation during both PTI and ETI in plant immune responses involves the potential involvement of small RNAs, specifically miRNAs (Llave *et al.*, 2002; Brodersen *et al.*, 2008; Bologna and Voinnet, 2014). Moreover, miRNAs, acting as cellular messengers, can be secreted into target cells through vesicles or binding proteins, regulating multiple target genes (Chen *et al.*, 2012b; O'Brien *et al.*, 2018). miRNAs play a direct role in targeting transcripts of major R gene classes, such as NLR and ARLPK (atypical receptor-like pseudokinase) genes crucial for plant defence (Jagadeeswaran *et al.*, 2009; Padmanabhan *et al.*, 2009; Shivaprasad *et al.*, 2012; Boccara *et al.*, 2014; Niu *et al.*, 2016b). In tomatoes and other solanaceous plants, members of the miR482/2118 family play a role in targeting numerous nucleotide binding site-LRR (NBS-LRR) resistance protein genes. The pathogen-encoded RNA silencing suppressor has the capacity to trigger the removal of miR482/2118 in a non-specific manner, thereby alleviating the inhibition on their NBS-LRR gene targets. This regulatory mechanism, as supported by expression profiling in both wild and cultivated tomatoes, ensures that R proteins are expressed at a low level in the absence of pathogen infection but can be rapidly induced upon encountering a pathogen (De Vries *et al.*, 2018). This balance allows for an effective defence strategy in which resistant proteins are poised for swift activation in response to pathogen invasion (Eckardt, 2012).

There are several reports supporting the significant contribution of plant miRNAs to PTI and ETI responses against pathogens (Seo *et al.*, 2013; Deng *et al.*, 2018). Exemplifying their role in plant defence, miR393 regulates immunity against *Pseudomonas syringae* by suppressing auxin signalling (Navarro *et al.*, 2006). Furthermore, miR160, miR825, miR398b, and miR773 have been identified as regulators of immunity against bacterial infection in *Arabidopsis* (Fahlgren *et al.*, 2007; Jagadeeswaran *et al.*, 2009; Antony *et al.*, 2010). The regulatory role of miRNAs in potato, especially concerning their impact on gene expression and defence mechanisms, is a noteworthy subject of discussion. miRNAs in potatoes exhibit a significant role in responding to various pathogens and environmental stresses (Zhang *et al.*, 2009; Hwang *et al.*, 2011; Chen *et al.*, 2015; Bonar *et al.*, 2018; Kondhare *et al.*, 2018). miRNAs influence growth, development, and anthocyanin biosynthesis (Zhang *et al.*, 2009; Bonar *et al.*, 2018). Besides established involvement in drought, salt, and cold stresses, (Hwang *et al.*, 2011; Chen *et al.*, 2015), specific miRNAs, such as stu-miR482d-3p and stu-miR397-5P, have been identified as regulators of virus PVA resistance by targeting PR genes (Li *et al.*, 2017). Additionally, miR160 in potato has been found to regulate *Pi* infection and activate SAR by modulating the SA-mediated resistance response (Natarajan *et al.*, 2018). The involvement of miRNAs in potato defence is further emphasised by their role in targeting R genes within the potato genome, as observed in the case of *Pi* miR8788 targeting potato

StABH1 to promote late blight disease (Hu *et al.*, 2022). Additionally, sRNA sequencing in potato indicates that the transient expression of miR396 facilitates *Pi* colonisation by targeting the *MULTICYSTATIN* gene (Luo *et al.*, 2023). These findings collectively stress on the multifaceted role of miRNA regulation in orchestrating defence responses and gene expression in the context of potato.

The role of miR396 in various stress conditions, including drought (Liu *et al.*, 2008), salt (Liu *et al.*, 2008), cold (Zhou *et al.*, 2008), and hypoxia stress (Zhang *et al.*, 2008), has been established. In terms of pathogen resistance, miR396 evolutionarily targets the *GROWTH-REGULATING FACTOR* (*GRF*) family of transcription factors across plant species, influencing growth, hyposensitivity response, and water saving pathways (Karlova *et al.*, 2013; Gao *et al.*, 2015; Zhang *et al.*, 2015). miR396 overexpression leads to the repression of *GRF* expression (Liu *et al.*, 2009) and the regulation of *GRF*s affects cell division and differentiation during leaf development (Liu *et al.*, 2009; Wang *et al.*, 2011). GRFs also have a proposed role in host reprogramming, resulting in an enhanced immune response like increased H_2O_2 accumulation, and callose deposition upon pathogen perception (Soto-Suárez *et al.*, 2017). The overexpression of miR396 in transgenic *Arabidopsis* plants resulted in a reduction in stomata density, as evidenced by findings from Liu *et al.* (2009). This observed decrease in stomata density suggests that the up-regulation of *miR396* or the down-regulation of its target, *GRF*, may serve as a mechanism employed by plants to mitigate stomatal density. The reduction in stomata density further led to a decrease in transpiration rate (Wang *et al.*, 2007a), subsequently reducing water loss and promoting an increase in relative water content during periods of drought stress (Liu *et al.*, 2009). However, there were no reports of *GRF*s targeted by miR396 in potato. *MIM396 Arabidopsis* plants, with reduced miR396 activity exhibit enhanced resistance to necrotrophic and hemibiotrophic fungal pathogens through a superactivation of defence responses, suggesting a priming event during pathogen infection (Soto-Suárez *et al.*, 2017). miR396, targeting the hypersensitive response protein, was also downregulated upon *Rs* infection in resistant peanut variety indicative of its negative regulation in plant defences (Zhao *et al.*, 2015). Many of miR396 targets as listed in **Table 1** are interesting candidate genes participating in defence responses in potato.

Table 1. Compiled list of predicted miR396 targets in potato based on Zhang *et al.* (2009) and Zhang *et al.* (2013).

Targeted protein	Target function	Accession ID
Ubiquitin activating enzyme	Transcription factor	TC103286
NBS-LRR disease resistance protein homologue	Disease resistance response	BF460277 , TC98672
Membrane-associated salt-inducible protein	Stress responses	CK716485
Prohibitin/PHB1	Stress responses	TC93512
Multicystatin precursor (MC)	Stress responses	BG600937 , TC109112
Glycerol-3-phosphate acyltransferase (GPAT)	Metabolism	CK274104 , TC95254
Cysteine protease	Metabolism	TC92741
Zinc metalloproteinase-like	No data	CN464599
DNA methyltransferase	No data	BE340450
Hypothetical protein SBB1	No data	BE922572
Resistance gene-like	No data	TC111603
BT1 (BTB and TAZ domain protein 1) protein binding/transcription regulator	No data	TC93491 , TC93492
Endomembrane protein EMP70	No data	TC99218
MYB transcription factor	Transcription factor	CK270158, CK861831, C108832, TC94134
MYC-type transcription factor	Transcription factor	TC104774
ATP sulphurylase	Metabolism	BG596849.1, BI176927.1
Protein disulfide isomerase	No data	PGSC0003DMT400006756
GRIP and coiled-coil domain-containing protein	No data	PGSC0003DMT400046956
Tospovirus resistance protein A	No data	PGSC0003DMT400016105
DNA binding protein	No data	PGSC0003DMT400084792
Multicystatin	Stress responses	PGSC0003DMT400015250
Multicystatin	Stress responses	PGSC0003DMT400015251
Multicystatin	Stress responses	PGSC0003DMT400069147
Multicystatin	Stress responses	PGSC0003DMT400069145
Multicystatin	Stress responses	PGSC0003DMT400069146
Multicystatin	Stress responses	PGSC0003DMT400069145
Multicystatin	Stress responses	PGSC0003DMT400069146
DNA binding protein	No data	PGSC0003DMT400049891
DNA binding protein	No data	PGSC0003DMT400049895
DNA binding protein	No data	PGSC0003DMT400049896

Furthermore, CRISPR/Cas9 technology has been employed to directly generate miRNA knockout mutants, demonstrating its efficacy for manipulating miRNA function in plants (Zhao *et al.*, 2016a; Zhou *et al.*, 2017). This approach allows for precise modifications, including single-base pair indels in mature miRNA regions or large deletions abolishing miRNA function (Zhou *et al.*, 2017).

It is promising to edit *miR396* to see its potential effect on defence responses in potato upon being subjected to *Rs* and *Pi*.

3.6 Sulphate transporters

While studying various plant susceptibility factors where the mutation could cause resistance against *Rs* and *Pi*, the prospect of limiting sulphur availability to the pathogens also turned up. As it was previously mentioned, *Xanthomonas* switches on plant genes with TAL effectors including sugar and sulphur transporters to feed itself (Garcia-Ruiz *et al.*, 2021). Therefore, knock out of such transporters seemed an obvious choice for increasing plant resistance against pathogens. Sulphur plays a crucial role in plant biology, serving as an essential element involved in various biological processes such as the biosynthesis of sulphur-containing amino acids, cysteine and methionine, conferring resistance against diseases and pests, and participating in the detoxification of reactive oxygen species, xenobiotics, and heavy metals (Leustek *et al.*, 2000; Saito, 2000; Xiang *et al.*, 2001; Takahashi *et al.*, 2011; Álvarez *et al.*, 2012). In the soil and water, sulphur commonly exists in the form of sulphate ions (SO_4^{2-}) (Takahashi *et al.*, 2011). Higher plants have evolved a family of sulphate transporters, known as SULTRs, to mediate the absorption of sulphate from the soil and its distribution throughout the organism (Smith *et al.*, 1995; Yoshimoto *et al.*, 2002). In plants, the *SULTR* gene family is thought to encode integral membrane proteins functioning as secondary active transporters. These proteins facilitate the movement of protons (H^+) along a favourable electrochemical gradient, powering the transport of anions against an unfavourable electrochemical gradient (Smith *et al.*, 1995; Wang *et al.*, 2021). The water-soluble sulphate ion (SO_4^{2-}), is taken up from the soil by a family of proton-coupled and high-affinity sulphate transporters expressed in root hairs and several cellular compartments of the root (**Figure 4A**). The assimilated sulphate is then transported by low-affinity transporters via the vascular system to the shoots and the reproductive organs for further metabolism (**Figure 4A and B**) (Saito, 2000). The end products of this processing are sulphur-containing amino acids (Cyseine, Methionine), glutathione, glucosinolates, sulphated enzymatic cofactors, etc.

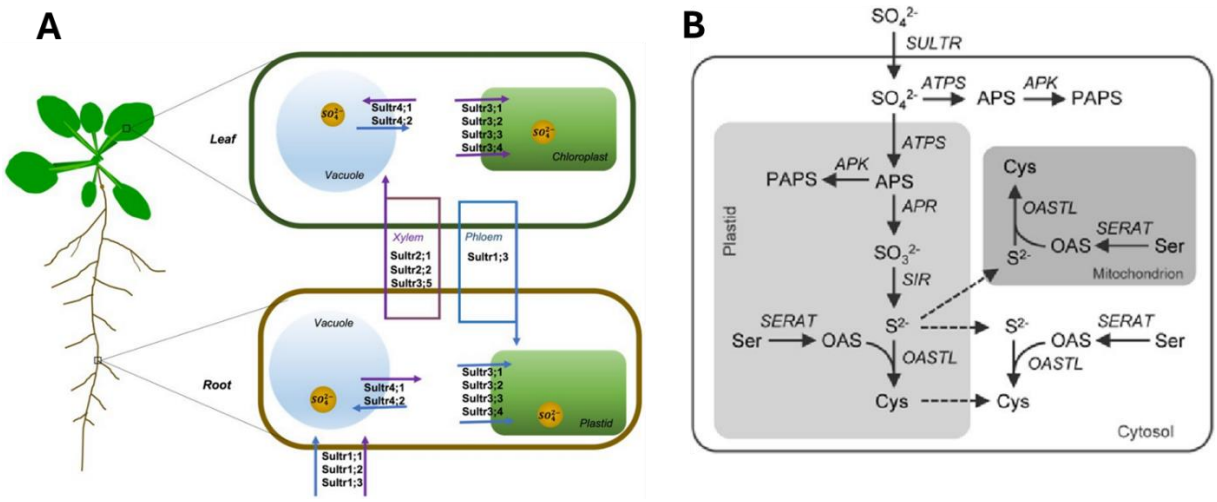


Figure 4. High-affinity sulphate transporters in various cell types of plant roots (A) and general overview of the sulphur metabolism in higher plants (B). (Wang *et al.*, 2022a)

The SULTR family, named for its involvement in sulphate uptake from the soil and internal movement within the plant, comprises various isoforms with diverse functions (Takahashi *et al.*, 2000; Kataoka *et al.*, 2004a). The dynamic functions of SULTRs are influenced by their distinct localisation patterns and substrate affinities, impacting physiological processes such as stomatal regulation, callose deposition, and defence hormone signalling (Zörb *et al.*, 2022). SULTRs are categorised into four subfamilies, exhibiting approximately 60% amino acid sequence similarity. The SULTR1 and SULTR2 subfamilies facilitate sulphate uptake in the root and transport from the root to the shoot (Takahashi *et al.*, 2000; Shibagaki *et al.*, 2002). These transporters have been extensively studied, with the SULTR3 subfamily playing a crucial role in sulphate uptake into chloroplasts, influencing ABA biosynthesis and the stress response (Cao *et al.*, 2013; Chen *et al.*, 2019b). SULTR3 is also the largest sub-group and includes isoforms with functions in phosphate transport and seed phytate accumulation (Zhao *et al.*, 2016b; Yamaji *et al.*, 2017). Individual SULTR3 isoforms localise to the plasma membrane, endoplasmic reticulum, and chloroplast membrane (Ye *et al.*, 2011; Raboy *et al.*, 2014; Ding *et al.*, 2020). Additionally, the SULTR4 subfamily is localised to vacuolar membranes, releasing stored sulphate into the cytosol (Kataoka *et al.*, 2004b).

Research in *A. thaliana* has elucidated the multifaceted roles of SULTR isoforms. Notably, some isoforms contribute to molybdate and phosphate transport, while others are involved in the ABA biosynthetic pathway, seed phytate accumulation, and pathogen susceptibility (Fitzpatrick *et al.*, 2008; Cernadas *et al.*, 2014; Zhao *et al.*, 2016b; Yamaji *et al.*, 2017; Batool *et al.*, 2018). The functionality of group 3 sulphate transporters remains elusive. Specifically, SULTR3;5 has been observed reinforcing the function of SULTR2;1 in facilitating sulphate translocation from roots to shoots, resulting in increased sulphate accumulation in the roots of *sultr3;5* mutants under low sulphur conditions (Kataoka *et al.*, 2004a). Another study indicated elevated sulphate levels

and decreased free cysteine content in *Arabidopsis* seeds of single defective mutants belonging to group 3 sulphate transporters. Despite unaffected total sulphur supply, this points to a reduction in sulphur assimilation and reduction in these mutants (Zuber *et al.*, 2010; Cao *et al.*, 2013). SULTR3;1 is primarily localised in the chloroplast and is characterised by a predicted protein structure encompassing 12 transmembrane helices and a Sulphate Transporter and Anti-Sigma factor antagonist (STAS) domain also comprising the conserved threonine residue Thr-578 in its STAS domain similar to what is required for SULTR1;2 transport (Rouached *et al.*, 2005; Cao *et al.*, 2013). The knockout mutants of group 3 sulphate transporters exhibited diminished chloroplast sulphate emphasising its role in sulphate uptake across the chloroplast envelope membrane (Cao *et al.*, 2013). However, the existence of a backup system for sulphate uptake into plastids is evident due to the redundant functions of all SULTR3s (Chen *et al.*, 2019b).

The synthesis of ABA is intricately linked to Cys availability, as ABSCISIC ALDEHYDE OXIDASE 3 (AAO3), a pivotal enzyme in ABA biosynthesis, relies on Cys as the sulphur donor for its molybdenum cofactor sulphuration catalysed by sulphurase ABA3. Notably, *sultr3;1* exhibited reduced AAO3 activity and reduced ABA levels under normal and salt stress conditions (Cao *et al.*, 2014). In a related study, Cys was identified as a trigger for ABA biosynthesis in *Arabidopsis*, elucidating the drought-sensitive phenotype of Cys-synthesis-depleted mutants and providing insights into the mechanism of sulphate-induced stomatal closure (Batool *et al.*, 2018). Additionally, *SULTR*s, including *SULTR3;6*, a susceptibility gene (Garcia-Ruiz *et al.*, 2021) are implicated in regulating ABA signalling and influencing stomatal closure, suggesting their intricate involvement in plant-pathogen interactions (Du *et al.*, 2014; Batool *et al.*, 2018). Furthermore, *SULTR3;6* has been identified as a key player in bacterial leaf streak susceptibility in rice, with its transcriptional activation by the Tal2g virulence factor of *X. oryzae* pv. *oryzicola* contributing to disease development therefore qualifying as a major susceptibility gene (Cernadas *et al.*, 2014; Garcia-Ruiz *et al.*, 2021; Scinto-Madonich *et al.*, 2023). Uncovering the role of sulphate transporters, particularly group 3 sulphate transporters might not just be a scientific resource but might also lead to susceptibility genes and associated resistance to pathogens.

4. MATERIALS AND METHODS

4.1. Pathogen strains

Table 2. Bacterial strains used in the study

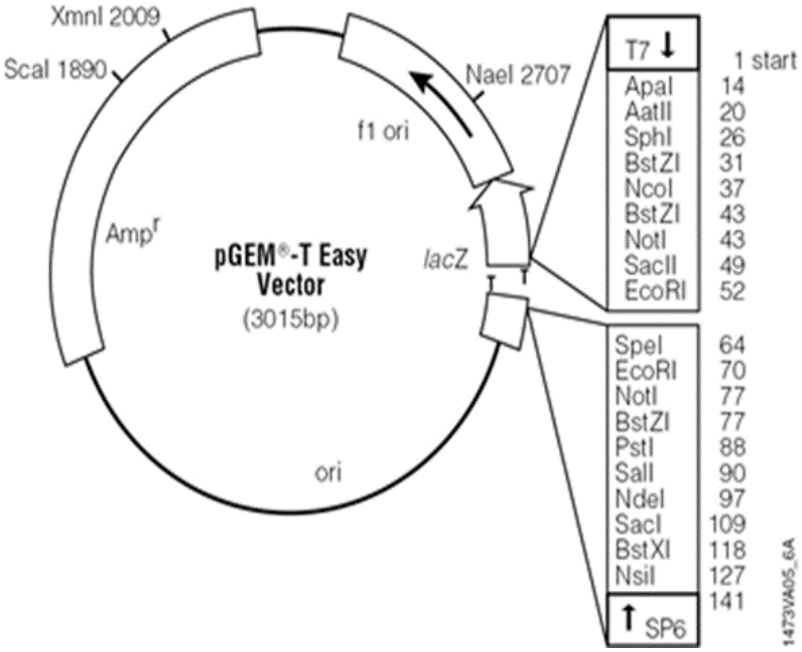
Bacterial strain	Genotype
Escherichia coli Top10	F- mcrA (mrr-hsdRMS-mcrBC) 80lacZ M15 lacX74 recA1 ara 139 (ara-leu)7697 galU galK rpsL (StrR) endA1 nupG.
Agrobacterium tumefaciens LBA4404	Ach5 (RIF R) Ti pAL4404 (strepr) Octopine

4.2. Vectors

Table 3. Vectors used in the study

Name	Type
pGEM-T Easy	Cloning vector
pKSE401	Binary CRISPR/Cas9 vector for *A. tumefaciens* mediated transformation
pCBC-DT1T2	Template for making expression cassette

A



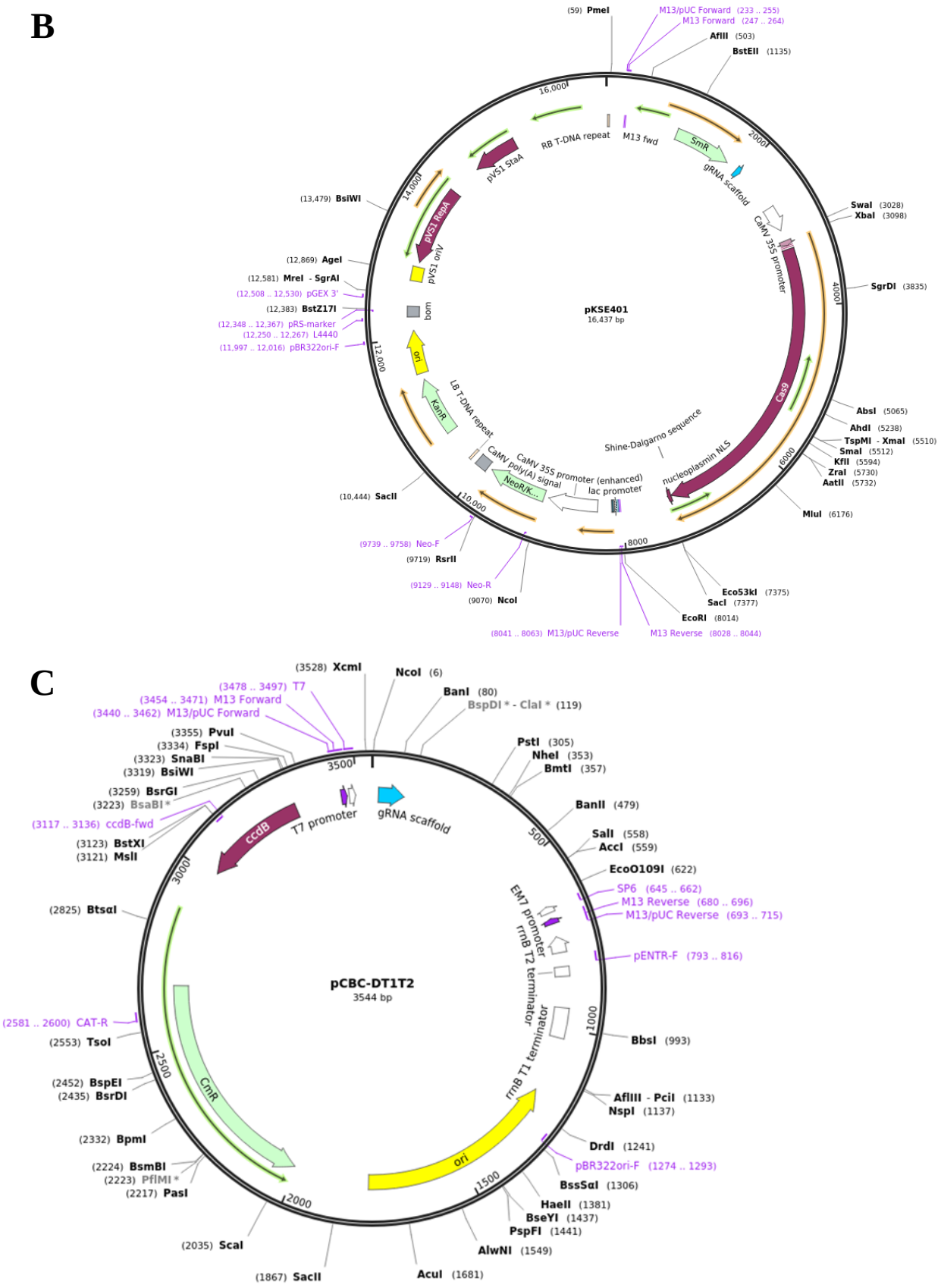


Figure 5. Plasmid map of the vectors used pGEM-T Easy (A) pKSE401 (B) and pCBC-DT1T2 (C)

4.3. Plant material

Table 4. Plant materials used in the study

No.	Name of variety	Origin	Resistance type
1	*Solanum tuberosum* L. 'Désirée'	WUR, Wageningen, The Netherlands	Partially resistant to *Pi*, susceptible to *Rs*
2	*Solanum tuberosum* L. 'Balatoni Rózsa'	Potato Research Centre, Keszthely	Moderately susceptible to *Pi*. No data for *Rs*
3	*Solanum tuberosum* L. 'Botond'	Potato Research Centre, Keszthely	Moderately resistant to *Pi*. No data for *Rs*
4	*Solanum tuberosum* L. 'Hópehely'	Potato Research Centre, Keszthely	Susceptible to *Pi*
5	*Arabidopsis thaliana* Col-0 ecotype	Nottingham Arabidopsis Stock Centre	Susceptible to *Rs*
6	*At*SULTR3.1 (SULTR3.1 T-DNA insertion line in *Arabidopsis thaliana* Col-0 ecotype)	Nottingham Arabidopsis Stock Centre: 4839483; SALK_023190C	No data
7	*At*SULTR1.2 (SULTR1.2 T-DNA insertion line in *Arabidopsis thaliana* Col-0 ecotype)	Nottingham Arabidopsis Stock Centre: 4839483; SALK_122974	No data

Table 5. Potato accessions tested for *Rs* resistance as listed by Jose *et al.* (2023)

No.	Name of Variety	Taxonomy	Accession ID	Reference, source
1	'Calalo Gaspar'	*S. stenotomum* ssp. *stenotomum*	CIP 700670	CIP Global Root & Tubers Base, Martin, (1979)
2	'Cruza 148'	*Solanum* hybrid (*S. demissum*)	CIP 720118, PI 619136	CIP database, Martin, (1979); Jackson (2012)
3	BW-5	*Solanum* hybrid (*S. demissum*)	CIP 379695.4	Schmiediche (1983)

4	BW-6	*Solanum* hybrid (*S. phureja*)	CIP 381064.3	Schmiediche (1983); Tung *et al*. (1990a)
5	BW-1.7	*Solanum* hybrid (*S. phureja*)	CIP 377831.7	Schmiediche (1983); Montanelli (1995)
6	'Monona'	*S. tuberosum*	AV 48	Stevenson *et al*. (1965); ECPD (2011)
7	'Ontario'	*S. tuberosum*	AV 25	Blodgett and Stevenson (1946); Jaworski *et al.* (1980)
8	'Kinga'	*Solanum* hybrid (*S. phureja*)	CIP 377852.1	Schmiediche (1983); French *et al.* (1998)
9	MS-42.3	*Solanum* hybrid (*S. phureja*)	CIP 800928	CIP (1979); Feng *et al.* (2003)

4.4. Plant growth conditions

Potato plants were grown under controlled laboratory conditions in 500-mL containers filled with MS medium (Murashige and Skoog, 1962) with vitamins (Duchefa Biochemie, North Holland, Netherlands, M0222). The medium included 2% sucrose and agar 7 g/L. The temperature was maintained at 23°C, and the plants were exposed to a 16-h light period with a light intensity of 100 μmol m^{-2} sec^{-1}. The plants were initially propagated from either nodal segments or apical stem cuttings. Plants were multiplied (20/genotype) for *Rs* infection from nodal segments. The apical part was then cut and grown on Phyta-tray vessels (P5929-250EA; Sigma-Merck, Darmstadt, Germany) containing MS medium with 0.8% agar. After 7 days, when the plants started rooting, they were transferred to rectangular plates (688102, Greiner Bio-One, Kremsmünster, Austria) with the same MS medium as above (five plantlets/plate) and subjected to infection after 7 days. 1/4th of the media was cut off to allow the aerial part of the plants not to be covered with the media. Additionally, sterile filter paper (MN 615) 0.16 mm thick, were cut according to ¾ of the media in the plate and put on top to absorb excess moisture. The plates were sealed with 3M micropore tape (Euromedic Trading Ltd, Budapest, Hungary). The propagation and multiplication of the resistant lines, as per literature, listed in **Table 5** for *Rs* resistance analysis and RNA isolation were carried out in Dr. Zsófia Bánfalvi's laboratory according to Stiekema *et al.* (1988) (Institute of Genetics and Biotechnology, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary).

To initiate the growth of *A. thaliana* ecotype Columbia (Col-0; wild-type) and *AtSULTR3;1*, *AtSULTR1;2* mutants, the seeds were first surface sterilised and then subjected to a 48-h stratification process at 4°C. After the stratification, the seeds were planted on Petri dishes containing ½ MS medium (Sigma-Merck, Darmstadt, Germany) with sucrose 10 g/L and a final pH of 5.8. Once germination occurred around the 7^{th} to 10^{th} day, the seedlings were transferred to Jiffy pots and initially kept covered at 21°C under short-day conditions, consisting of 8-h of light and 16-h of darkness, while maintaining high relative humidity for 3 days. After this initial period, the covering was removed, and the seedlings continued to grow under the same conditions. These plants were irrigated with ¼ strength MS (pH=5.6, salts only no vitamins) to support their growth.

For seed production, the germinated seedlings were transplanted into pots filled with soil (Composana Ltd., Halle, Germany) comprising raised bog peat, perlite, minerals, and organic fertilisers, and they were grown under a 16-h light followed by an 8-h dark cycle at 23°C. The plants were cultivated in controlled environments using Conviron (Winnipeg, Canada) chamber retrofitted with Viola L40 led lighting, Panasonic (MLR-352) or SANYO (MLR-351) environmental test chambers fitted with 800W fluorescent lamps, with two different lighting conditions available: a 16-h light followed by an 8-h dark cycle, or an 8-h light followed by a 16-h dark cycle. Both lighting conditions provided a consistent light intensity of 100 µmol m^{-2} sec^{-1}. The temperature was maintained at 21°C during the light period and 18°C during the dark period, with a relative humidity of 75%.

4.5. CRISPR constructs

To design a specific guide for the target gene, the gene of interest was initially identified on http://spuddb.uga.edu/, and the exons were duly annotated. Subsequently, the CRISPR target was designed within the first three exons of the target gene, using a 20bp-NGG-Sp Cas9, SpCas9-HF1, eSpCas9 1.1 Protospacer Adjacent Motif (PAM) parameters on the http://crispor.tefor.net/crispor.py platform. The CRISPOR program also screened the *S. tuberosum*-Phytosome V9 genome, as of December 2012 for potential off-targets. A single target was carefully chosen based on its low off-target score and high specificity. To facilitate testing of the mutant plants, primers were designed on https://primer3.ut.ee/ to amplify the exon of the selected target.

For PPO mutants, the genomic DNA was isolated from ´Désirée' and ´Balatoni Rózsa' potato plants using the GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Scientific, Waltham, MA, USA, K0792). Subsequently, specific sections of *PPO* genes with root and tuber specificity (*Pot32*, *33*, and *72*) were amplified from the potato genomic DNA. The amplification was carried out using the general primers PPOallF-R presented in **Supplementary Table 2** which amplify the first half of the three genes. The Phire Plant Direct PCR Master Mix (Thermo

Scientific, Waltham, MA, USA, F160S) was used for amplification. Subsequently, the PCR products were cloned into the vector pGEM-T Easy using the CloneJET PCR Cloning Kit (Thermo Scientific, Waltham, MA, USA, K1231). The cloned sequences were subjected to sequencing. The obtained sequences were blasted in the Solgenomics database (https://solgenomics.net/tools/blast/) to identify the *PPO* genes. Based on the sequence information, a single CRISPR target was designed to target all three PPO genes (*Pot32*, *Pot33* and *Pot72*) simultaneously.

CRISPR constructs for all target genes were created by following a standardised protocol. For single guides, the CRISPR targets were ordered as oligos in 5' to 3' orientation that excluded the NGG motif in both their forward and complementary strands, but included *Bsa*I overhangs, like oligo no. 3 and 4 for PPO listed in **Supplementary Table 2.** These oligos, each at a concentration of 50 μmol/L, were annealed by incubating them at 65°C for 5 min and slowly cooling them to room temperature before being integrated into the binary vector pKSE401, positioned behind the AtU6-26 promoter and in front of the gRNA scaffold, as originally described by (Xing *et al.*, 2014), employing the components listed in **Table 6** under the conditions listed in **Table 7.** The construct was assembled in *E. coli* and then introduced into the LBA4404 *A. tumefaciens* strain following the method described by Weigel and Glazebrook, (2006). To ensure the presence of the oligo in the construct, the primers U6-26p-F, as published by Xing *et al.* (2014) and listed in **Supplementary Table 2** at no. 5 and the CRISPR-target lower strand, were used in PCR. The final constructs were subjected to sequencing using the pJET1.2-F primer.

For editing requiring two guides, such as miR396, the miR396 sequence was obtained from https://mirbase.org/hairpin/MI0025984, and the CRISPR target with NGG PAM was designed on both the leader and passenger strands using CRISPOR. A PCR was conducted using the specific primers numbered from 7 to 10, as detailed in **Supplementary Table 2**, encompassing both target sequences and *Bsa*I sites on the pCBC-DT1T2 vector as a template. The PCR product containing both targets, each with an Atu6 individual promoter, was then incorporated into the pKSE401 vector, following the procedures outlined in **Table 6** and **7**.

4.6. Plant transformation

Four-week-old plant leaves were subjected to transformation using LBA4404 having an optical density (OD_{600}) of 0.6-0.8 and carrying the genetic construct. Horizontal incisions were made on the leaf surfaces, which were then floated on the top of the *Agrobacterium* suspension containing liquid MS (Duchefa M0222) medium upside down. After a two-day incubation period in darkness, the leaves were transferred to a kanamycin-selective Callus Induction Medium (CIM; **Table 8**) supplemented with timentin to stop agrobacterial growth for one week and subsequently, they were moved to Shoot Induction Medium (SIM) as specified in **Table 9**, with weekly subculturing to facilitate shoot regeneration. Throughout the CIM and SIM stages, the leaves were

exposed to a 16-h light and 8-h dark cycle at 23°C. The transgenic lines were later transferred to a medium containing MS with vitamins for rooting with kanamycin selection (75 µg/mL) following the methodology proposed by Dietze (1995).

Table 6. Components for building CRSIPR targets into the vector

Components	Amounts (total vol 15 µL)
Annealed oligo (50 µmol/L) / PCR product 200 ng	2 µL
pKSE401	50 ng
Buffer G (10X) (Thermo Scientific)	15 µL
ATP (10 mM)	1 µL
T4 Ligase 5U/µL	0.8 µL
*Bsa*I (*Eco*31I)	0.7 µL

Table 7. Temperature conditions for building CRSIPR targets into the vector

Temperature (°C)	Time (min)
37	2
16	5 *50 cycles
80	5
4	Infinity

Table 8. Callus Induction Medium

Components	Amounts
MSB5 (Duchefa)	4.4 g/L
Glucose	16 g/L pH: 5.8
Milli-Q water	up to 1 L
NAA (Naphthaleneacetic acid) (5 mg/mL)	1000 µL/L
BAP (6-Benzylaminopurine) (1 mg/mL)	100 µL/L
Timentin (150 mg/mL)	3 mL/L
Kanamycin (50 mg/mL)	1500 µL/L

Table 9. Shoot Induction Medium

Components	Amounts
MSB5 (Duchefa)	4.4 g/L
Sucrose	16 g/L pH: 5.8
Milli-Q water	up to 1 L
NAA (Naphthaleneacetic acid) (0.2 mg/mL)	100 µl/L
Trans-zeatin riboside (2 mg/mL)	1000 µl/L
Gibberellic acid (0.2 mg/mL)	100 µl/L
Timentin (150 mg/mL)	3 mL/L
Kanamycin (50 mg/mL)	1500 µl/L

4.7. Characterisation of mutants

Genomic DNA was isolated from plant samples using the AquaGenomic solution provided by Multitarget Pharmaceutical (Salt Lake City, UT, USA). In this process, 250 µL AquaGenomic solution was added to 30 mg of leaf samples and ground together. Subsequently, 30 µL isopropanol was added, followed by heating at 60°C for 10 minutes. After centrifugation and washing, following the manufacturer's protocol, the DNA was eluted in Tris-EDTA, and its concentration was assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The presence of the transgene was checked by running a PCR on the genomic DNA with U6 promoter Forward primer (no. 5 in **Supplementary Table 2**) and Target Lower strand (no. 4 for PPO and nos. 11 and 12 for miR396T1 and miR396T2, respectively in **Supplementary Table 2**). The *Agrobacterium* chromosomal *picA* gene was amplified by PCR using primers 13 and 14 to detect *Agrobacterium* contamination (Yusibov *et al.*, 1994). The presence of *zCas9* was checked using primer no. 15 and 16 in **Supplementary Table 2** The specific exon containing the target sequence was then amplified from the genomic DNA. For the *PPO* gene, the primers 1 and 2 listed in **Supplementary Table 2** were used, while for *miR396*, the primers 17 and 18 targeting the *miR396* gene were employed. Additionally, primers 19 and 20 were used for Chr7, amplifying the segment of the chromosome to which *miR396* belongs (**Supplementary Table 2).** A portion of the amplified PCR product was separated on a gel, and another portion was purified and cloned into the pJET1.2/blunt vector using the CloneJET PCR Cloning Kit following the manufacturer's instructions.

The resulting plasmid was subsequently introduced into competent *E. coli* Top10 cells for replication and plated onto LB media with ampicillin selection. A colony PCR was conducted using pJET1.2 sequencing primers (primers 21 and 22 in **Supplementary Table 2**), and the positive colonies were cultured. Plasmids were extracted from the culture using the GeneJET Plasmid Miniprep Kit (Thermo Scientific, Waltham, MA, USA, K0502) and the concentration was checked using the NanoDrop spectrophotometer. Plasmids corresponding to 450 ng were then sent for sequencing to Macrogen (Amsterdam, The Netherlands), utilising the pJET1.2 forward sequencing primer. The edits around the CRISPR target were subsequently analysed using Notepad++ (http://notepad-plus.sourceforge.net/).

For *Arabidopsis*, the genomic DNA was extracted using the GeneJET Plant Genomic DNA Purification Mini Kit. The homozygosity of the mutants *AtSULTR3;1* and *AtSULTR1;2* were tested by running a PCR on their genomic DNA using primers 23 and 24 and primers 26 and 27 (**Supplementary Table 2**) for *AtSULTR3;1* and *AtSULTR1;2*, respectively, which attach to the neighbouring region of the T-DNA insertion in the genes such that they only give the product in the wild type. The database http://signal.salk.edu/tdnaprimers.2.html suggested the insertion is at the position chr3 19255485 (Cao *et al.* 2012) for *AtSULTR3;1* and chr1 29329170 for *AtSULTR1;2*. A second PCR was run using primers 23 and 25 or 24 and 25 *AtSULTR3;1* and 25, 26 and 25, 27 for *AtSULTR1;2* as listed in **Supplementary Table 2** that attach to the T-DNA and the gene which gives a product only in the mutant lines.

4.8. Examination

4.8.1. Optimisation of *Ralstonia* infection

In vitro infections were performed on potato plantlets with each genotype involving twenty plantlets placed across four rectangular Petri dishes. The Petri dishes, containing four-six, three-week-old plants, were placed in a vertical position and incubated at a constant 24°C with a 16-h photoperiod at a light intensity of 45-95 µmol m^{-2} s^{-1} for an additional 10-12 days before inoculation. The *Rs* strain UW551 contained the pDSK-GFPuv plasmid (Wang *et al.*, 2007b), which carries the green fluorescent protein (GFP) reporter gene controlled by the constitutive *psbA* promoter (Jose *et al*., 2023). The concentration of bacterial suspension was set so that the majority of the control plants were in a wilted state on the 14^{th} day after infection (5-7 × 10^8 CFU ml^{-1} (OD_{600} = 0.8). For inoculations, 350 µL of the transgenic *Rs* strain was delivered to the cut sites at the root tip of each plant. The level of infection was monitored once a day for 2 to 21 days post-inoculation (dpi) for all the genotypes. Images were captured under visible light to track the progression of disease symptoms, and under UV light using the iBright CL1500 Imaging System (Thermo Fisher Scientific, Waltham, MA, USA) to visualise the spread of GFP-expressing

bacteria within the plants. The total count of wilted plants was determined and the experiment was repeated twice for consistency.

The above experiments and the modification of UW551 strain were conducted by Dr. Zoltán Bozsó (Plant Protection Institute, Centre for Agricultural Research, Hungarian Research Network, Budapest, Hungary).

To infect about six weeks old *Arabidopsis* plants grown in Jiffy pots (23℃ in short day conditions, 11-h/13-h light/dark), both control and mutants, 20 plants/genotype were exposed to *Rs* strain GMI1000 with an optical density of 0.2 set at 600 nm (OD_{600}= 0.2, ca. 10^8 CFU/mL). Before inoculations, the roots of the plants were cut 1 cm from the bottom of the Jiffy and the plants were immersed for 30 min in the bacterial suspension. After inoculations, the plants were incubated at 27℃, in short day conditions (11-h/13-h light/dark). The progression of the disease was observed every day for 2 to 19 dpi across all genotypes. A disease index was used to categorise the severity into five distinct grades as Phiri *et al.* (2024): Grade 0 indicated the absence of leaf wilting, Grade 1 represented up to 25% of the total leaf area showing wilting, Grade 2 indicated up to 50% leaf wilting, Grade 3 signified up to 75% leaf wilting and Grade 4 was assigned from 76% to complete leaf wilting or when the entire plant succumbed to the infection. Throughout the observation period, photographs were taken, and the total count of wilted plants was recorded. This experiment was repeated once more.

4.8.2. ImageJ

Bacterial quantities were assessed by measuring the GFP fluorescence emitted from all plants of each genotype at every time point, following the methodology outlined by (Shihan *et al.*, 2021). The images were processed by first inverting them, and then the background was removed using default settings. A specific region of interest (ROI), corresponding to each plant in the Petri plate, was defined. The mean grey area within this ROI was determined and subsequently adjusted by subtracting the mean grey area of the ROI from an area devoid of plants. This allowed for the normalisation of the fluorescence measurements.

4.9. Optimisation of *Phytophthora* infection

To optimise *Phytophthora* infection conditions, a table was created (**Supplementary Table 1**) to explore various combinations of explant types and inoculum concentrations. In the case of whole plant infection assays, potato plants were cultivated in pots with peat-containing soil within a glasshouse, maintaining regular ambient light conditions of 12-h of daylight per day with a temperature of 20°C. When the plants reached 8 weeks of age, five plants per genotype were subjected to *Pi* isolate P1217 together with Dr. József Bakonyi (Plant Protection Institute, Centre for Agricultural Research, Hungarian Research Network, Budapest, Hungary).

The *Pi* pathogen was cultivated and rejuvenated on approximately 7 mm thick potato tuber slices (‘Hópehely’ cultivar). These slices were inoculated on the abaxial side with 0.5 cm diameter mycelial agar plugs obtained from colonies growing at 18–20°C on V8 juice agar, following the method described by (Dhingra and Sinclair, 1985) The infested tuber slices were kept in an environment with 100% relative humidity at 18–20°C for a week, allowing the pathogen to colonise the upper side of the tuber slices. Subsequently, sporangia were washed off in distilled water, and filtered to remove debris, and the inoculum concentration was adjusted to 1.5×10^4 sporangia/mL.

For whole-plant infections, the plants were sprayed with a total of 115 mL of this inoculum suspension upon the release of zoospores. Inoculated plants were maintained at 17°C in nearly 100% relative humidity and complete darkness within a climate chamber for the first 24-h and subsequently, they were placed under a 12-h light/day regime, light intensity of 50 μmol m^{-2} sec^{-1}.

For tuber assays, tubers measuring approximately 2x3 cm were collected from sixteen-week-old potted plants cultivated in the greenhouse. The infection was carried out based on the Eucablight protocol (Colon *et al*., 2004). The tubers were washed with tap water, disinfected in 70% ethanol for 45 seconds, and rinsed with sterile distilled water. Slices of about 7 mm thickness were then cut from the disinfected tubers, rinsed in sterile distilled water, and dried in a sterile laminar box. The slices were subsequently inoculated on the abaxial side with 0.5 cm diameter mycelial agar plugs derived from a colony growing at 20°C on V8A agar. The inoculated tubers were placed in plastic containers with moistened paper towels at the bottom to maintain 100% relative humidity and kept in the dark at 20°C for 5 days.

4.10. RNA extraction

To investigate the underlying reasons for the resistance observed in ´Calalo Gaspar’ and ´Cruza 148’ compared to the susceptible ´Désirée’, the following procedure was used: Total RNA was extracted from 150 mg of frozen root tissue from 2-week-old potato plants that were either inoculated with *Rs* or remained uninoculated, according to the method described by (Stiekema *et al.*, 1988). The quality, purity, and integrity of the total RNA samples were assessed using agarose gels, a NanoDrop spectrophotometer, and a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Half volumes of the samples were employed for cDNA synthesis after purifying mRNA with poly-T oligo-attached magnetic beads. The RNA samples were treated with RQ1 RNase-free DNase (Promega, Madison, WI, USA) followed by random-primed reverse transcription to generate cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA, K1621) according to the manufacturer’s instructions. The success of the DNase treatment and reverse transcription was checked by running a PCR on DNase-treated RNA as well as on cDNA as templates.

4.10.1. RNA-seq

RNA extraction for the resistant lines ‘Calalo Gaspar’ and ‘Cruza 148’, and susceptible ‘Désirée’ was followed by paired-end, non-directional library construction using the NEBNext Ultra RNA Library Prep Kit for Illumina from New England Biolabs (Ipswich, MA, USA). After quantification with a Qubit fluorometer (Thermo Fisher), the samples underwent real-time PCR and custom sequencing. All these activities were carried out using an Illumina NovaSeq 6000 platform by Novogene (Nanjing, China).

The bioinformatic analysis comprised several steps: quality control to eliminate adapter sequences, poly-N sequences, and low-quality data using fastp (Chen *et al.*, 2018), resulting in a high number of clean reads (**Supplementary Table 3**); mapping of the data to the reference genome sequence of *S. tuberosum* group Phureja DM1–3 516 R44 (v6.1); Pham *et al.*, 2020) and gene annotation using HISAT2 software (Kim *et al.*, 2015) and StringTie for reference annotation and novel gene prediction (Pertea *et al.*, 2015); quantification of gene expression in FPKM (Fragments Per Kilobase of transcript sequence per Million base pairs sequenced) and correlation analysis; differential expression analysis using DeSeq2 (Love *et al.*, 2014) with a log2(FoldChange) threshold of ≥1 and p-values adjusted for False Discovery Rate (FDR) according to Benjamini and Hochberg (1995); and analysis of gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for differentially expressed genes (DEGs) using the ClusterProfiler R package (Wu *et al.*, 2021). All these analysis were performed by Novogene. Based on the GO, KEGG, and DEG provided by Novogene, the different lines ‘Calalo Gaspar’ and ‘Cruza 148’, and susceptible ‘Désirée’ were compared.

4.10.2. Validation of the RNA-seq data by real-time quantitative PCR

The cDNA samples derived from the extracted RNA were diluted three-fold, and 0.6 µl of this dilution was used as a template in 10 µL qPCR reactions. RT-qPCR assays for selected genes in silenced lines with primers 28-37 as listed in **Supplementary Table 2** were performed using the GoTaq® 1-Step RT-qPCR System kit (Promega Corporation, Madison, WI, USA, A6101). The standard cycling conditions for the three-step amplification began at 37°C for 15 min, followed by 95°C for 10 min. Subsequently, there were 40 cycles consisting of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with a final extension step at 60°C for 30 seconds.

For normalisation, the expression of the two housekeeping potato genes, *β-TUBULIN* (Yin *et al*., 2016; accession No. DQ228319.1 in NCBI) and *ELONGATION FACTOR 1α* (AB061263.1 in NCBI) were used as references. The expression levels of these housekeeping genes were assessed using Ef1qPCRF-R and BtubF-R primers as listed in **Supplementary Table 2**, pimer no. 38-41, with an annealing temperature of 60°C. The transcription level was calculated using the 2^{-}

$^{\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with efficiency correction, as described by (Pfaffl, 2004).

The qPCR experiments were conducted in triplicate using a 7500 Fast PCR System from Applied Biosystems, located in Waltham, ME, USA. Each reaction mixture consisted of 0.4 µl of cDNA, 1 µl of the primer pair (at a concentration of 10 µM), 5 µl of Fast SYBR™ Green master mix (Thermo Fisher Scientific, 4385612), and 3.6 µl of water, resulting in a total volume of 10 µL. The temperature profile involved an initial denaturation step at 95°C for 20 seconds, followed by 40 cycles of 5 seconds at 95°C and 30 seconds at 60°C. The expression levels of the target genes were determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), with an efficiency correction step based on the approach outlined by (Pfaffl, 2004). Data analysis was carried out using ANOVA, followed by Tukey's *post-hoc* test.

4.11. miRNA extraction, reverse transcription qPCR assay

The effect of the caused *miR396* gene mutation on the expression of *miR396* itself and on its predicted target genes from the literature were quantified. The studied miRNA sequences included the *stu-miR396* leader strand: UUCCACAGCUUUCUUGAACUU (Zhang *et al.*, 2009) and the *stu-miR396* passenger strand: UCCAAGAAAGCUGUGGGAAA (own prediction from the potato gene sequence based on sequence alignments with other species). While the studied predicted miR396 target gene sequences were taken from (Zhang *et al.*, 2009, 2013) and (Luo *et al.*, 2023). All of miR396 targets examined were predicted targets of the *miR396* leader strand, with the exception of the Nbs-lrr resistance protein PGSC0003DMG400007999 that was studied due to sequence similarity to the passenger strand, as indicated by BLAST searches.

Potato plants were grown for the analysis in pots in peat-containing soil in a glasshouse under normal spring light conditions (around 12-h of light per day) at around 20°C temperature. Total RNA (including miRNAs) was extracted from leaves (150 mg/sample) of one-month-old plants using the high binding capacity Zymo Direct-zol RNA Miniprep Kit according to the manufacturer's instructions. Genotypes included *miR396* mutants (´Botond' miR396/6; ´Botond' miR396/8; ´Botond' miR396/9; ´Désirée' miR396/3; ´Désirée' miR396/4; ´Désirée' miR396/6 and ´Désirée' miR396/10) along with the non-mutant original varieties (´Botond', ´Désirée'). The reverse transcription and qPCR method was carried out based on the publications of Varkonyi-Gasic *et al.* (2007), Yin *et al.* (2021), and Székely *et al.* (2023). Stem-loop primers (**Table 10**) for miRNA reverse transcription have been designed having a complementary region to the last 6 bases of the miRNAs, according to Varkonyi-Gasic *et al.* (2007). Reverse transcription of the microRNAs and messenger RNAs was carried out in the same reaction using step-loop and oligodT primers as described by Yin *et al.* (2021). A 20 µL reverse transcription reaction contained 2 µg total RNA; 1-1 µL 1 µM stem loop primer for each of the 4 miRNAs and components of the

RevertAid First Strand cDNA synthesis kit including 1 µL oligodT, 4 µL 5×reaction buffer, 2 µL dNTP, 1 µL RNase inhibitor and 1 µL reverse transcriptase. The following temperature program was applied: 30 min at 16°C. 60 min at 42°C and 5 min at 70°C.

Table 10. Stem-loop RT primers

Name	Sequence (5'-3')
miR396Lstemloop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAGTTC
miR396Pstemloop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTTTCCC

Three-fold diluted cDNA was used as template in qPCRs. The miRNA-specific forward qPCR primers (no. 42 and 43, **Supplementary Table 2**) all contained a non-specific GC-rich 5' end as well the 3' end containing other parts of the miRNA (excluding the last 6 bases). The reverse primer (no. 44, **Supplementary Table 2)** of the miRNA qPCRs was not specific to the miRNAs but to the stem-loop region, as suggested by (Székely *et al*., 2023). Target gene specific primers (45-64) from **Supplementary Table 2** were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). qPCRs were run in triplicates in the CFX96 Touch – Real-Time PCR Detection System (Bio-Rad, CA, USA) using the PCRBIO SyGreen Blue Mix (PCR Biosystems, London, UK). Reaction efficiencies were calculated using the standard curve method and optimised until reaching E>=1.8 (80%). Gene expression was normalised for the geometric mean of two housekeeping genes, *β-TUBULIN* and *ELONGATION FACTOR 1α* as it was described earlier (Jose *et al.*, 2023). Expression levels of the studied genes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with an efficiency (E) correction step applied according to (Pfaffl, 2004).

4.12. Browning Assay

The potato roots for browning assay were obtained from four-week-old *in vitro* plants grown from apical cuttings in tubes containing solid MS medium with vitamins (Duchefa Biochemie, M0222) under the same conditions as described in section 4.4. For analysing the browning response of tubers, three-week-old *in vitro* grown potato seedlings with roots were potted in 1L-pots into the soil comprising raised bog peat, perlite, minerals, and organic fertilisers (Composana Ltd.) and incubated with 100% relative humidity for the first 5 days and grown further in an automated greenhouse (Global Glasshouse Ltd., Szentes, Hungary) at a humidity of 60–90% and 12-h photoperiods with illumination provided by Groxpress 600 W E40 lamps at 2050 K colour temperature (Sylvania, Budapest, Hungary). Tubers were harvested from three-month-old potato plants. The browning assay of tubers and roots of PPO mutants 'Désirée' Line 14 and 17 and 'Balatoni Rózsa' Line 12 and 25 including the controls 'Désirée' and 'Balatoni Rózsa' was

performed on the entire root system of 5 *in-vitro* grown plants in tubes per genotype. Three potato tuber slices/genotype (randomly selected from the harvest of 5 plants per genotype), each 5 mm in thickness were used for the experiment. A 100-µL volume of a substrate solution, which contained 0.01 M disodium tyrosine, was carefully applied to the test samples. The samples were then allowed to incubate in the dark at 30°C for a period of 1 to 2 h, following the procedure outlined by Busch, (1999). The tubers and roots were then photographed to record differences.

The browning was quantified by designating one ROI per tuber (with three ROIs per genotype) and creating a histogram using ImageJ. The ROI remained constant across genotypes, with values ranging from 1 to 255, where 255 represented the lightest and 1 indicated the darkest colour intensity. In the case of roots, the ROI was established for one complete set of roots per plant, and this was kept consistent across roots of other genotypes. The resulting histogram was plotted using the same intensity scale. Additionally, the reduction in observed browning was measured by subtracting the ROI value from 255 (representing darkness/browning) and dividing the outcome by 255 to express the reduction as a percentage.

4.13. PPO enzyme activity measurement

Triplicate samples of potato roots (four-week-old 10 *in vitro* plants) and tuber samples (harvested from 3 plants/genotype) of PPO mutants (‘Désirée’ Line 14 and 17, ‘Balatoni Rózsa’ Line 12 and 25) together with control (‘Désirée’ and ‘Balatoni Rózsa’), each weighing 0.1 g, were collected from every genotype. Enzyme activity measurements were conducted according to the protocol detailed in González *et al.* (2020). For tuber samples, the extraction buffer included 1% Triton X-100, while for root samples, it contained 0.01% SDS. Statistical analyses, such as ANOVA and Student's *t*-test, were employed to determine the significance levels.

4.14. Metabolic screening by targeted ultra-performance liquid chromatography – tandem mass spectrometry

For analysis of differential metabolic regulation among the *Rs* resistant and susceptible lines, one gram of leaf and root tissues was obtained from the two *Rs*-resistant cultivars, namely ‘Calalo Gaspar’ and ‘Cruza 148’, as well as from the *Rs*-susceptible ‘Désirée’, at two time points: 0 dpi and 6 dpi. Additionally, 0.1g samples of leaf and root tissues from four replicates were collected from both ‘Désirée’ and ‘Balatoni Rózsa’ genotypes, including control and transgenic lines grown *in vitro* for the PPO experiment. The samples were immediately frozen in liquid nitrogen and stored at -80°C until homogenisation with liquid nitrogen using a mortar and pestle. Portions of 0.1 g homogenized frozen fresh weight (FW) plant material were transferred to 1.5-mL safety Eppendorf tubes and stored at -80°C until extraction. High-performance liquid chromatography (HPLC)-grade chemicals and ultra-performance liquid chromatography-mass

spectrometry (UPLC-MS)-grade acetonitrile from VWR (Radnor, PA, USA) were used for the extraction and elution steps. Non-labelled reference materials were acquired from Sigma-Aldrich (Darmstadt, Germany).

The measurement for metabolic analysis was conducted using UPLC-MS/MS, following a method previously published (Cseh *et al.*, 2024) by Kamirán Áron Hamow (Agricultural Institute, Centre for Agricultural Research, Hungarian Research Network. Martonvásár, Hungary). This method was developed based on previous work, with an expansion of its scope by the number of components surveyed.

The ClustVis web tool, accessible at https://biit.cs.ut.ee/clustvis/, was utilised for several analytical tasks, including Principal Component Analysis (PCA), while box plots for the *Rs* resistant lines ('Calalo Gaspar' and 'Cruza 148') and 'Désirée' were generated using BoxPlotR accessible at http://shiny.chemgrid.org/boxplotr/. The 90% confidence interval of means was also added. The same tools were used to visualise the variations in the metabolic profiles between the *PPO*-edited lines and the control. Student's *t*-test, were employed to determine the significance levels.

4.15. Suberisation study

Suberisation assessment was conducted on seven-day-old seedlings from the mutants *AtSULTR3;1, AtSULTR1;2* and Col-0, which had been cultivated on solid half-strength Murashige and Skoog (MS) plates (Sigma) without the inclusion of sucrose. These seedlings were vertically grown in growth chambers maintained at 22°C, under continuous illumination at an intensity of 100 µE. To visualise suberin on the root surface *in vivo*, a staining procedure was performed using Fluorol Yellow, following the protocol described by Lux *et al.* (2005). The roots were immersed in a freshly prepared solution containing 0.01% (w/v) Fluorol Yellow 088 (Santa Cruz Biotechnology, Inc.,Dallas, Texas, USA, CAS:81-37-8) dissolved in lactic acid at 70°C, and were incubated for 1-h before examination under a microscope.

4.16. Confocal microscopy

Roots from both non-infected and *Rs*-infected potato plants of cultivars 'Calalo Gaspar', 'Cruza 148', and 'Désirée' were harvested at 6 dpi (n=5 for each treatment and cultivar). For *A. thaliana-Rs* resistance analysis, root and shoot samples were taken from the infected Col-0 ecotype and *AtSULTR3;1* at 7 dpi and from uninfected plants. A 10-mm segment was carefully excised from the middle region of each root, located approximately 5 cm from the root tip. Shoots slices were cut from the central axis. The root and shoot segments were fixed in a solution containing 60 mM phosphate buffer (pH 7.2) and 4% formaldehyde. After fixation, they underwent a series of washing steps, dehydration in various ethanol solutions, and gradual infiltration with LR White

resin (Agar Scientific Ltd., Stansted, UK). The resin was solidified through polymerisation at 55°C for 48-h. The experiment was done with the help of Dr. Attila Fábián. Subsequently, 1-µm thick cross sections were sliced from the resin blocks using an Ultracut-E microtome (Reichert-Jung, Heidelberg, Germany). The sections were stained with safranin O (for cell walls of 'Calalo Gaspar', 'Cruza 148', and 'Désirée'), Calcofluor White (for cell walls of Col-0 ecotype and *AtSULTR3;1*) and Fast Green FCF (as a counterstain), employing reagents from Searle Diagnostic (High Wycombe, UK) and Sigma-Aldrich, respectively. The stained sections were then mounted in 50% glycerol and visualised using a TCS SP8 confocal laser scanning microscope (Leica Microsystems GmbH, Wetzlar, Germany). The excitation and detection parameters were set as follows: Safranin O was excited at 514 nm, with signals detected in the range of 600 to 720 nm, Calcofluor White was excited at 405 nm and detected at 410-500 nm, while Fast Green FCF was excited at 633 nm, with signals detected in the range of 640 to 780 nm. Microscopic images were captured using the Leica Advanced Fluorescence software v3.1.5.16308 (Leica Microsystems) by Dr. Attila Fábián without any further image processing.

The confocal laser-scanning microscopy images were captured using a Leica SP8 microscope with Leica Application Suite X (LAS X) program v3.1.5.16308. The specific excitation and detection settings were as follows: fluorol yellow was excited at 488 nm, and the detection range was set to 500–530 nm (as indicated by Ursache *et al.*, 2021). To quantify the suberisation in the roots of 7-day-old *AtSULTR3;1* and Col-0 seedlings, the relative fluorescence intensity of fluorol yellow dye was measured on the acquired pictures using the LAS X software. Relative fluorescence intensities were measured on 20 micrographs per genotype and treatment, using 1 ROI per micrograph.

5. RESULTS AND THEIR DISCUSSION

5.1. Molecular analysis of *Rs*-resistant potato cultivars

5.1.1. Identification of *Rs*-resistant accessions

Five cultivars and four breeding lines known to possess *Rs* resistance in the field (**Table 5**) were initially chosen and propagated for testing their resistance to *Rs* in an *in vitro* inoculation bioassay. To perform the bioassay, a highly virulent *Rs* strain, UW551 (R3B2, PIIB-1), transformed with a *GFP* reporter gene, was employed to inoculate *in vitro* potato plants. After conducting an initial screening of the nine accessions, along with the *Rs*-susceptible commercial cultivar 'Désirée' (DES) used as a control, the four top-performing accessions were 'Calalo Gaspar' (CG), 'Cruza 148' (CR), BW-5, and 'Monona'. These four accessions were subjected to a repeated comparative bioassay. Among them, CG and CR exhibited remarkable resistance, with a 100% survival rate (**Figure 6A and B**) at 15- and 21-days post-inoculation (dpi) compared to only 58% and 32% survival of the susceptible control DES at 15 dpi and 21 dpi, respectively (**Figure 6A and B**). The *Rs*-resistant cultivars CG and CR remained green with minimal bacterial penetration, while the susceptible control wilted and the *Rs* bacteria spread throughout the plants (**Figure 6C**). The uninfected control plants showed vigorous growth (not shown).

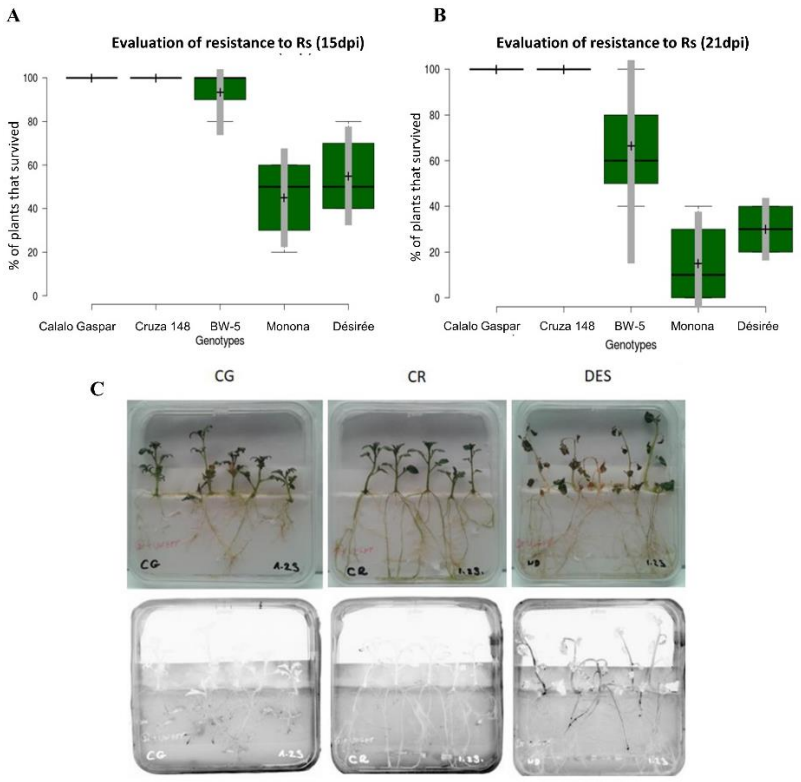


Figure 6. Survival of four selected potato accessions in an *in vitro Rs* inoculation assay at 15 dpi (A) and 21 dpi (B). Visual evaluation of resistance to *Rs* infection (21 dpi) in the *in vitro* inoculation bioassay under visible light for the observation of wilting (upper row) and UV light for the detection of GFP-expressing *Rs* bacteria (lower row). CG, 'Calalo Gaspar'; CR, 'Cruza 148'; DES, 'Désirée'(C) (Jose *et al.*, 2023).

Among the resistant genotypes, CG is a traditional diploid landrace originating from Peru, belonging to the species *S. stenotomum*. This species is considered the ancestor of cultivated potatoes and is cross-compatible with *S. tuberosum* cultivars (Jackson *et al.*, 1978; Gottschalk, 1984). Its *Rs* resistance was transferred to *S. tuberosum* through breeding (Martin and French, 1985) and somatic hybridisation (Fock *et al.*, 2001). CR is categorised as a 'tuberosum type' and originates from Toluca, Mexico. It was the first cultivated potato to exhibit tolerance to bacterial wilt (Jackson *et al.*, 1979). Subsequently, it has been routinely employed as a moderately *Rs*-resistant cultivar in screening breeding lines due to its consistently low wilt percentage in standardised field experiments (Priou *et al.*, 2001). Our *in vitro* inoculation tests corroborated these prior findings for these two cultivars, which were thus selected for further molecular analyses to unravel the cause for the displayed resistance.

5.1.2. Transcriptome analysis of the *Rs*-resistant and susceptible cultivars

Co-expression and differential expression of potato genes

We conducted a transcriptome analysis to examine the gene expression patterns in the *Rs*-resistant cultivars CG and CR, as well as the *Rs*-susceptible cultivar DES, during the early stages of infection. Total RNA was isolated from the roots of *in vitro* plants, both non-infected and *Rs*-inoculated at 2 dpi. In total, we evaluated 16 samples, consisting of three biological replicates for both CG and DES, and two biological replicates for CR. The RNA isolation and the sequencing process yielded high-quality RNA with good RNA integrity numbers (RIN) and a high frequency of clean (ranging from 95.8% to 97.6%) and uniquely mapped reads (85.4% to 88.8%). The error rates across all samples remained below 0.03% (**Supplementary Table 3**).

The co-expression Venn diagrams revealed that approximately 15,000 genes were commonly expressed in the non-infected roots of all cultivars, with around 600-700 uniquely expressed genes each case (**Figure 7A and B**). The *Rs* infection did not significantly alter these proportions. However, the impact of *Rs* infection on each cultivar's transcriptome varied considerably (comparing each infected to its own control), with only 580 differentially expressed genes (DEGs) commonly identified, while 2,142, 1,242, and 616 unique DEGs were observed in CG, CR, and DES, respectively (**Figure 7C**). In total, the number of DEGs influenced by *Rs* infection was 4,011 (1,988 upregulated and 2,023 downregulated) in CG, 2,766 (1,443 upregulated and 1,323 downregulated) in CR, and 2,043 (1,163 upregulated and 880 downregulated) in DES (**Figure 7 D-F**).

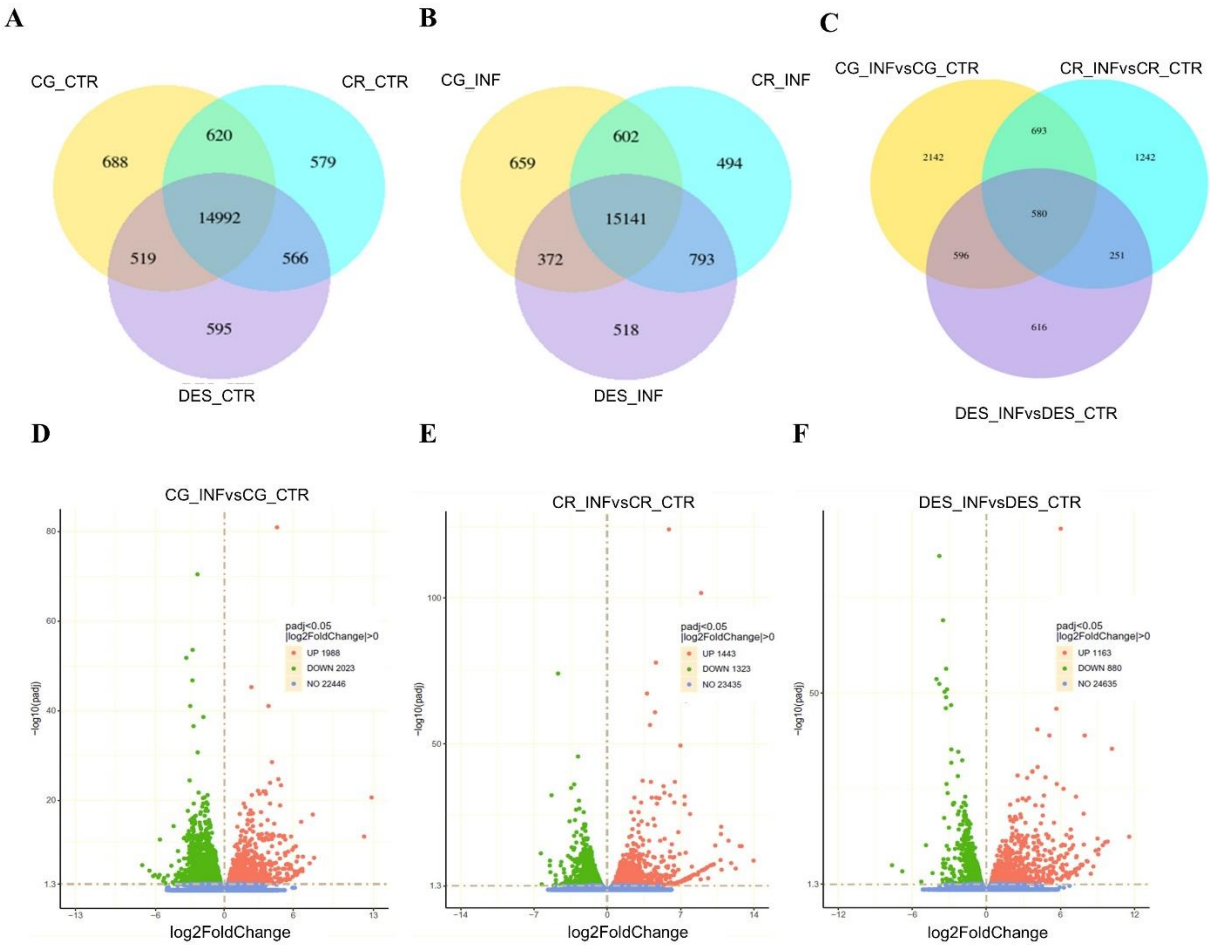


Figure 7. Number and distribution of co-expressed (A, B) and differentially expressed (C-F) genes in the roots of 'Calalo Gaspar' (CG), 'Cruza 148' (CR), and 'Désirée' (DES). Co-expression of genes in non-infected control (CTR) roots (A). Co-expression of genes in *Rs*-infected (INF) roots at 2 dpi (B). Differentially expressed genes upon *Rs* infection at 2 dpi (C). Volcano plots show the distribution of significantly (padj<0.05) upregulated (red) and downregulated (green) genes and those with unchanged expression (blue) in the roots of CG (D), CR (E), and DES (F).

Gene regulations and pathways contributing to Rs resistance

Gene ontology (GO) enrichment analysis (**Figure 8**) uncovered conspicuous expression patterns in response to *Rs* infection in the potato cultivars. Ribosome-related genes and those associated with ribonucleoprotein complexes were upregulated in all three cultivars. Further, a substantial number of genes linked to oxidative stress responses were downregulated in the CG cultivar. In contrast, both CR and DES exhibited induction of cell wall and chitin metabolic and catabolic processes upon *Rs* infection. However, CR uniquely displayed downregulation of carbohydrate metabolism.

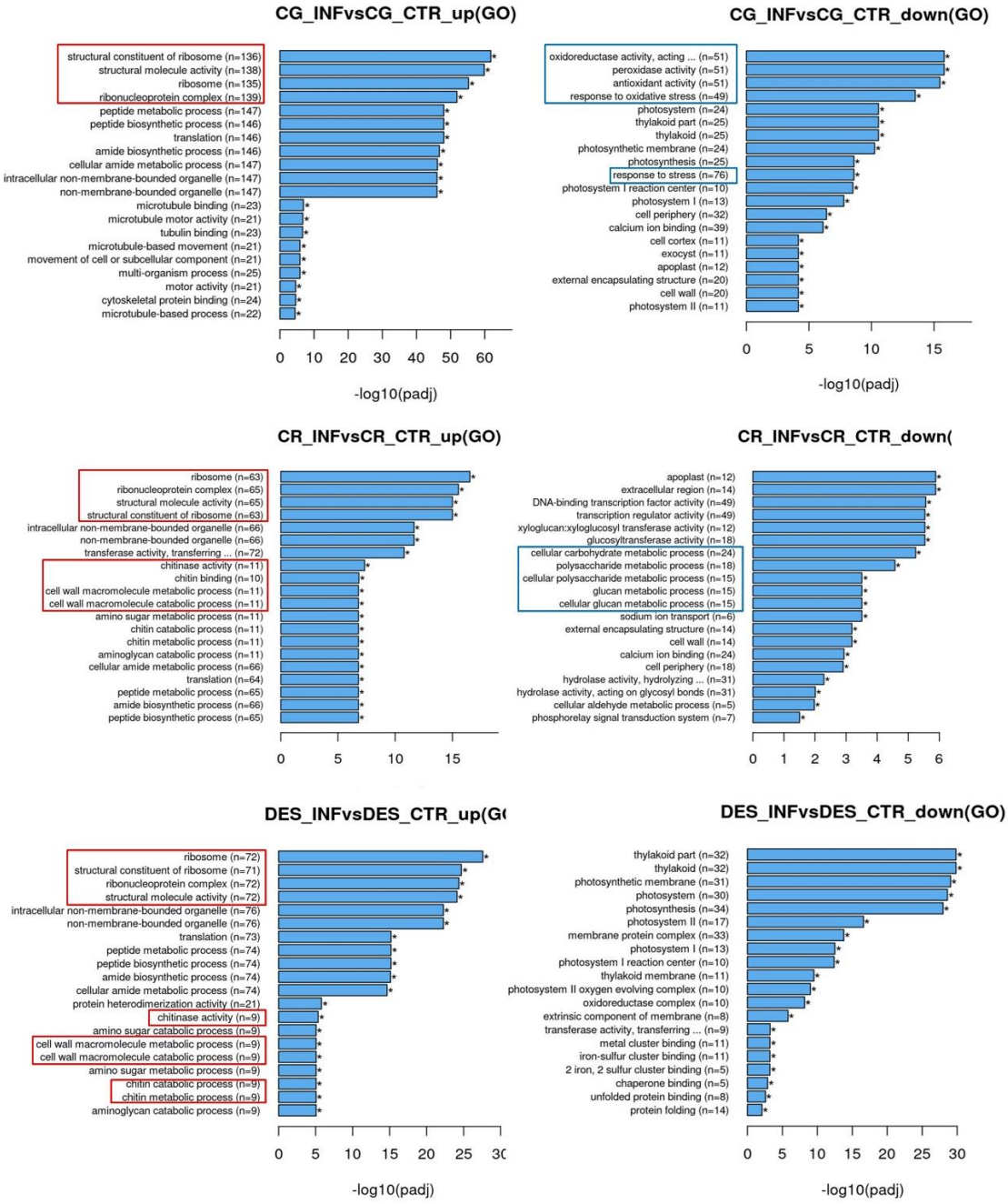


Figure 8. Upregulated and downregulated GOs upon *Rs* infection (2 dpi) in the roots of 'Calalo Gaspar' (CG), 'Cruza 148' (CR), and 'Désirée' (DES). Asterisks indicate that all enrichments are significant. Categories with enrichments specific to each cultivar are boxed (Jose *et al.*, 2023).

KEGG pathway analysis (**Figure 9**) reinforced some aspects of GO enrichment findings. Specifically, the phenylpropanoid and plant-pathogen interaction pathways were downregulated in CG, while in CR the phenylpropanoid pathway was upregulated, and the plant-pathogen interaction pathway was similarly downregulated. Further examination of the downregulated phenylpropanoid biosynthesis genes in CG revealed that most of these genes (45 out of 62) were peroxidases, while only 17 genes, including *PAL* (*PHENYLALANINE AMMONIA-LYASE*), were involved in multiple enzymatic steps of phenylpropanoid biosynthesis. In CR, 37 upregulated

genes fell into the same category, with 27 encoding peroxidases and 10 encoding different synthesis enzymes.

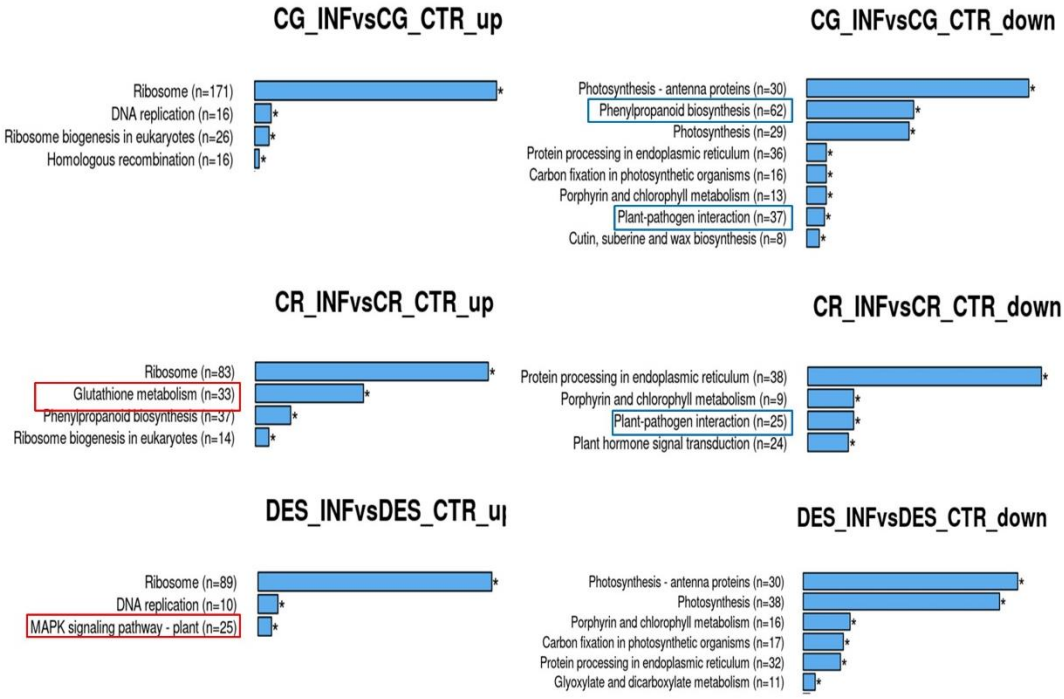


Figure 9. KEGG pathway analysis upon *Rs* infection (2 dpi) in the roots of 'Calalo Gaspar' (CG), 'Cruza 148' (CR), and 'Désirée' (DES). Asterisks indicate that all enrichments are significant. Pathways with enrichments specific to each cultivar are boxed (Jose *et al.*, 2023).

Notably, CR displayed increased levels of lignin-forming anionic peroxidases and suberisation-associated anionic peroxidases, in line with the heightened intensity of cell wall metabolic and catabolic processes observed in the GO analysis too. A distinctive feature was the upregulation of glutathione metabolism in CR, involving genes such as *GLUTATHIONE S-TRANSFERASE*, *γ-GLUTAMYLCYSTEINE SYNTHETASE*, and *ASCORBATE PEROXIDASE*. Moreover, the endochitinase gene *ChiB* and *PR1 (PATHOGENESIS-RELATED PROTEIN 1*) were highly activated in all three cultivars. In DES, the mitogen-activated protein (MAP) kinase signalling pathway was activated. However, several genes of this pathway were downregulated in the *Rs*-resistant cultivars, especially in CG. Some specific genes, such as *MEKK1* (*MAP KINASE KINASE KINASE A*) and *MAPK9* (*MAP KINASE 9*), were uniquely downregulated in CG, while *MEKK7* was repressed in CR only, and *MEKK EDR1* (*ENHANCED DISEASE RESISTANCE 1*) was downregulated in both *Rs*-resistant cultivars (Jose *et al.*, 2023). This analysis suggests that the response to *Rs* infection varies between different potato cultivars, with distinct patterns of gene expression and pathway activation, reflecting their varying levels of resistance. Peng *et al.* (2021) reported an upregulation of glutathione metabolism in eggplant roots and stems upon *Rs* infection. An increase in *GLUTATHIONE S-TRANSFERASE* expression was also observed in *S. commersonii*, in both *Rs*-resistant and *Rs*-susceptible accessions (Zuluaga *et al.*, 2015), while it

was induced more than 10-fold in a *Rs*-resistant tomato cultivar compared to a susceptible cultivar (Ishihara *et al.*, 2012). Transcriptome analysis of *Rs*-resistant tobacco seedling roots suggested that glutathione and flavonoids are likely the main substances conferring early resistance to *Rs* infection (Gao *et al.*, 2019). This hypothesis was further supported by Li *et al.* (2021), who demonstrated that glutathione metabolism and phenylpropanoid pathways are the primary resistance pathways to *Rs* infection in tobacco. DEGs related to glutathione metabolism were also identified in the stems of a highly *Rs*-resistant tobacco variety (Pan *et al.*, 2021). Glutathione serves as the most abundant antioxidant in cells and plays a crucial role in protecting cell membranes and biomolecules from damage caused by ROS (Dorion *et al.*, 2021). Thus, it is likely to contribute to the *Rs* resistance of the CR cultivar. Apart from functional enrichment analysis, (GO, KEGG) we also manually analysed the DEG tables and based on literature evidence, we highlighted genes which might have a role in the resistance against *Rs*. The research also highlighted other pathways influencing resistance to *Rs* infection. In particular, WRKY transcription factors, which play a pivotal role in plant resistance to phytopathogens, were downregulated in CG and CR (Jose *et al.*, 2023). However, the role of WRKYs can be complex and context-dependent, with some acting as positive regulators of immunity while others as negative regulators. Additionally, the MAPK signalling pathway was activated in the *Rs*-susceptible DES, suggesting that modulating MAPK activity may be a strategy to reduce losses caused by *Rs*.

Validation of transcriptome data

To confirm the findings from the RNA-seq data, we conducted RT-qPCR experiments with five randomly selected genes (three upregulated and two downregulated in each cultivar) in the three potato cultivars. The expression trends and rankings of these tested genes were consistent between the RNA-seq and RT-qPCR results, yielding a high correlation coefficient ($R^2 = 0.9102$), affirming the reliability of the transcriptome data (**Figure 10 and Supplementary Table 4**).

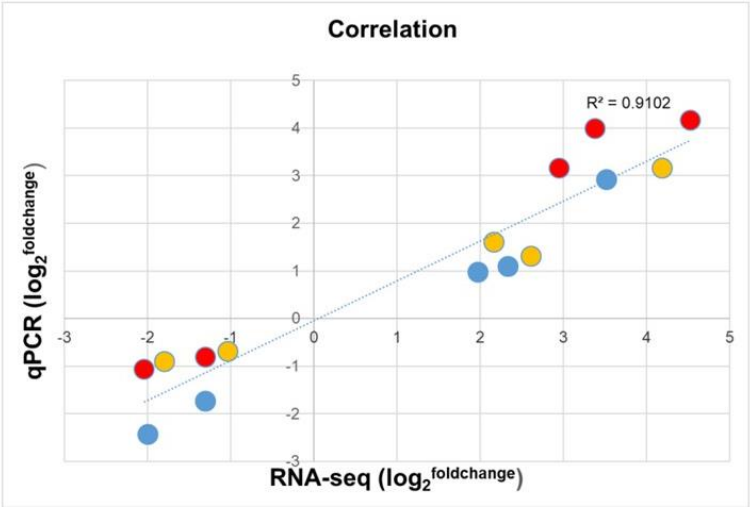


Figure 10. Differential expression of selected genes in the roots of non-infected and *Rs*-infected 'Calalo Gaspar' (CG), 'Cruza 148' (CR), and 'Désirée' (DES). The average fold change of biological replicates in expression levels detected by RNA-seq are compared to the fold change of the same biological replicates detected by RT-qPCR and are plotted on the x- and y-axis, respectively. Expression of *AGMATINE HYDROXYCINNAMOYLTRANSFERASE 1* (Soltu.DM.11G024290.1), *PATHOGENESIS-RELATED PROTEIN*, *STH-2* (Soltu.DM. 09G027710.1), *WOUND-INDUCED PROTEIN*, *WIN1* (Soltu.DM. 01G036460.1), *ABSCISIC ACID AND ENVIRONMENTAL STRESS-INDUCIBLE PROTEIN*, *TAS14* (Soltu.DM. 02G024670.1) and *L-ASCORBATE PEROXIDASE 2* (Soltu.DM. 09G006560.1) in CG (blue), CR (red) and DES (yellow) (Jose *et al.*, 2023).

5.1.3. Metabolomic analysis of *Rs*-resistant and susceptible cultivars

Plants have long been known to produce a wide range of secondary metabolites, and it is established that plants with higher concentrations of these secondary metabolites may exhibit greater resistance to various biotic and abiotic stresses. Many of these defence compounds are phenolics and flavonoids produced via the phenylpropanoid pathway. Furthermore, plant hormones, particularly ethylene (ET), JA, SA, and ABA, play critical roles in orchestrating responses to stress in conjunction with growth-promoting compounds, IAA (Mierziak *et al.*, 2014; Verma *et al.*, 2016; Zaynab *et al.*, 2018; Yadav *et al.*, 2020).

To investigate the significance of these compounds, we conducted a comprehensive study in three potato cultivars (CG, CR, and DES) by quantifying 30 specific metabolites mainly from the phenylpropanoid pathway and the plant hormones ABA, SA, JA, and IAA. We collected samples

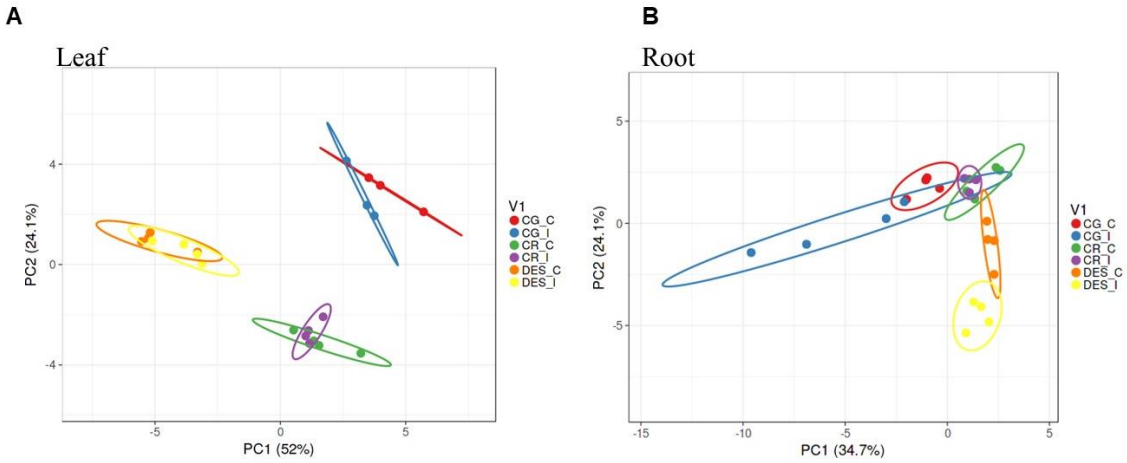


Figure 11. Principal component analysis of differences in the concentration of secondary metabolites between non-infected and *Rs*-infected 'Calalo Gaspar' (CG), 'Cruza 148' (CR), and 'Désirée' (DES) leaves (A) and roots (B) at 6 dpi. The data were obtained from four biological replicates of the roots of each cultivar and leaves of CG and DES and three biological replicates from the leaves of CR. Each biological replicate contained pooled samples of five plants.

from roots and leaves of both non-infected and *Rs*-infected potato plants at 6 dpi and subjected them to analysis using UPLC-MS/MS.

To better understand these metabolic variations, we performed PCA analyses and the results revealed that the profiles of these compounds could effectively distinguish samples according to the cultivar and the infected status (**Figure 11**). In general, *Rs* infection led to more characteristic changes in the metabolite patterns in the roots than in the leaves. Also, these changes were more pronounced in CG and DES compared to CR, where the PCA showed no significant difference between non-infected and *Rs*-infected CR roots (**Figure 11B**).

Taking a closer, component-specific look at the metabolite differences (**Figure 12, Supplementary Table 5 and 6**), based on evident observable changes, we found that while the concentration of the phenolic acid, chlorogenic acid was higher in the *Rs*-resistant cultivars than in DES, it showed a similar tendency to increase in the roots of each cultivar following *Rs* infection. Although the derivatives of chlorogenic acid only showed a significant increase in CG roots (Jose *et al.*, 2023). The increase in the concentrations of chlorogenic acid derivatives and quercetin derivatives was a distinctive characteristic of CG. Chlorogenic acids are known for their abundance in potato tubers and other plant organs and play vital roles in antimicrobial activity, antioxidant activity, and the synthesis of suberin and lignin, crucial components in fortifying cell walls against pathogen attacks (Kabir *et al.*, 2014; Valiñas *et al.*, 2015; Yang *et al.*, 2016; Zhang *et al.*, 2018). It is worth noting that the regulation of the synthesis of these metabolites can differ among different plant species and their responses to stress. For example, studies in other species have shown variations in the accumulation of flavonoids in response to stress, with different effects on resistant and susceptible genotypes (Li *et al.*, 2021; reviewed by Wang *et al.*, 2022b).

.

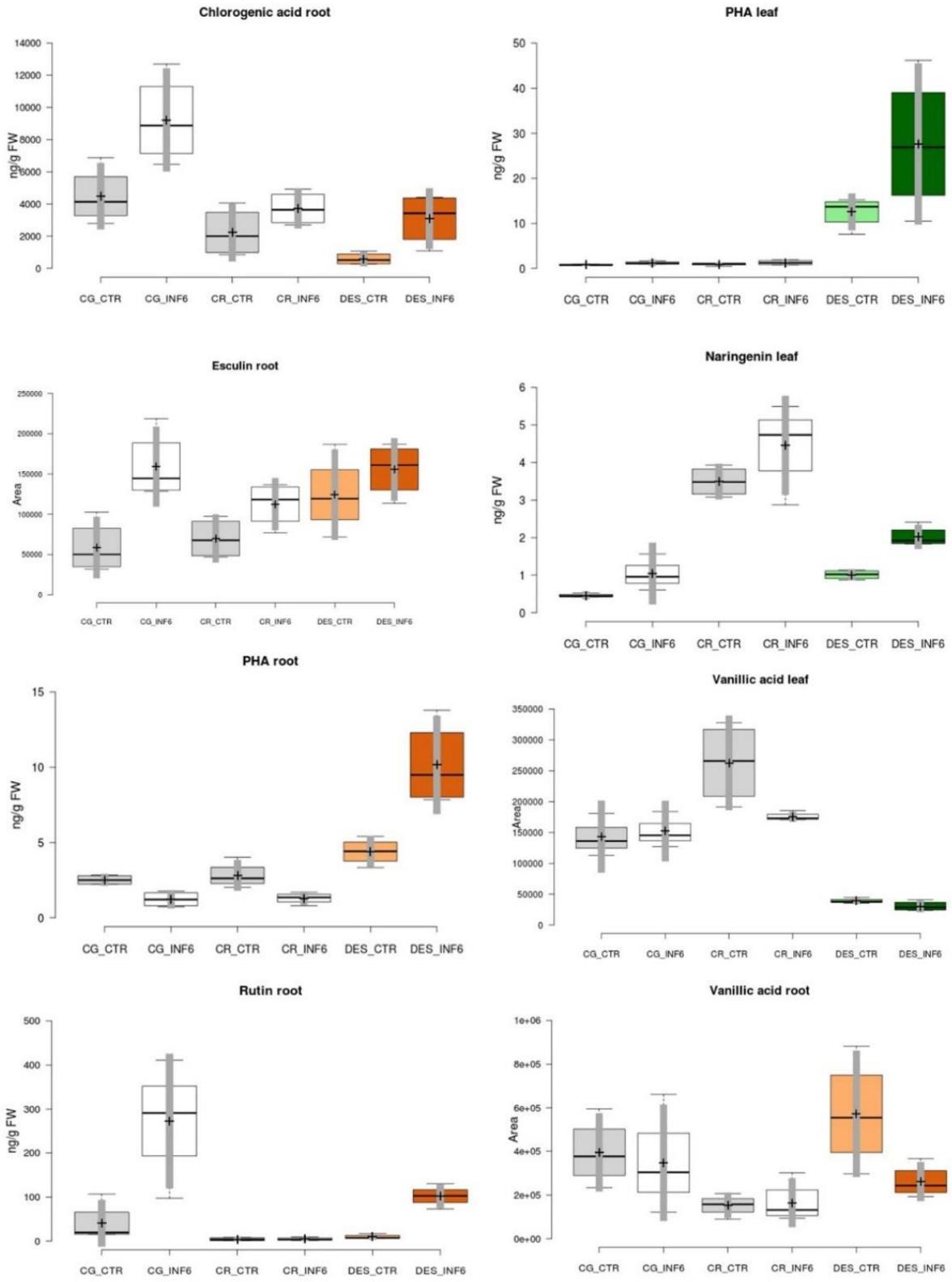


Figure 12. Concentration differences of five selected secondary metabolites between non-infected and *Rs*-infected 'Calalo Gaspar', 'Cruza 148', and 'Désirée' roots (brown) and leaves (green) at 6 dpi. CG_CTR, 'Calalo Gaspar' control (grey); CG_INF6 , 'Calalo Gaspar' infected (white); CR_CTR, 'Cruza 148' control (grey); CG_INF6, 'Cruza 148' infected (white); DES_CTR, 'Désirée' control; DES_INF6, 'Désirée' infected.

The concentration of the antibacterial compound esculin also increased in the roots of all the cultivars upon infection. Dihydrophaseic acid (PHA) in DES roots showed a different pattern

than in CG and CR roots, where it increased upon infection, whereas it decreased in the resistant lines. Comparing the controls of the genotypes, PHA was in lower basal amounts in the leaves of the resistant cultivars indicative of its inverse relation to resistance. Increased level of phaseic acid, particularly in the leaves of DES, probably contributing to stomatal closure (Kriedemann *et al.*, 1975), was also observed and could be a part of the basal defence. This is also in line with the increased amount of ABA found especially in DES (decreased in CR, unchanged in CG: **Figure 13**). Drought-stress protective pathways were also activated in the sensitive peanut genotype during *Rs* infection, possibly as response to water shortage (blocking of xylem elements) and wilting caused by the bacteria (Zhao *et al.*, 2015).

In leaves, naringenin, a flavonoid, increased in amount upon infection in all the cultivars, but particularly in CR. CG had a characteristic increase in the concentration of flavanols (quercetin and its derivatives, rutin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside) upon infection in the roots, indicating that flavanols might be the major contributors to the resistance response in CG (**Supplementary Table 5**) Flavonoids have both antioxidant and antipathogenic properties and are stress generated metabolites (Blount *et al.*, 1992; Dai *et al.*, 1996; Mierziak *et al.*, 2014) which can significantly contribute to resistance (Li *et al.*, 2021). Vanillic acid, a derivate of vanillin with antioxidant and antibacterial effects (Maisch *et al.*, 2022), was reduced in amount upon *Rs* infection in CR leaves, however, it remained unchanged in CG and DES leaves, whereas in the roots, a strong reduction of vanillic acid level was observed in DES. In contrast, the vanillic acid concentration was elevated in CG and CR. Since the primary infection site of *Rs* is the root, the root metabolome might have pivotal importance in resisting the pathogen (Qian *et al.*, 2020; Maisch *et al.*, 2022).

Upon the quantitative analysis of selected plant hormones, it was observed that *Rs* infection led to an increase in SA levels in the roots of each cultivar indicating that SA might be a part of the basal defence response to *Rs,* as suggested by Lowe-Power *et al.* (2016). The IAA level was high in CR roots, but it only increased slightly by *Rs* infection in CG and DES. The basal JA amount was higher in DES leaves than in CG and CR leaves and decreased in each genotype upon *Rs* infection (**Figure 13**).

Numerous recent studies have identified metabolic and signalling pathways involved in defence against *Rs* in various plant species (Lowe-Power *et al.*, 2016; Wang *et al.*, 2019; Pan *et al.*, 2021). For example, DEGs related to ABA, JA, IAA, and ET signalling cascades were identified in *Arabidopsis* roots at 4 dpi (Zhao *et al.*, 2019). In tomato, the JA- and ET-mediated signalling pathways were found to be associated with *Rs* resistance when elicitors were infiltrated together with bacteria into the leaves (Kawamura *et al.*, 2009). Similarly, Baichoo and Fakim

(2017) observed the expression of marker genes associated with JA, SA, and ET signalling pathways in different tomato species.

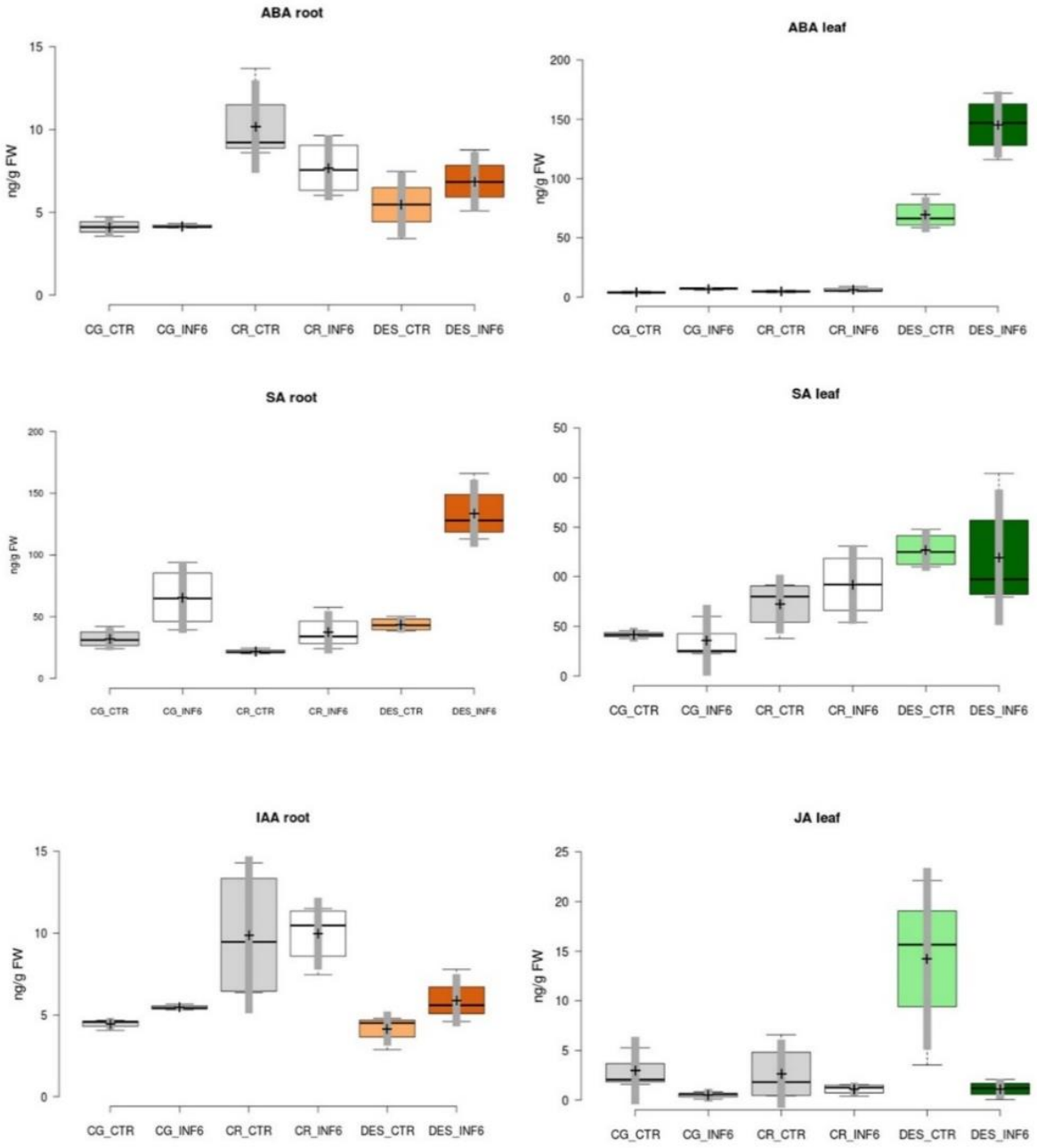


Figure 13. Concentration differences of the ABA, SA, JA, and IAA hormone levels between the non-infected and *Rs*-infected 'Calalo Gaspar', 'Cruza 148', and 'Désirée' roots (brown) and leaves (green) at 6 dpi. CG_CTR, 'Calalo Gaspar' control (grey); CG_INF6, 'Calalo Gaspar' infected (white); CR_CTR, 'Cruza 148' control (grey); CG_INF6, 'Cruza 148' infected (white); DES_CTR, 'Désirée' control; DES_INF6, 'Désirée' infected.

They concluded that SA and ET signalling plays a significant role in defence against *Rs*, while JA signalling does not. In addition, French *et al.* (2018) proposed that roots contribute to *Rs* resistance through genome-wide transcriptomic changes, leading to the activation of defence genes and alteration of IAA pathways. In our *in vitro* experiment, the expression of thousands of genes was altered at 2 dpi in roots, but no significant GO terms related to plant hormone signalling

pathways were identified. Nevertheless, we detected changes in the concentration of four plant hormones (ABA, SA, JA, and IAA) at 6 dpi in the infected roots of three cultivars. It is worth noting that there is no consensus on the role of plant hormones in defence against *Rs*. For instance, the JA-insensitive *Arabidopsis* mutant *jar1-1* is susceptible to *Rs* infection, but the loss-of-function JA receptor mutation *coi1-1* enhances plant defence against *Rs* (Hirsch *et al.*, 2002; Hernández-Blanco *et al.*, 2007). Similarly, though SA-deficient *Arabidopsis* transgenic plants do not show significant differences in wilt symptom development, an overexpression of *NahG* (SA hydroxylase) restores susceptibility to *Rs* in the *Rs*-resistant *wat1* mutant (Denancé *et al.*, 2013). Therefore, increased concentrations of ABA, SA, JA, and IAA alone may not be sufficient to prevent *Rs*-induced wilting in DES plants. We have to reiterate that the level of SA increased in roots of all of the tested cultivars during the infection, but the level of JA (present in very low amounts, data not shown) and ABA only increased in the susceptible DES. Thus, similar to tomato (Baichoo and Fakim, 2017), JA may be dispensable for *Rs* resistance in potato.

5.1.4. Lignification of root cell walls influences *Rs* resistance

The GO analysis of transcriptome data (**Figure 8)** revealed that *Rs* infection led to an increase in cell wall metabolic processes in the roots of CR and DES. The metabolomic analysis also pointed to the possibility of cell wall reinforcement as a point of difference between the cultivars owing to chlorogenic acid among others. Therefore, we employed confocal microscopy to further investigate the reinforcement of the cell wall in *Rs*-infected roots compared to non-infected roots. In this analysis, root cross-sections were stained with safranin to visualise the level of lignification (**Figure 14**).

In all three cultivars (CG, CR, and DES), the central parts of the roots (the stele), and primarily the xylem, exhibited lignification even in the absence of *Rs* infection (**Figures 14A-C**). However, the area and intensity of the red staining (indicating lignin) were the lowest in DES (**Figure 14C**). In addition to the central stele, the innermost layer of the cortex in the *Rs*-resistant CG also displayed some lignin (**Figure 14A**). The presence of *Rs* infection did not alter the extent of lignification in CG (**Figure 14D**). However, it significantly increased lignification throughout the stele in CR (**Figure 14E**), and even more dramatically in the xylem of DES (**Figure 14F**). Also, a large quantity of *Rs* bacteria was detected in DES (**Figure 14F**). This suggests that the basal level of lignification in DES may not have been sufficient to prevent *Rs* invasion.

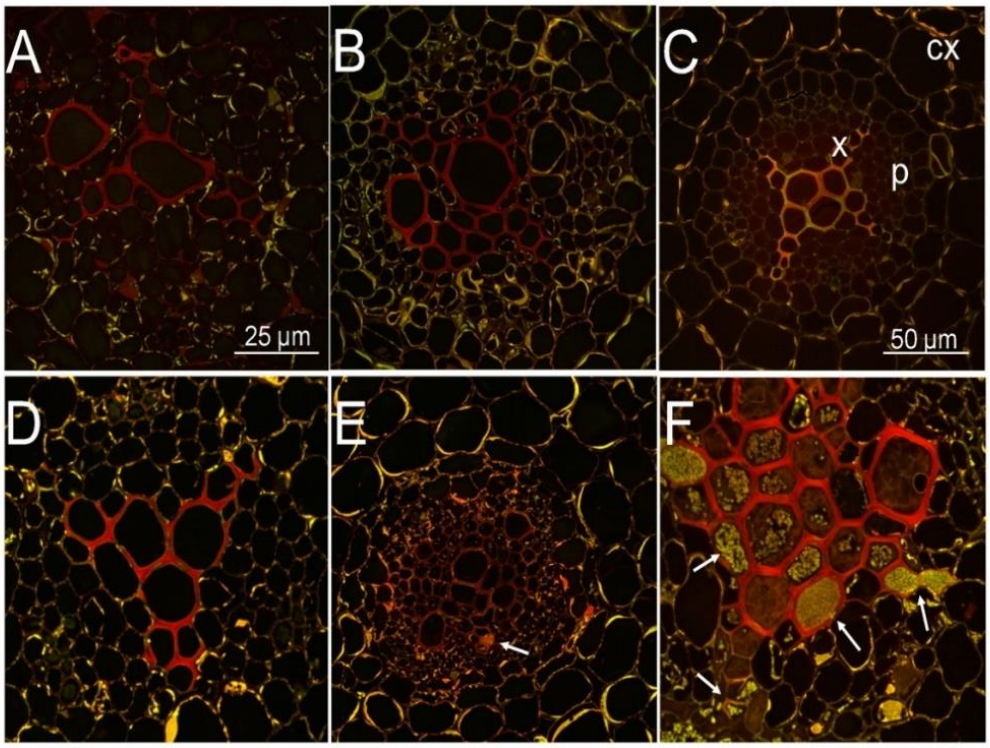


Figure 14. Confocal microscopy images of potato root cross-sections from non-infected control (A-C) and *Rs*-infected (D-F) plants at 6 dpi. Samples from 3-week-old, *in vitro* plants were stained with safranin for lignin and Fast Green for contrast. 'Calalo Gaspar' control (A), 'Cruza 148' control (B), 'Désirée' control (C), 'Calalo Gaspar' infected (D), 'Cruza 148' infected (E), 'Désirée' infected (F). cx, cortex; p; parenchyma; x, xylem; arrows, Rs bacteria (Jose *et al.*, 2023).

Lignin, a crucial secondary metabolite, is synthesised within the phenylalanine/tyrosine metabolic pathway in plant cells. The biosynthesis pathway of lignin in higher plants initiates with PAL and terminates with peroxidase (POD) and laccase. Accordingly, the expression of two *PAL* transcript variants and multiple *POD*s was significantly upregulated in CR roots upon *Rs* infection (Jose *et al.*, 2023). Lignin plays a pivotal role in plant response to various biotic and abiotic stresses, with regulation involving MYB transcription factors and microRNAs (reviewed by Cesarino, 2019). In this study, a substantial number of *MYB* DEGs, ranging from 30 to 55, were identified in each cultivar. Notably, in CG, which maintained undisturbed lignification, the majority of *MYB*s (40 out of 55 or 73%) exhibited a slight downregulation. In contrast, CR and DES displayed a characteristic increase in the number of upregulated *MYB*s to balance the distribution (Jose *et al.*, 2023).

The cell wall serves as the initial physical defence layer of plants against pathogens. Studies on *Arabidopsis* mutants with impaired cell wall-cellulose synthesis have shown that alterations in primary and secondary cell wall formation can confer resistance against vascular pathogens, including *Rs* (Hernández-Blanco *et al.*, 2007; Denancé *et al.*, 2013; Kesten *et al.*, 2017). Therefore, our study could also highlight differences in cell wall fortification as a resistance response to *Rs* infection which differed between the resistant cultivars as also mentioned by Nakaho *et al.* (2000). It is worth noting that the resistant CR demonstrated a self-protective mechanism against bacterial

invasion through cell wall reinforcement. On the other hand, CG exhibited primarily a metabolic response (as discussed in the previous chapter). Nonetheless, both strategies appear to be viable in countering the pathogen.

5.2. Candidate susceptibility genes that could contribute to pathogen resistance in potato

Based on the transcriptomic data and simultaneous analysis of relevant literature, we screened for candidate susceptibility genes from the DEGs that were downregulated among the resistant lines contrary to susceptible DES and could be potentially used for CRISPR/Cas9 editing to confer resistance against the pathogens *Rs* and *Pi* as listed in **Table 11**. We identified candidate susceptibility genes including genes from **Table 11** the editing of which might confer resistance to *Rs* and might even contribute to broad-spectrum resistance, including defence against the hemibiotrophic pathogen *Pi*. The candidate genes range from *WRKY* transcription factors to genes involved in cell wall building and ion transporters that can facilitate infection (**Table 11**). The three candidates from this combined analysis, namely *POLYPHENOL OXIDASE* (*Pot32*, literature), *stu-miR396* (literature) and *SULPHATE TRANSPORTER* 3;1 (transcriptome data) is described in detail in the upcoming sections.

Table 11. List of candidate susceptibility genes selected from transcriptome data and literature analysis for CRISPR/Cas based genome editing

S. No.	Target gene	Gene id	Gene_chr	Gene start	Gene end	Gene strand	Cultivar	No. of generated trg. lines	No. of confirmed, sequenced CRISPR genome-edited lines	No. of lines tested for Rs resistance	No. of lines showing effect (tolerance/ susceptibility)	Additional comments
	Genes discussed in detail											
1	Sulphate transporter 3.1	AT3G51895.1	chr03	19251287	19255922	+	Arabidopsis thaliana (Col)	0	0	1 T-DNA insertion mutant: SALK_023190C	1	The potato homologue was there in our own transcriptome data, in resistant varieties, supported by literature too. Very strong Rs resistance found in all tests.
	Ongoing work											
2	Sulphate transporter 3.1	Soltu.DM.06G034550.1 Soltu.DM.09G024940. Soltu.DM.09G020160.1 in SPUD DB	chr09	60833361	60838774	-	Désirée	none	none	no data	no data	Resistance validated in Arabidopsis; Construct designed for potato, transformed
3	Probable WRKY transcription factor 71 in potato, the Arabidopsis homologue is WRKY28	Soltu.DM.02G014150.1; AT4G18170.1 (TAIR)	chr02	28805293	28806931	-	Arabidopsis thaliana (Columbia)	20	5 OX lines	no data	no data	The gene was there in our own transcriptome data, in resistant varieties. Arabidopsis homologue overexpressed in Arabidopsis; resistance to be validated
4	Serine/threonine-protein phosphatase PP1 StTOPP6	Soltu.DM.06G014600.1	chr06	40053020	40059222	-	no data	none	none	no data	no data	Construct being designed for editing in potato
5	WRKY transcription factor 22	Soltu.DM.01G034200	chr01	73800897	73802758	+	Balatoni Rózsa	none	none	no data	no data	Construct designed for potato for editing, transformed
	Completed work											
6	DMR6-LIKE OXYGENASE 1	Soltu.DM.03G030400	chr03	54868368	54891227	+	Botond	10	4	4	2	The gene was there in our own transcriptome data, in resistant varieties, supported by literature too. Delayed symptom development has been observed in first tests, in 2 tested lines out of the 4
7	WAT1-related protein	Soltu.DM.03G000570.1	chr03	617947	622736	+	Balatoni Rózsa	3	0	None	None	The gene was there in our own transcriptome data, in resistant varieties, supported by literature too. Regenerants, bot no mutants. Too AT-rich first exon, Cas9 target could not have been designed, Cas12 would have been the solution. It was our first attempt with Cas12, but we failed.

5.3. Studying the effect of *PPO* knockout in tetraploid potato on resistance to *Rs* and *Pi*

5.3.1. Generation of *PPO* mutant lines

The process of potato transformation including the vector construct with the CRISPR targets of the *Pot32 PPO* gene is presented in **Figure 15**. The pKSE401 vector harbouring the editing construct was mobilised into *A. tumefaciens* (LBA4404) and its uptake was confirmed with colony PCR. The *Agrobacterium* harbouring the *Pot32*-editing construct was used for leaf transformation of the potato cultivars ‘Désirée’ (DES) and ‘Balatoni Rózsa’ (BR).

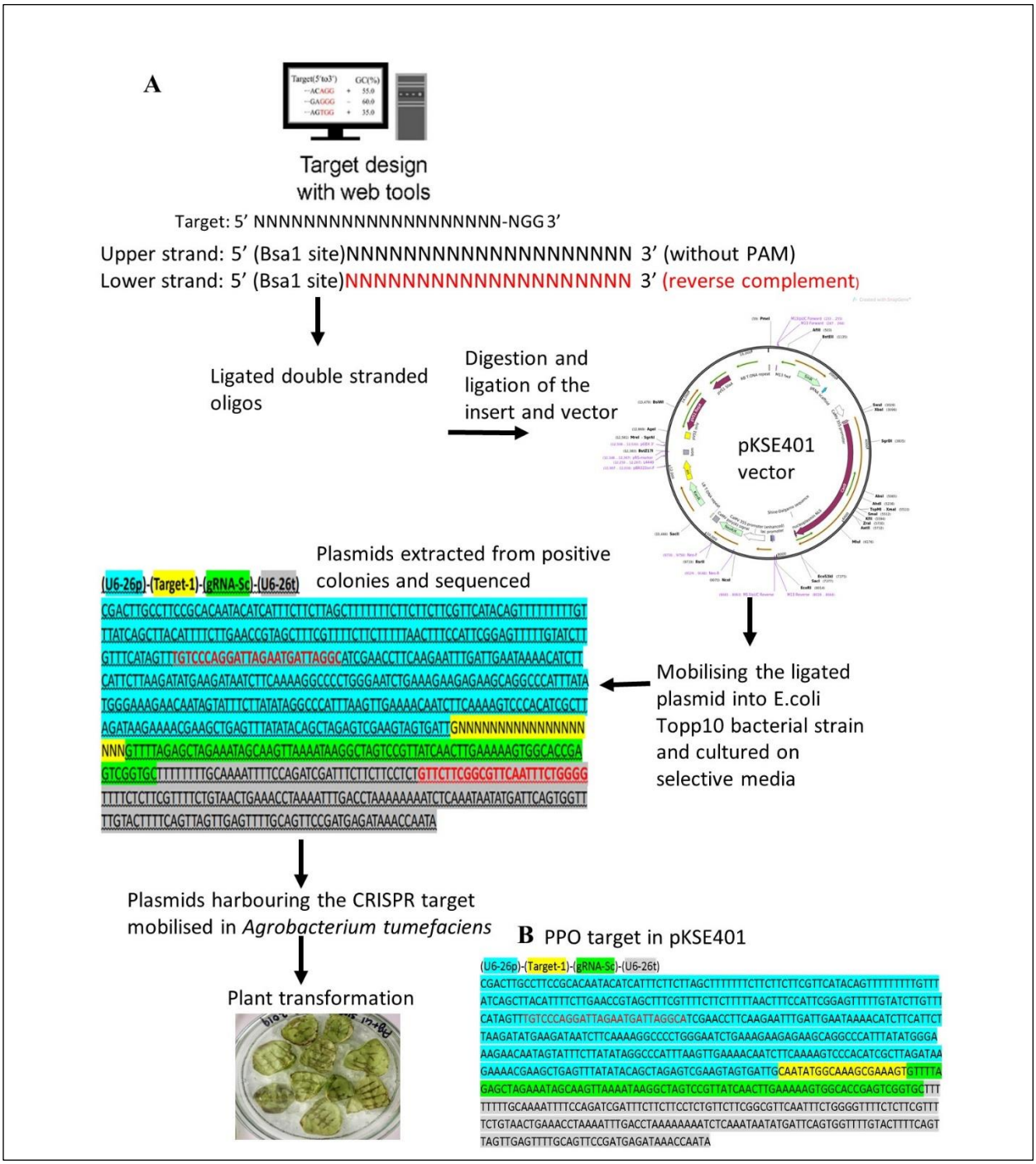


Figure 15. Systematic building CRISPR/Cas constructs for plant transformation (A). The CRISPR target for the *PPO* gene was designed and assembled into the pKSE401 vector highlighted in yellow (B).

Among the 20 transformed plant lines/genotype generated, two DES lines (referred to as D14 and D17) and two lines from BR (referred to as BR12 and BR25) were selected for further examination to understand the effect of *PPO* knockout across genotypes. All four of these lines carried the sgRNA-coding oligo (**Supplementary Figure 1**) and were devoid of *Agrobacterium* contamination (**Supplementary Figure 2**). Additionally, they contained various mutations at the target site within the *Pot32 PPO* gene (**Supplementary Figure 3**). D14 and D17 only had mutations in two of four alleles, BR12 had monoallelic mutation for this gene, while BR25 had mutations in three alleles of *Pot32* contrary to other lines (**Supplementary Figure 3**). These plants also exhibited a few mutations in other *PPO* genes, such as *Pot33* and *Pot72*. Especially, BR25 showed distinct mutations (**Supplementary Figure 3**). Phenotypically, all edited lines maintained the cultivar-specific morphological characteristics, however, displayed a slightly smaller plant height compared to the controls. An exception to this trend was observed in BR25, which exhibited rapid growth, attaining a tall plant height.

5.3.2. Reduced browning and PPO activity in *PPO*-knockout mutants

All four examined lines, particularly D17 and BR25, displayed significantly reduced browning in their tubers compared to the respective controls (**Figure 16A,B** and **Supplementary Table 7**), indicating a decrease in PPO activity. D14 and D17 had 10.9% and 33.8% reduction, respectively, in browning compared to the control, while BR12 and BR25 had 10.9% and 13.2% reductions, respectively, related to their control. Three of the mutant lines (D14 and D17 and BR25) also exhibited reduced (7.6 %, 11.5 % and 8.6%) browning in their roots when compared to their unedited controls (**Figure 16A,B** and **Supplementary Table 7**). However, BR12 did not show such kind of decrease.

Reduced PPO enzymatic activity was observed in the tubers of all edited lines except BR12. BR25 displayed the most significant difference, indicated by a *p*-value of 0.0005 (**Figure 16B**). Measurement of PPO enzymatic activity confirmed the reduced PPO activity in the roots of D14 and D17 in comparison to the control, and similar results were observed for BR25 and BR12 (**Figure 17B**).

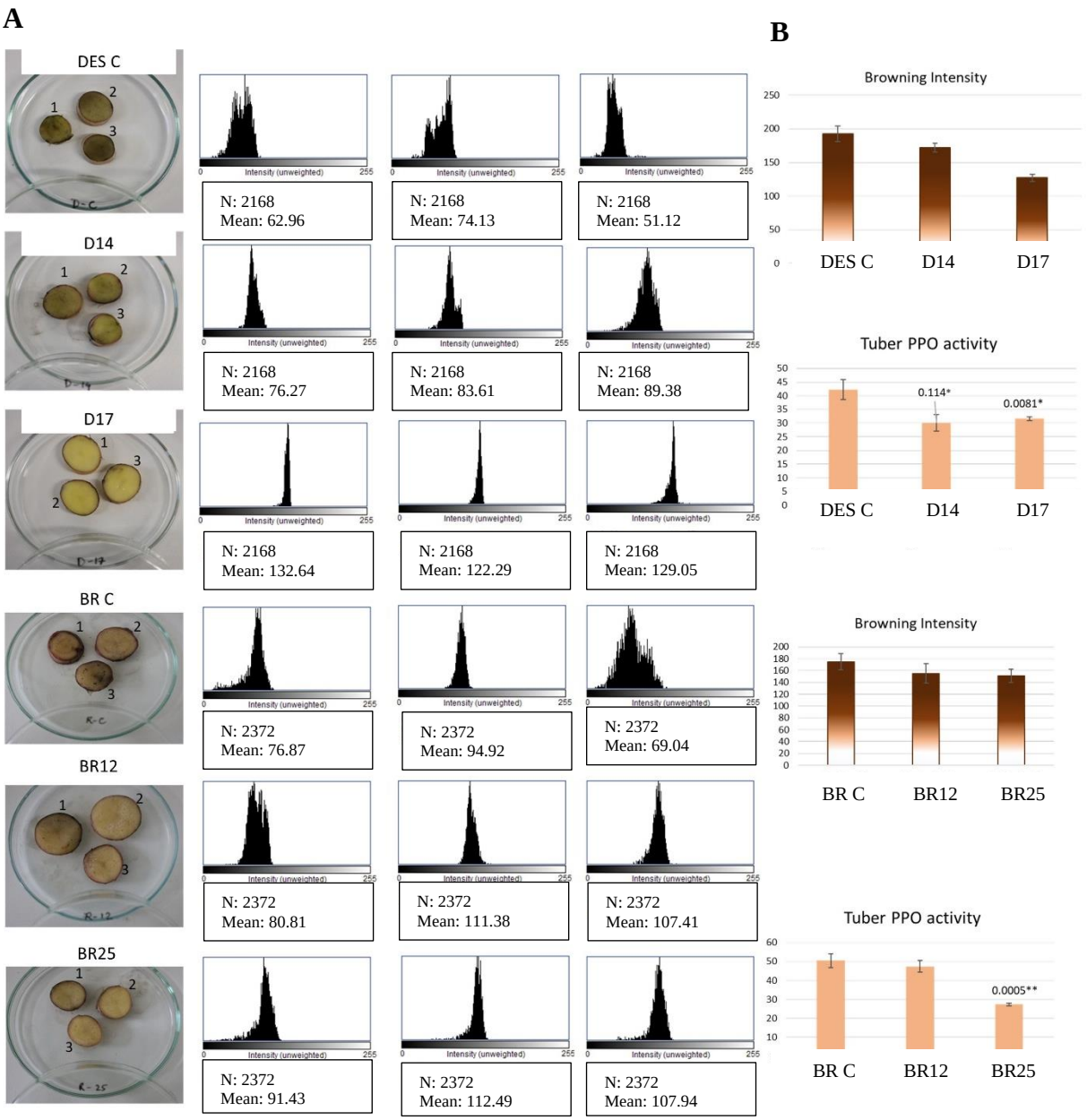


Figure 16. Quantification of browning assay done on tubers of control and *PPO* mutants with substrate solution containing 0.01M disodium tyrosine and PPO enzyme activity in tubers against 15 mM 4-methylcatechol. Browning as observed on the tuber slices (Column 1) of ´Désirée' control (DES C) and *PPO*-mutants ´Désirée' Line14 and 17 (D14, D17), 'Balatoni Rózsa' control (BR C) and PPO-mutants, ´Balatoni Rózsa' Line12 and 25 (BR 12 and BR25), with the histogram of the three slices (Cloumn 2-4; left-right) depicting the degree of browning (A). PPO browning intensity and enzyme activity against 15 mM 4-methylcatechol of potato tubers of ´Désirée' control (DES C) and *PPO*-mutants ´Désirée' Line14 and 17 (D14, D17), 'Balatoni Rózsa' control (BR C) and PPO-mutants, ´Balatoni Rózsa' Line12 and 25 (BR 12 and BR25) (B). * indicates the level of significance of p-value<0.05

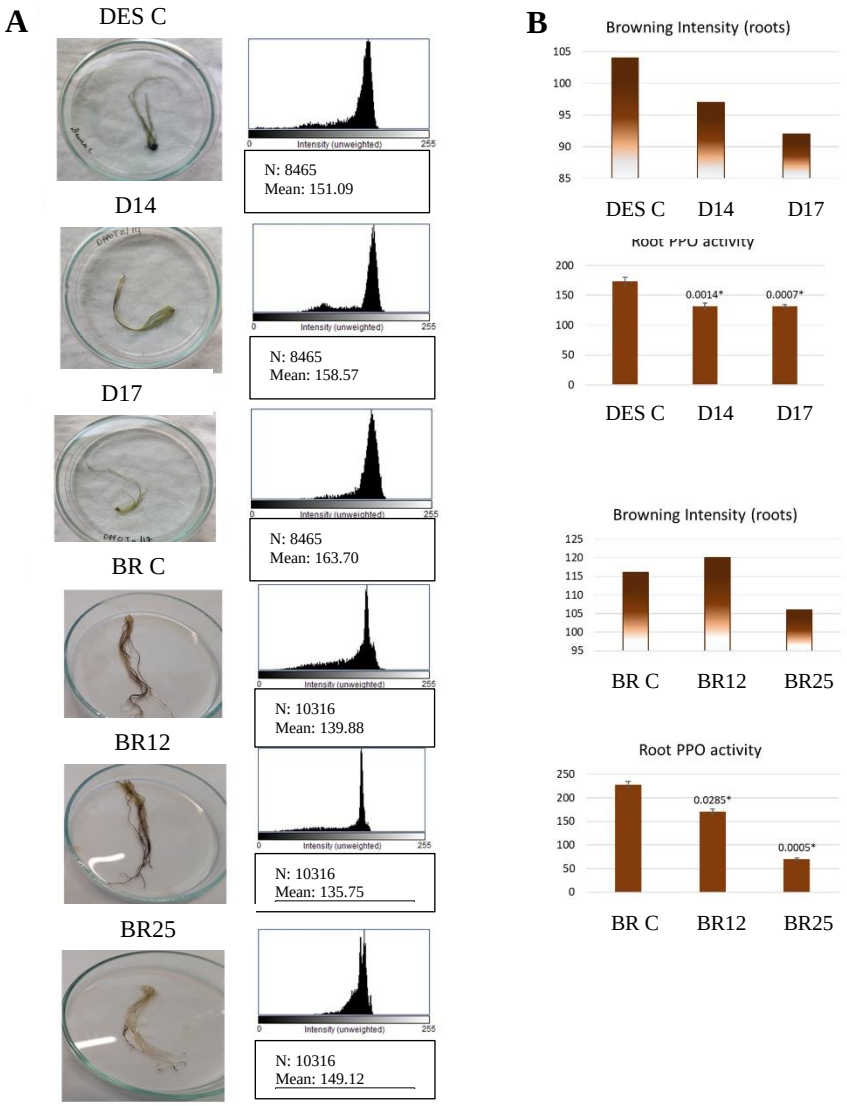


Figure 17. Quantification of browning assay done on roots of control and *PPO* mutants with substrate solution containing 0.01M disodium tyrosine and PPO enzyme activity in tubers against 15 mM 4-methylcatechol. Browning as observed on whole roots (top-bottom) of ´Désirée' control (DES C) and *PPO*-mutants ´Désirée' Line14 and 17 (D14 and D17), 'Balatoni Rózsa' control (BR C) and *PPO*-mutants, ´Balatoni Rózsa' Line12 and 25 (BR 12 and BR25, with the plotted histogram depicting the degree of browning (A) PPO enzyme activity against 15 mM 4-methylcatechol and quantified browning intensity of potato roots of ´Désirée' control (DES C) and *PPO*-mutants ´Désirée' Line14 and 17 (D14 and D17), 'Balatoni Rózsa' control (BR C) and PPO-mutants, ´Balatoni Rózsa' Line12 and 25 (BR 12 and BR25) (B). * indicates the level of significance of p-value <0.05

One of the collateral advantage of this study with PPO was the enhancement of the commercial quality of potatoes by reducing browning, which was successfully achieved by disrupting *PPO* genes. While this approach was previously employed by various groups to decrease PPO activity in potatoes using methods like RNA interference or CRISPR-related techniques (Bachem *et al.*, 1994; Llorente *et al.*, 2010, 2011; Chi *et al.*, 2014; González *et al.*, 2020) our dual-genotype approach provides a more comprehensive understanding of the role of PPO in potatoes and extends the applicability of this knowledge to the Hungarian cultivar, BR.

5.3.3. Metabolomic analysis of edited PPO lines

Our objective was the detection of the metabolic changes related to the introduced *PPO* mutations. Previously, it was demonstrated that downregulation of *PPO* genes by RNAi leads to diverse metabolic alterations in potato tissues (Araji *et al.*, 2014; Llorente *et al.*, 2014; Shepherd *et al.*, 2015). These studies delineated a redirection of the metabolic pathway and demonstrated elevated levels of specific metabolites such as chlorogenic acid, fraxin, naringenin, phaseic acid and taxifolin. Surprisingly, however, the levels of well-known PPO substrates, including caffeic acid, did not exhibit a significant increase in amount in any of our *PPO* mutants (**Supplementary Figures 4ABC**). Nevertheless, our UPLC-MS/MS measurement revealed variations in the concentrations of 26 diverse phenolic compounds between the control and *PPO*-edited potato plants (**Supplementary Tables 8**). The PCA analysis resulted in detection of a marked divergence in the root metabolome for most *PPO* mutants (D14, D17, and BR25; **Figure 18C and D**). Intriguingly, this contrast in metabolomic profiles was not observed in the leaf metabolome, except for BR25 (**Figure 18A and B**), which might be explained with the selection of tuber-specific *PPO* genes for targeted mutagenesis.

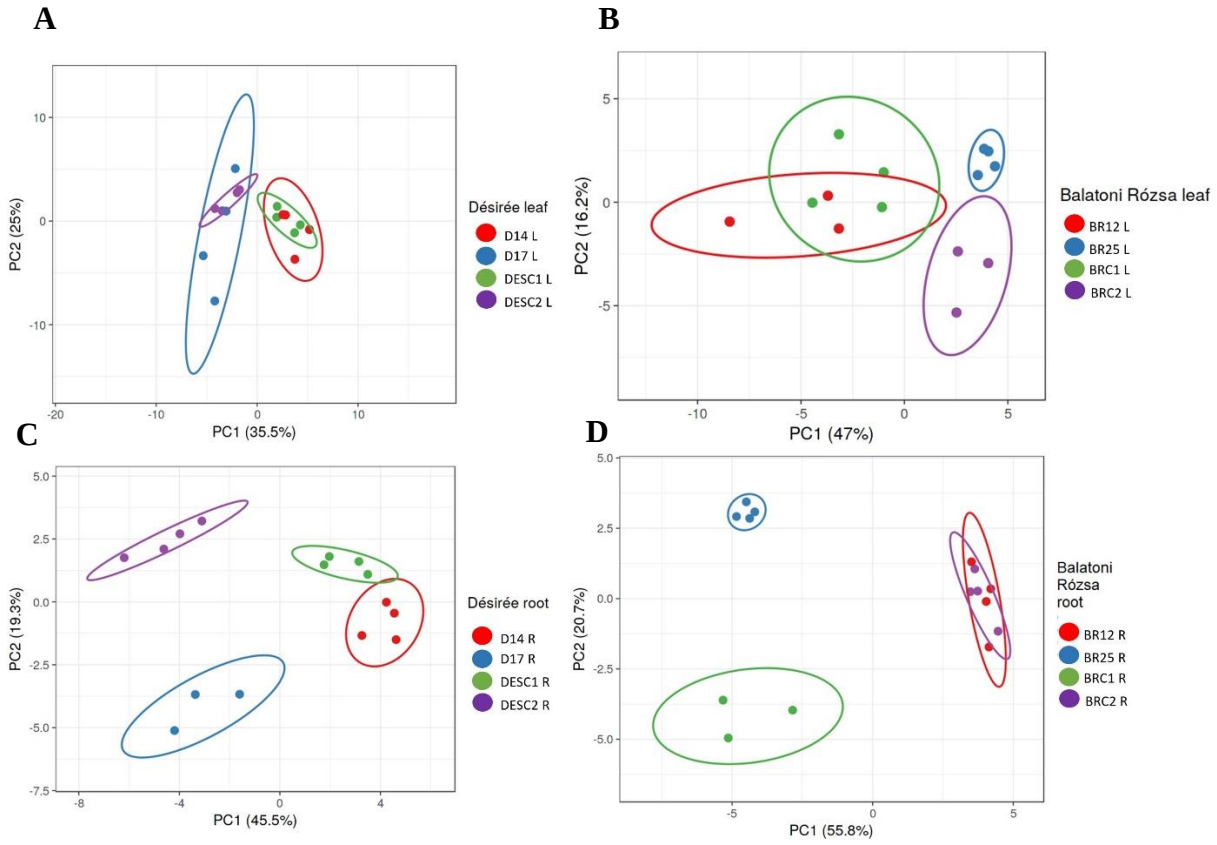


Figure 18. Principal component analysis of 26 metabolites. In leaves (A) and roots (C) of ´Désirée' control DESC1 and DESC2 (4 technical replicates each) and *PPO* mutants D14 and D17 (4 replicates each), and in leaves (B) and roots (D) of ´Balatoni Rózsa' control BRC1 and BRC2 (4 technical replicates of each) and *PPO* mutants BR12 and BR25 (4 replicates each).

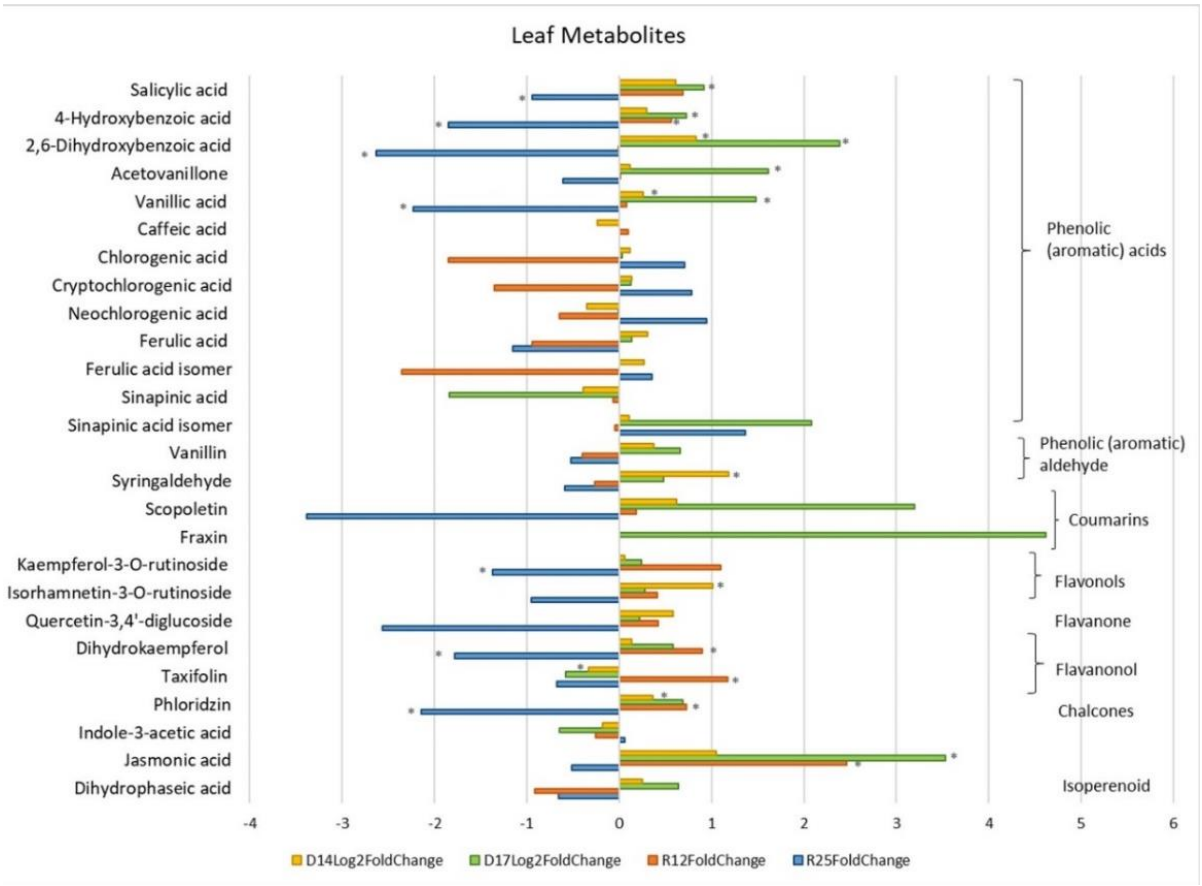


Figure 19. Differential regulation of 26 metabolites calculated by the Log2Fold change with respect to the control in the leaves of the mutant lines 'Désirée' Line 14 (D14), 'Désirée' Line 17 (D17), 'Balatoni Rózsa' Line 12 (R12) and 'Balatoni Rózsa' Line 25 (R25). * indicates significant changes at $p \leq 0.05$

We also assessed the impact of *PPO* gene knockout on the phenolic metabolome across different genotypes (**Figure 19, 20 and Supplementary Figures 4ABC**). The root and leaf metabolome highlighted certain metabolites that changed similarly in three (D14, D17 and BR12) of four edited lines plausibly accounting to *PPO* knockout which involved increase of flavonols (isorhamnetin-3-O-rutinoside and quercetin-3,4'-diglucoside). However, roots and leaves of BR25 specifically had significantly reduced amounts of flavonoids including naringenin, and taxifolin (**Figure 19 and 20**). In line with PCA results, leaves of BR25 exhibited diminished levels of certain metabolites (e.g., dihydrokaempferol, 2,6-dihydroxybenzoic acid, and phloridzin) compared to its control, a trend not observed in other mutants. (**Supplementary Figure 4C**). Phaseic acid levels were elevated in roots of D14 and D17, BR25 while, vanillic acid and indole-3-acetic acid showed lower levels in D17 and BR25 (**Figure 20**). The genotype specific differences upon *PPO* knockout was observed in flavonoids like naringenin, dihydrokaempferol and taxifolin, recognised as a PPO substrate in walnut (Panis and Rompel, 2020), which increased in the roots of D14 and D17 and decreased in BR12 and BR25 relative to their controls. Dihydrophaseic acid also characteristically increased in the roots and leaves of edited DES lines and decreased in BR12 and BR25 (**Figure 19 and 20)**. Notably, the root metabolomes of the two potato cultivars exhibited

marked distinctions, emphasising the impact of *PPO* knockout. It is worth noting that BR12 did not exhibit significant differences from its control.

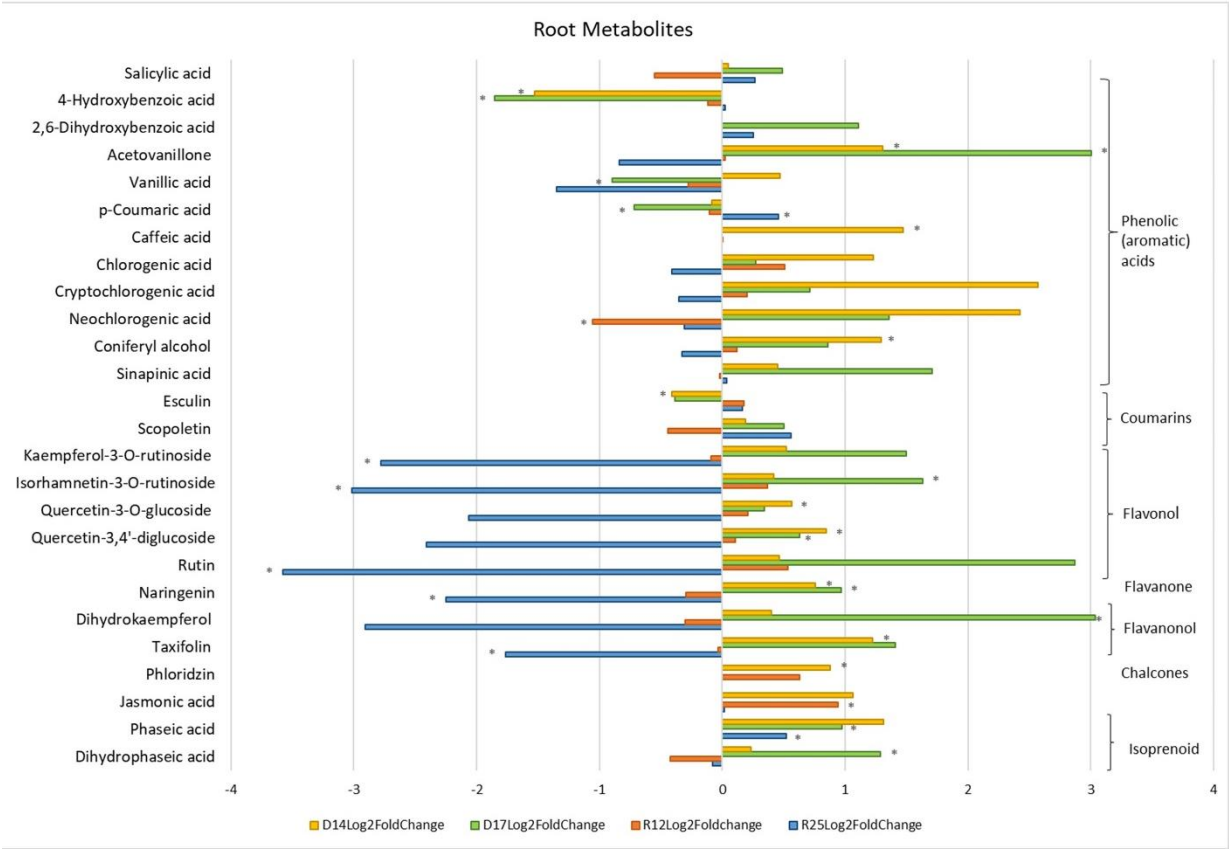


Figure 20. Differential regulation of 26 metabolites calculated by the Log2Fold change with respect to the control in the roots of the mutant lines 'Désirée' Line 14 (D14), 'Désirée' Line 17 (D17), 'Balatoni Rózsa' Line 12 (R12) and 'Balatoni Rózsa' Line 25 (R25). * indicates significant changes at $p \leq 0.05$

The impact of *PPO* silencing on hormonal levels was not previously documented. Various *PPO* genes exhibit responses to stress-related hormone signalling, albeit with variations across species and within *PPO* gene families (Constabel and Ryan, 1998). As reviewed by Taranto *et al.* (2017), these genes were induced by SA, JA, systemin, and, in certain species, by GAs (Taneja and Sachar, 1974; Stewart *et al.*, 2001). While Araji *et al.* (2014) observed necrotic lesions on *PPO*-silenced walnut leaves and suggested a role for SA in programmed cell death induction, our findings did not align with this hypothesis, as significant elevated SA levels were not detected. Nevertheless, our study underscores the occurrence of regulatory changes due to *PPO* silencing. Remarkably, we observed heightened levels of JA predominantly in the DES mutants and various forms of GAs in BR *PPO*-knockout plants. JA is recognised for enhancing secondary metabolism, whereas GAs were reported to inhibit flavonoid biosynthesis (Hinderer *et al.*, 1984). In line with this, the increased metabolite production was predominantly observed in the DES lines following *PPO* knockout. However, when shoot tip samples were analysed, both BR lines exhibited elevated

levels of GA8, and GA20 was increased only in BR25, suggesting a genotypic differential response (**Supplementary Figure 5**). This differential response may have contributed to the observed rapid growth phenotype in BR25. Our hypothesis posits that plants, possibly through redox regulation, could sense the effects of *PPO* knockout, prompting a negative feedback regulation. This regulatory mechanism, in turn, instigates the synthesis of JA and GA hormones, thereby activating alternative defence mechanisms against pathogens in the absence of PPOs. Elevated levels of phaseic acid, a degradation product of ABA, was observed across most mutants. While literature on phaseic acid's role in *Rs* resistance is limited, as one of the catabolic product of ABA alongside dihydrophaseic acid (Ye *et al.*, 2012), (its elevated levels in the susceptible DES mutants) coupled with extremely low amounts of ABA (could not be quantified) across genotypes, align with literature in mediating *Rs* basal response.

Comparisons with our previous studies on the *Rs*-resistant lines CG and CR and the susceptible DES, revealed insightful patterns. Dihydrophaseic acid exhibited an inverse relationship with resistance in roots of resistant lines contrary to increased amounts in D14 and D17 *PPO* mutants, and rutin, associated with *Rs* resistance, showed a decrease in the leaves of *PPO* mutants BR25 and D17 contrary to CG. These findings highlight the intricate interplay of these metabolites in defence mechanisms against *Rs*.

5.3.4. Increased susceptibility of *PPO* mutants toward *Rs* infection

While monitoring the GFP-tagged *Rs* strain UW551, we observed that the *PPO*-edited D14 and D17 displayed higher susceptibility to *Rs* compared to the unedited DES control. This augmented susceptibility was manifested by the increased number of wilted plants and an earlier spread of the bacteria to the shoots (**Figure 21, Supplementary Table 9 and Supplementary Figure 6**). Similarly, BR25 exhibited enhanced susceptibility compared to the BR control. In this case, the plants wilted and succumbed to the rapidly spreading *Rs* infection at an earlier stage (about one week) than the DES control (**Figure 22 and Supplementary Figure 7**). Conversely, no significant disparities in symptom intensity or timing were noted in BR12 in comparison to its control (**Figure 22**).

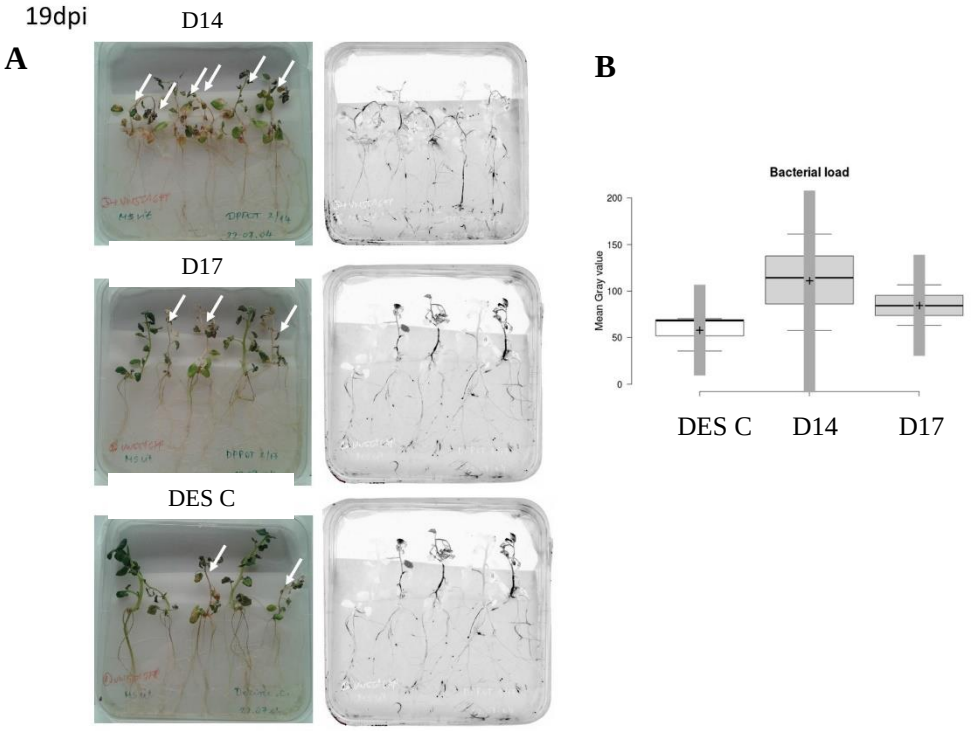


Figure 21. Visual evaluation of 'Désirée' and its *PPO* mutants in response to *Rs* infection and bacterial spreading (19 dpi) in the *in vitro* inoculation bioassay under visible light for the observation of wilting (1st column) and UV light for the detection of *GFP*-expressing *Rs* bacteria (2nd column). Arrows point to the wilted plants. (Z. Bozsó, NÖVI) (A). The spreading of *GFP*-expressing UW551 *Rs* strain was quantified by fluorescence on inverted colour images as the Region of Interest (ROI). The plots were created with the mean fluorescence intensity values obtained upon analysis using the ImageJ software (B).

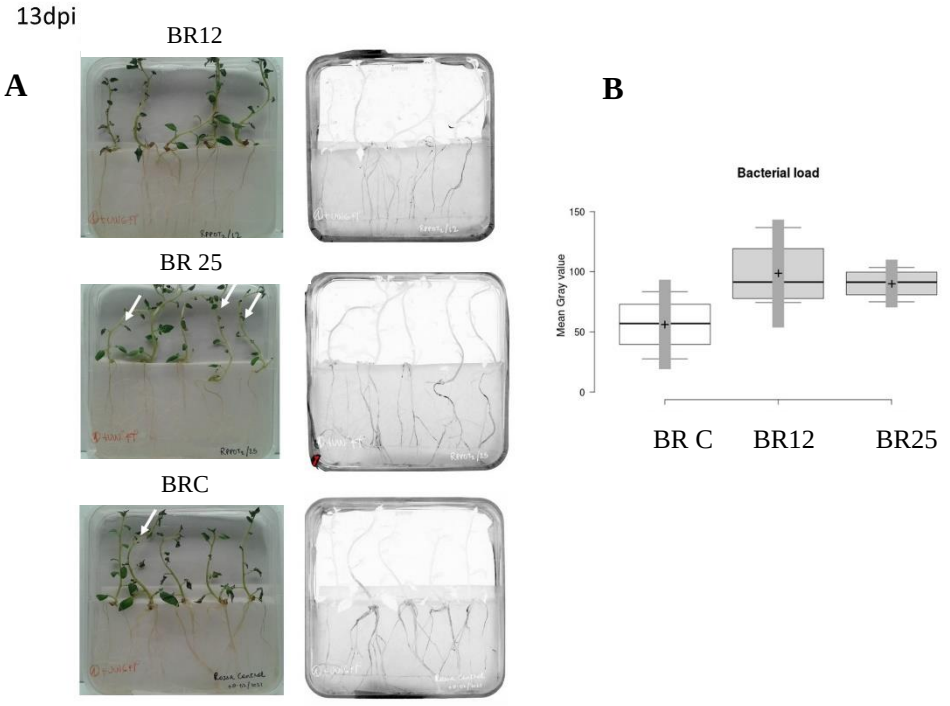


Figure 22. Visual evaluation of 'Balatoni Rózsa' and its *PPO* mutants in response to *Rs* infection and bacterial spreading (13 dpi) in the *in vitro* inoculation bioassay under visible light for the observation of wilting (1st column) and UV light for the detection of *GFP*-expressing *Rs* bacteria (2nd column). Arrows point to the wilted plants. (Z. Bozsó, NÖVI) (A). The spreading of *GFP*-expressing UW551 *Rs* strain was quantified by fluorescence on inverted colour images as the Region of Interest (ROI). The plots were created with the mean fluorescence intensity values obtained upon analysis using the ImageJ software (B).

As symptoms appeared earlier in BR compared to DES, the latter were evaluated at 19 dpi, and the former genotypes were assessed at 13 dpi. In addition to offering representative images, we conducted a quantitative assessment of disease progression by counting the number of wilted plants (**Supplementary Table 9**), which further substantiated the susceptibility of D14, D17, and BR25, in comparison to their respective controls. D14, D17 mutants had a wilting percentage of 87% and 60%, respectively, surpassing the control's 40%. Similarly, BR25 displayed increased susceptibility, with wilting percentages of 73% compared to control's 13%. It is noteworthy that repeating the experiment consistently yielded the same results.

Higher quantity of Rs in PPO mutants than control lines

We quantified bacterial growth by assessing the fluorescence emitted by *GFP*-tagged bacteria in UV-images, and these measurements revealed elevated fluorescence in 3 plates comprising 5 plants/genotype in all of the *PPO*-edited lines compared to their respective control lines (**Figure 21B and 22B**). The levels were significant with a *p* value of 0.03 in both DES mutants (**Supplementary Table 9**). It emphasises the pivotal role of PPOs in plant defence mechanisms against the *Rs* pathogens.

A special focus of our investigation was to develop strategies for fortifying plants against *PPO*-expressing pathogens. Initially, we hypothesised that *Rs*, armed with its own PPO enzyme (Hernández-Romero *et al.*, 2005), might endure the quinones generated by PPOs but could prove susceptible to specific antimicrobial compounds (e.g., phloridzin, naringenin, taxifolin, *p*-coumaric acid) produced in higher quantities in the absence of PPOs. Surprisingly, our findings diverged from this hypothesis. Instead, it became evident that PPO activity in potatoes serves as a protective mechanism against *Rs*, akin to the observed protection against *Pseudomonas* bacteria (Li and Steffens, 2002).

In elucidating the heightened susceptibility observed in *PPO* mutants to *Rs*, we noted a significant decrease in the concentration of vanillic acid, a potent phenolic acid, in two mutants, one from each potato cultivar (D17 and BR25). As discussed earlier, vanillic acid possesses robust antibacterial, antioxidative, and antibiofilm properties (Maisch *et al.*, 2022). Interestingly, in our work (Jose *et al.*, 2023), the levels of vanillic acid only decreased in the susceptible DES roots during *Rs* infection. The diminished levels of vanillic acid in *PPO* mutant D17 and BR25 may have contributed to their increased susceptibility. In contrast, other metabolites were produced in higher quantities in some edited lines, including taxifolin, phloridzin, naringenin, and dihydrophaseic acid in the edited DES lines, and 4-hydroxybenzoic acid and *p*-coumaric acid in BR25. Although these compounds have demonstrated potential as effective antimicrobial agents (Reen *et al.*, 2018), the susceptibility of our mutants to *Rs* persisted. This paradoxical outcome

could be attributed to the loss of PPO activity, emphasising the significance of PPO as a more effective defence mechanism against *Rs*. Metabolite regulations appear to be intricately linked to the extent of mutation in the *PPO* gene. BR12, a monoallelic mutant, exhibited the least changes, while D14 and D17, each possessing two intact alleles, showed an increased production of metabolites, potentially as a compensatory response to the loss of PPO function. In contrast, BR25, with three out of four alleles definitively mutated, encountered challenges in metabolite regulation.

This discovery has spurred a fundamental inquiry at the core of our investigation, i.e., what holds greater significance as a defence mechanism against pathogens: PPOs or simple (poly)phenols? Our research affirms the pivotal role of PPOs as a potent resistance mechanism against *Rs*. As highlighted earlier, while the accumulation of polyphenolic compounds may contribute to the defence response by potentially serving as precursors for essential compounds, their effectiveness appears contingent on several factors. These factors include their spatial and temporal regulation, as well as their collaborative effects with other enzymes like PPO in achieving a robust and comprehensive resistance.

PPOs likely fulfil this role by generating cytotoxic quinones that have the capacity to bind to proteins and amino acids, thereby denying pathogens essential resources (Felton *et al.*, 1992). Additionally, PPOs are actively involved in lignification and the formation of cell walls (Cao *et al.*, 2020; Bai *et al.*, 2023). As highlighted in our previous work, lignification has already been recognised for its importance in providing resistance against *Rs* (Jose *et al.*, 2023). Consequently, the significance of PPOs cannot be underestimated, and the intricacies of resistance through polyphenols cannot be disregarded. Our results suggest that the loss of PPO activity might have outweighed potential advantages arising from increased production of antimicrobial phenolic compounds and the biotic stress hormone JA in our edited lines. Additionally, elevating JA levels as a strategy to counter *Rs* infection may prove ineffective, given its known increase in susceptible genotypes at post-infection. Resistance to *Rs* in host species has been associated with elevated SA, chlorogenic acid, phenylpropanoid pathway components, antioxidants, and glutathione metabolism (Cao *et al.*, 2020; Chen *et al.*, 2022; Jose *et al.*, 2023). In the complex landscape of plant defence mechanisms, the role of PPOs stands as important and should not be underestimated.

5.3.5. *PPO* mutation does not affect the resistance to *Pi* infection

The response of whole plants as well as potato tubers to *Pi* varied. In the whole plant assay, the BR displayed delayed symptom development compared to DES (**Figure 23A**). At 2 dpi, BR plants remained largely unaffected, whereas leaf lesions had already appeared on DES. However, by 4 dpi, both cultivars showed signs of damage, and nearly all plants from all genotypes succumbed to the infection by 7 dpi. However, the *PPO*-knockout plants did not exhibit significant differences from the respective controls at any of the observed time points.

Figure 23. Disease symptoms of whole potato plants (A) and tubers (B) of 'Désirée' (DES C) and 'Balatoni Rózsa' (BR C) controls and *PPO* mutants in a *Pi* inoculation test. Timing in the test: potato tuber slices, 5 dpi; whole plants 2, and 4 dpi.

In the tuber infection tests, *Pi* colonised the slices with the mycelia emerging on the upper side of the slices as early as 2 dpi. By 5 dpi, the mycelium uniformly covered the entire surface of all tuber slices in both cultivars. Although the mycelium mat on BR appeared slightly denser than on DES, once again, the *PPO* mutants did not exhibit significant distinctions from their respective controls (**Figure 23B and Supplementary Table 10**). The pathogen prolifically generated sporangia on all tuber slices. Contrary to literature suggesting increased *Pi* resistance attributed to *PPO* knockout (Llorente *et al.*, 2014), our findings indicate no discernible effect of the knockout on the resistance response.

5.4. The effect of *miR396* knockout in tetraploid potato on resistance to *Rs* and *Pi*

5.4.1. Generation of *mir396* mutant lines

The two-target genome editing vector was generated using the protocol of Xing *et al.* (2014) (**Figure 15A**) the use of which resulted in the generation of 10 CRISPR/Cas9-edited mutant plant lines each in two commercial cultivars 'Désirée' (DES) and 'Botond' (Bt). The mutations at

the two target sites obtained by CRISPR/Cas9 editing were identified in the Bt mutant lines 6, 7, 8, and 9 with a comparison to the unedited control (**Supplementary Figure 8A**). The edited Bt lines were complete mutants and had uniform mutations across the two targets. Analysis of mutations at the two target sites of the edited DES lines 3, 6, and 10 with a comparison to the unedited control (**Supplementary Figure 8B**) indicates that in Line 3 the entire region between the two targets was deleted in some of the alleles. In line 10, the deletion was prominent around the first target, whereas in Line 6, there was least mutation and was predominantly around the second target.

5.4.2. Evaluating the expression of predicted target genes of miR396 in the control and *mir396* mutant lines

To evaluate the impact of *miR396* knockout, we conducted selected gene expression studies focusing on predicted miR396 targets listed in **Supplementary Table 11A** using primers listed in **Supplementary Table 11B**. (based on Zhang *et al.*, 2009, 2013). Across all edited lines, a consistent reduction in the expression of both *miR396* leader and passenger strands was observed (**Figure 24**). Contrary to the anticipated increased accumulation of target genes due to the absence of miR396 inhibition, most potential targets were downregulated in the mutant lines. Though deviating, the equal number of positive and negative miRNA-target regulations was reported previously (Lopez-Gomollon *et al.*, 2012). It is noteworthy that previous studies have emphasised the importance of considering sample characteristics when examining miRNA targets and mRNA expression profiles. Sample sizes, ranging from single cells to entire organisms, can yield significantly different results. The inverse or negative relationship can manifest temporally, where miRNAs and their targets are expressed in the same cells but at different times, or spatially, where they are expressed simultaneously but in different cells, contrasting regulations have also been observed (Voinnet, 2009; Lopez-Gomollon *et al.*, 2012). Accounting to the mixed samples comprising multiple cells, positive correlation usually indicates mutual exclusion (Kawashima *et al.*, 2009). However, some of our results confirm to the expectation that removal of the miRNA will increase the level of target mRNA. The expression of the LRR receptor-like serine/threonine-protein kinase (Soltu.DM.02G002890.1/ PGSC0003DMT400054175), multicystatin147 (Soltu.DM.06G028940.5/ PGSC0003DMT400069147) and in some lines, the potassium transporter (Soltu.DM.06G013620.1/ PGSC0003DMT400006057) was found in abundance in the edited lines relative to the control (**Figure 24**).

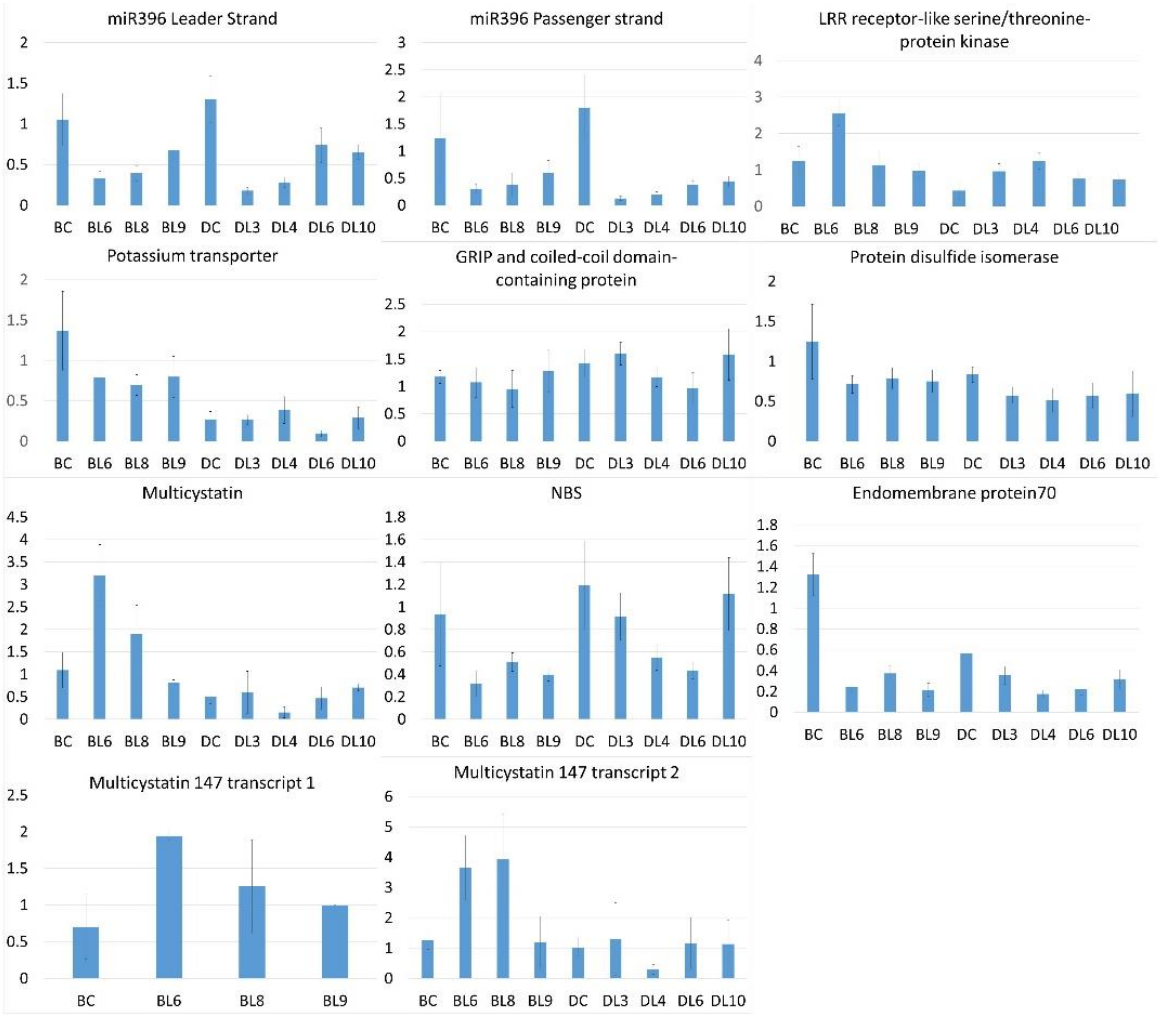


Figure 24. The relative expression of the *miR396* gene and its predicted targets in the *miR396*-edited lines of 'Botond' (BL6, BL8, and BL9) and 'Désirée' (DL3, DL4, DL6, and DL10) along with the respective controls (BC and DC).

5.4.3. Delayed symptom development of some *miR396* mutants toward *Rs* infection

Upon infecting the *miR396*-edited *in vitro* Bt Lines 6, 7, 8, and 9 and the control with the virulent *Rs* strain UW551, the edited Bt Line 8 and 9 showed a delayed symptom development in both repetitions of the experiment with reduced bacterial load (**Figure 25 and Supplementary Table 12A and B**), but eventually all plants succumbed to the infection. The display of resistance response was not prominent among the other edited lines despite sharing conserved mutations among the Bt lines. Among the DES lines, Line 3 and Line 10 showed delayed symptom development contrary to Line 6 relative to the control. As mentioned above, the CRISPR mutation varied among the lines with larger deletion in target 1 in Line 3 and 10 with significant deletion around target 2 in Line 6 (**Supplementary Figure 8B and 9**).

.

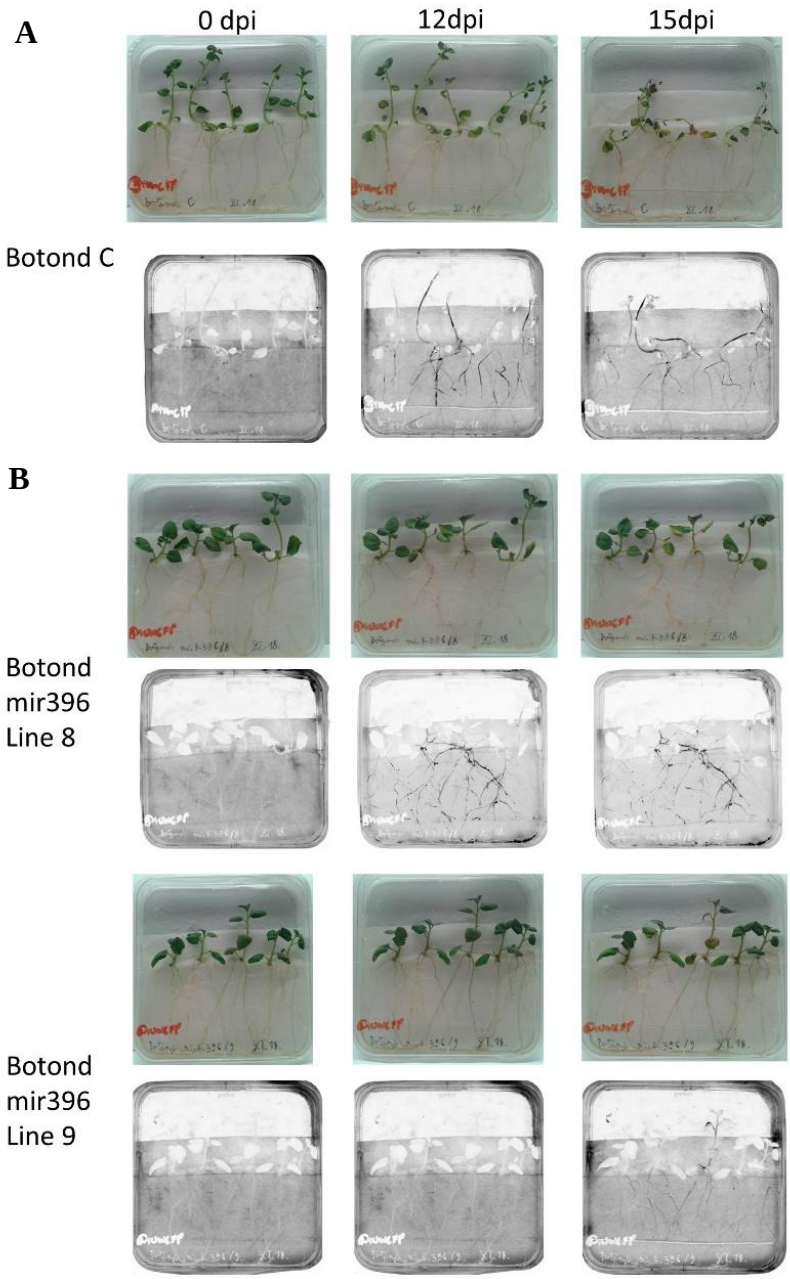


Figure 25. Visual evaluation of response to *Rs* infection (0, 12, and 15 dpi) in the *in vitro* inoculation bioassay under visible light for the observation of wilting (upper row) and UV light for the detection of *GFP*-expressing *Rs* bacteria (lower row). 'Botond' control (A) and 'Botond' *miR396* mutants, Line 8 and Line 9 (B). (Z. Bozsó, NÖVI)

As highlighted by (Zhao *et al.*, 2015), transcriptome analysis of resistant and susceptible peanut varieties exposed to *Rs* infection revealed a downregulation of miR396 in the resistant variety. The study further identified predicted targets of miR396, including a hypersensitive response factor associated with SA-mediated resistance response against (hemi)biotrophic pathogens (Grant and Lamb, 2006; Radojičić *et al.*, 2018), such as *Rs*. Consequently, an augmented resistance was anticipated in the *miR396* mutant plants. Surprisingly, the delayed resistance was only observed in Bt Line 8 and 9, while the DES mutants, specifically Line 3 and 10 exhibiting larger deletions at the CRISPR target site, displayed enhanced resistance (**Figure 25 and Supplementary Figure 8B and 9**).

5.4.4. Increased susceptibilty of *miR396* mutants toward *Pi* infection

The ‘Botond’ lines, subjected to gene editing and cultivated in a controlled greenhouse environment, displayed a markedly heightened susceptibility and swift progression of disease symptoms in the comprehensive plant-*Pi* assay, standing in stark contrast to the unaffected ‘Botond’ control group (**Figure 26**). While the control group remained unscathed even at 8 dpi, the mutant lines exhibited conspicuous leaf lesions (**Figure 26**). In the context of tuber assays, the increased susceptibility of ‘Botond’ edited lines could not be conclusively confirmed upon repetions as it showed no significant difference in colonisation by the mycelial mat of *Pi* compared to those from the ‘Botond’ control group. This could be attributed to the fact that the amount of miR396 was not quantified in the tubers prior edition and possibly has little to no effect even upon edition towads *Pi*. However, these collective observations based on whole plant assays strongly suggest a pivotal role of the *miR396* gene in regulating the defence response against *Pi*.

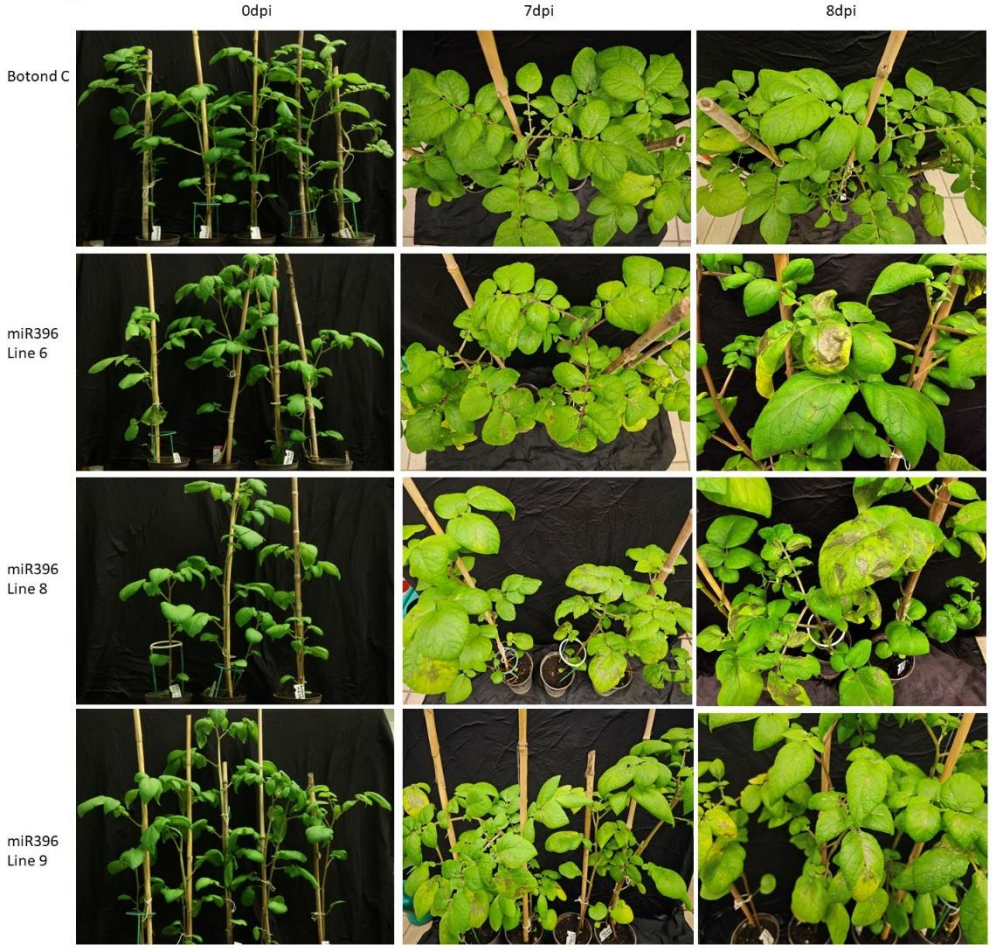


Figure 26. Visual evaluation of response to Pi infection (0, 7, and 8 dpi) in the whole plant bioassay for the observation of necrotic spots in ´Botond’ control and Botond Lines 6, 8, and 9.

Several studies have identified miRNAs as negative regulators of immunity against bacterial infection in *Arabidopsis* (Fahlgren *et al.*, 2007; Jagadeeswaran *et al.*, 2009; Antony *et al.*, 2010). In the realm of tomato defence mechanisms, miR482 was identified as a key player,

orchestrating host defence by targeting transcripts encoding coiled-coil-nucleotide-binding leucine-rich-repeat (CC-NB-LRR) proteins. This targeting triggers a cascade of events, leading to the generation of secondary small interfering RNAs (siRNAs) via RNA-dependent RNA polymerase 6 (RDR6). These secondary siRNAs, in turn, target additional mRNAs associated with defence-related genes, amplifying the plant's defence response (Shivaprasad *et al.*, 2012). Moreover, in tobacco, the regulatory roles of miR6019 and miR6020 were highlighted, as they target the immune receptor gene *N*, known for conferring resistance against the tobacco mosaic virus. This dual targeting mechanism by specific miRNAs further emphasises the complexity and versatility of miRNA-mediated regulation in plant immunity (Li *et al.*, 2012).

Recent evidence underscores the role of miR396 in the context of pathogen infections. A significant decrease in *miR396* expression was observed in wheat lines JD8 (susceptible) and JD8-Pm30 (resistant) infected with *Blumeria graminis* f. sp. *tritici*, emphasising its potential role in responding to this pathogen (Xin *et al.*, 2010). Similarly, the wheat accession Shan 4445 (susceptible) exhibited reduced *miR396* expression when confronted with *Blumeria graminis*, it being a biotrophic pathogen, suggesting a conserved mechanism in wheat-pathogen interactions (Wu *et al.*, 2015). Considering that *Rs* is also a (hemi)biotrophic pathogen, it is noteworthy that the effector RipAK of strain GMI1000 inhibits the hypersensitive response (Sun *et al.*, 2017), characteristic of the defence response towards biotrophic pathogens (Grant and Lamb, 2006)

Previous studies have asserted that the heightened expression of miR396a in tobacco leads to increased susceptibility to *P. nicotianae* infection (Chen *et al.*, 2015). More recently, miR396 was found to modulate defence responses against both hemibiotrophic and necrotrophic fungal pathogens in *Arabidopsis* (Soto-Suárez *et al.*, 2017). *Arabidopsis* plants with diminished miR396 activity, referred to as MIM396 plants, exhibited heightened resistance against both hemibiotrophic (*F. oxysporum* f. sp. *conglutinans, C. higginsianum*) and necrotrophic fungal (*P. cucumerina, B. cinerea*) pathogens. This enhanced resistance was characterised by a superactivation of defence responses, indicating a preparatory response triggered during pathogen infection (Soto-Suárez *et al.*, 2017). Contrary to these findings, our results do not align with the argument, as the knockout of *miR396* resulted in increased susceptibility to *Pi* infection. Differing and even antagonistic defence responses are known to exist for different types of pathogens (Glazebrook, 2005; Liao *et al.*, 2022), whether they are biotrophic or hemibiotrophic. In our study, we observed a positive effect of *miR396* knockout towards the (hemi)biotrophic *Rs* and an opposite result of increased susceptibility towards *Pi* inoculations. The differential regulation of the miR396 target genes, specifically the NBS-LRR receptor-like serine/threonine-protein kinase and multicystatin, might have directed the resistance response differently for both pathogens.

LRR receptors are often recognised as negative regulators of plant resistance to pathogens, with Clavata and Erecta being prominent examples (Dievart and Clark, 2004). Conversely, cysteine proteases, including caspases and papain-like proteases, are secreted by both potato and tomato during the early stages of *Pi* infection and are implicated in inducing programmed plant cell death – an effective defence against the early, biotrophic phase of *Pi* – or causing proteolytic damage to the hyphae (Avrova *et al.*, 1999; Kaschani *et al.*, 2010; Fernández *et al.*, 2012). In response, *Pi* secretes cystatin-like protease inhibitors (Tian *et al.*, 2007; Song *et al.*, 2009). The leaves pre-treated with protease inhibitors exhibit higher disease development compared to controls (Fernández *et al.*, 2012). Multicystatins, identified as potato cysteine proteases (Green *et al.*, 2013), further support the negative role of multicystatins in resistance against *Pi*, as indicated by the decreasing expression of multicystatin genes (Soltu.DM.06G028940 and Soltu.DM.06G028910) in the SPUD database for resistant potato cultivars after *Pi* infection and increased expression in our more susceptible edited lines. Nevertheless, the increased susceptibility of our *miR396* mutants with elevated multicystatin expression represents a novel finding as typically, protease inhibitors, including multicystatins, are considered to have a protective effect against pathogens by reducing the digestibility of the plant (Green *et al.*, 2013).

5.5. The *Arabidopsis thaliana* sulphate transporters and resistance response to *Rs*

5.5.1. *A. thaliana* mutant lines *AtSultr1;2* and *AtSultr3;1* display increased resistance to *Rs*

The *AtSultr3;1* sulphate transporter mutant plants showcased a notable increase in resistance to *Rs* (GMI1000) when compared to the Col-0 (Columbia ecotype) control. In parallel, the *AtSultr1;2* mutant also underwent *Rs* infection and also revealed enhanced resistance relative to the Col-0 control, but less compared to *AtSultr3;1*. The elevated resistance manifested through a significantly diminished incidence of wilted plants resulting in lower disease index and mortality rate (**Supplementary Table 13**). At 19 dpi, the *AtSultr3;1* mutants remained in a healthy state, while the Col-0 plants succumbed to the rapidly spreading *Rs* infection at an earlier stage with a higher disease index and mortality rate (**Figure 27**). Importantly, this consistent pattern was observed across multiple experimental repetitions.

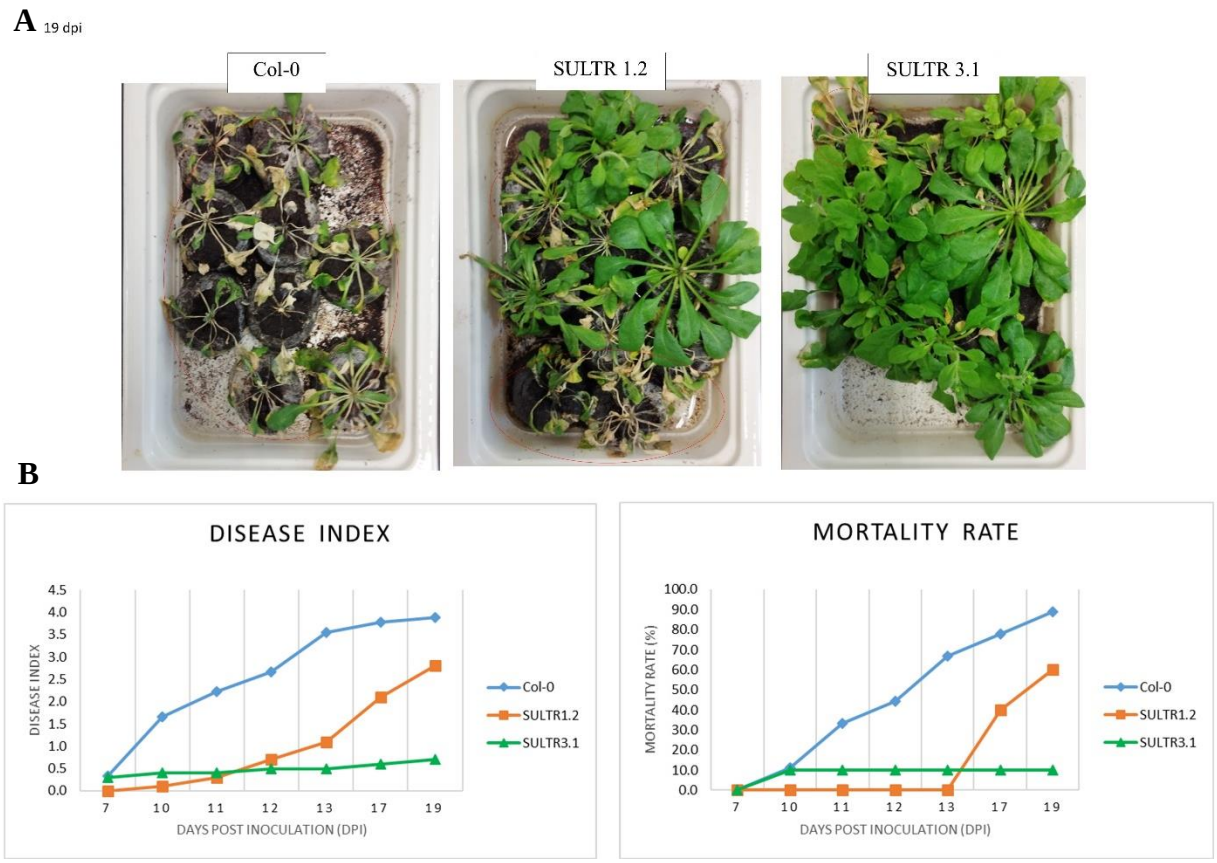


Figure 27. Visual evaluation of response to *Rs* infection at 19 dpi Jiffy soil inoculation for the observation of wilting in *Arabidopsis thaliana* Col-0 ecotype and sulphate transporter mutant lines in *Arabidopsis* Col-0 background *SULTR1;2* and *SULTR3;1*. Images captured by Z. Bozsó. (A). Disease index and mortality rate calculated based on the no. of wilted plants and the severity of wilting among the genotypes (B).

5.5.2. Greater suberisation of the roots of *AtSultr* mutants contributes to *Rs* resistance

Confocal microscopy analysis of roots stained with Fluorol Yellow in the *AtSultr1;2* and *AtSultr3;1* mutants and the control Col-0 ecotype revealed an elevated level of suberisation in the two mutants, particularly in the vicinity of the vascular bundles. Longitudinal observations of the roots using fluorescence microscopy depicted an increased fluorescence intensity in the endodermal region of both mutants compared to the control Col-0, providing clear evidence of enhanced suberisation (**Figure 28 and Supplementary Table 14**).

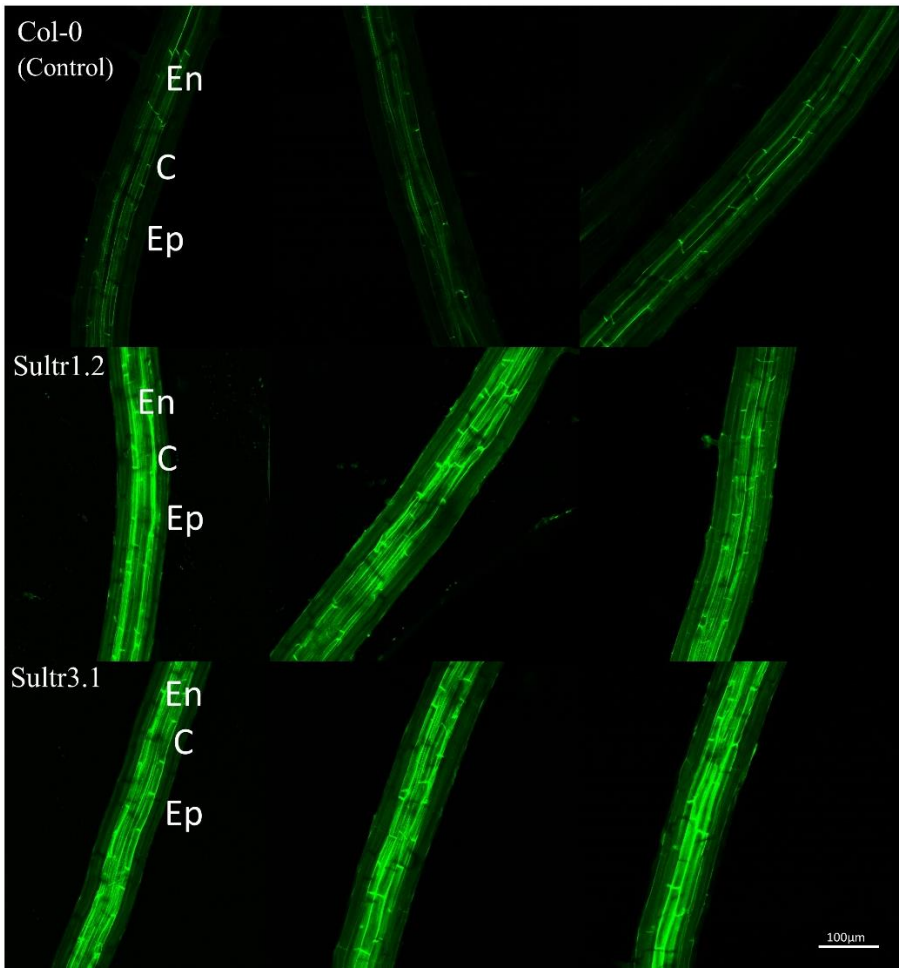


Figure 28. Confocal microscopy images to observe the level of suberisation in 7-day old roots of Col-0 ecotype (control) and the two sulphate transporter mutants (*AtSultr1;2* and *AtSultr3;1*). More intense Fluorol Yellow fluorescence is indicative of increased suberisation, particularly in the endodermis (En). C, cortex; Ep, epidermis

The cell wall, acting as both a structural support and a protective barrier, prevents water loss and pathogen entry. Suberin, a heteropolymer produced in response to various stresses, including biotic and abiotic factors, is also influenced by nutrient deficiencies, such as sulphur deficiency (Barberon *et al.*, 2016). The suberisation of the root endodermis, at the casparian strip, serves as a critical checkpoint for nutrient uptake. This structural alteration prompts a transition from an apoplastic route to a symplastic one, necessitating active uptake of nutrients by the plant for subsequent transport to the shoots. In the context of the root, the suberised endodermis serves as a protective layer, separating the stele from the rest of the root. This barrier acts as a defence mechanism against nematodes and protects the xylem from pathogen invasion, ensuring the overall health of the plant (Holbein *et al.*, 2019; Kashyap *et al.*, 2021).

The role of suberin varies among plant species. In potatoes, the aromatic domain of suberin was observed to contribute to resistance against pathogen penetration (Lulai and Corsini, 1998). The suberised endodermis functions as a protective barrier, hindering pathogens from infiltrating vascular tissues and spreading throughout the plant (Ranathunge *et al.*, 2008; Holbein *et al.*, 2019; Kashyap *et al.*, 2021). Notably, soybean shows a correlation between suberin extent and resistance

to fungi, with higher suberin content associated with delayed fungal hyphae growth, suggesting a potential mechanism for enhanced resistance (Ranathunge *et al.*, 2008). Considering *Rs* is a vascular pathogen requiring a clear path for infection, the consolidated endodermis in sulphate transporter mutants could obstruct its progression. However, the cause for resistance still needs to be explored further.

5.5.3. Structural differences via confocal microscopy

At 7 dpi, root sections from both the *AtSultr3;1* mutant and the Col-0 control exhibited colonisation by *Rs*. The pathogen was observed degrading the cell wall and advancing toward the xylem (**Figure 29**). Images of the controls and the *AtSultr3;1* mutant revealed distinctive features, with the mutant having a limited presence of *Rs* in the cortex, while in the Col-0 control colonisation extended across the cortex and into the xylem. However, these differences and their variation were not overly pronounced. Conversely, the shoot sections revealed more characteristic distinctions between the genotypes. *Rs* bacteria were not visible in the pith of the shoot cross-sections of the *AtSultr3;1* mutant, whereas they completely colonised the xylem in the Col-0 control (**Figure 30**). This stark contrast in *Rs* colonisation supports the resistance displayed by the *AtSultr3;1* mutant. Previous reports on *Arabidopsis* have documented cell wall reprogramming as a response to *Rs* infection (Zhao *et al.*, 2019), providing additional support for the observed resistance in the mutant lines. These findings underscore the importance of cell wall dynamics in plant defence mechanisms against pathogenic invaders.

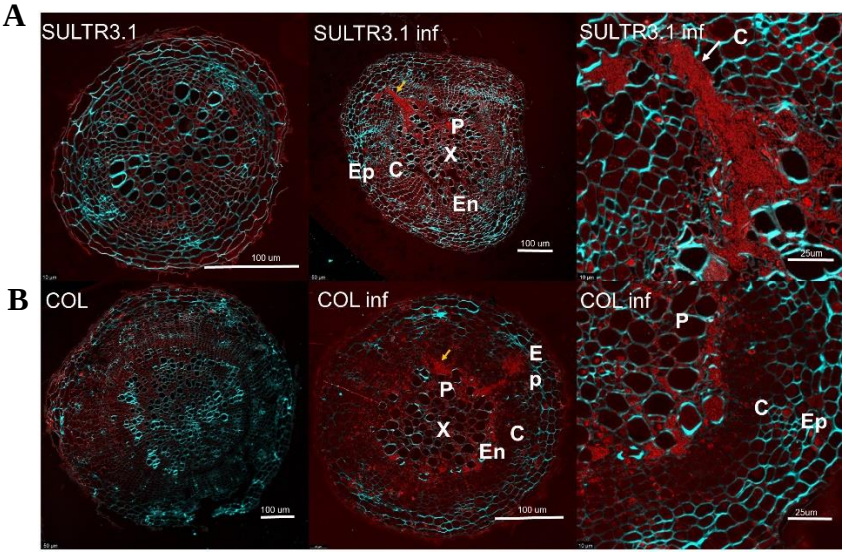


Figure 29. Confocal microscopy images of *A. thaliana* root cross-sections from non-infected (left panels) and *Rs*-infected plants (two right panels) of the *AtSultr3;1* mutant (A) and Columbia (COL) control (B) with a magnified sector (rightmost panels). Samples from 4-week-old, *in vitro* plants were stained at 7 dpi with Calcofluor white for cell walls and Fast Green for contrast. Ep, epidermis; C, cortex; En, endodermis; P, phloem; X, xylem; arrows, *Rs* bacteria

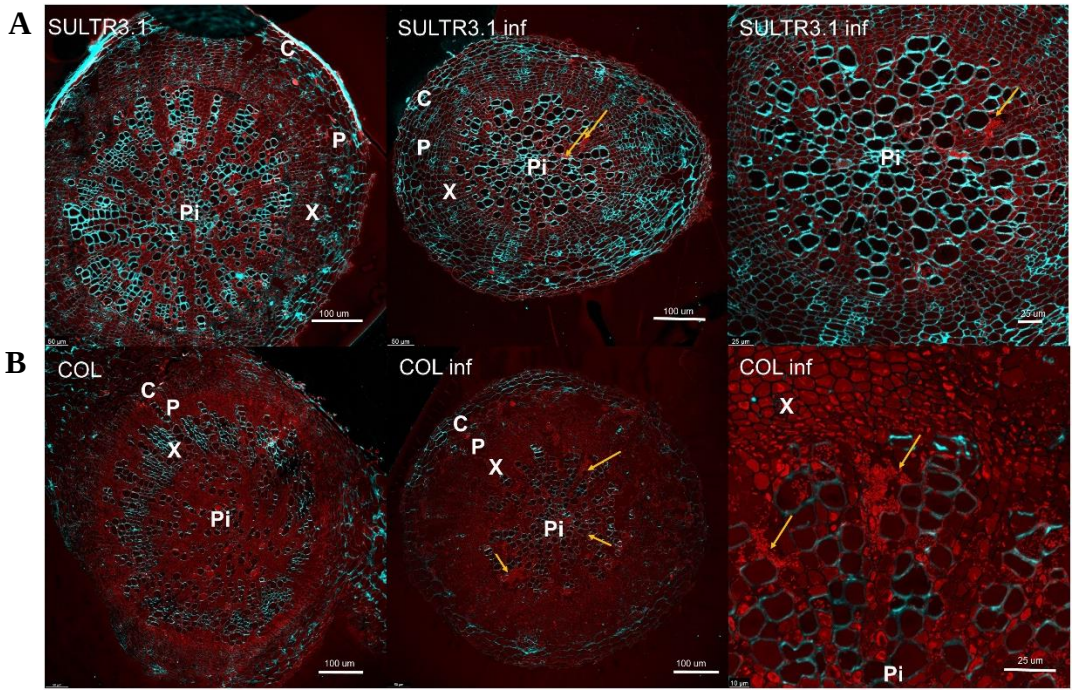


Figure 30. Confocal microscopy images of *A. thaliana* shoot cross-sections from non-infected (left panels) and *Rs*-infected plants (two right panels) of the *AtSultr3;1* mutant (A) and Columbia (COL) control (B) with a magnified sector (rightmost panels). Samples from 4-week-old, *in vitro* plants were stained at 7 dpi with Calcofluor white for cell walls and Fast Green for contrast. C, cortex; P, phloem; X, xylem; Pi, pith; arrows, *Rs* bacteria

5.5.4. The potential role of sulphate transpoters (SULTR) in pathogen resistance

In plants, the sulfate ion (SO_4^{2-}), is absorbed from the soil through a group of proton-linked and highly efficient sulfate transporters present in root hairs and various cellular regions of the root system. Initially, sulphate is imported from the soil into the root symplast, traverses the root, and reaches the central stele. Subsequently, it is further imported into the xylem, where it moves up the shoot, eventually being released into leaf cells. The final destination is the chloroplast, the primary site for sulphur reduction. Simultaneously, sulphate undergoes transportation across the tonoplast into the vacuole (Saito, 2000).

Of the two *SULTR* genes studied here, *SULTR1;2* is active in the external root cell layers (epidermis, cortex) whereas *SULTR3;1* is expressed in plastids and chloroplasts, primarily in the shoots. A related sulphate transporter *SULTR3;6* was formerly established as a susceptibility gene in rice for *Xanthomonas oryzae* pv. *oryzicola* causing the bacterial leaf streak (Cernadas *et al.*, 2014) by affecting ABA signalling, stomatal regulation, and promoting nutrients and water export into the apoplast (Scinto-Madonich *et al.*, 2023). It can be concluded from our data that the interaction between *Rs* effectors and SULTR3;1 increases the susceptibility of potato to the bacterial pathogen. Thus, the question arises of how the *SULTR3;1* mutation (and potentially its targeted editing) would confer *Rs* resistance.

The above work on rice and *X. oryzae* can provide some clues and may be applied to our case, too. Cernadas *et al.* (2014) established that a type 3 bacterial transcription activator-like (TAL) effector (Tal2g) can induce the expression of *SULTR3;6* in rice. Pathogenic fungi are also

known to influence the expression of other sulphate transporter genes in several hosts (Petre *et al.*, 2012; Fu *et al.*, 2016). Why would bacteria and other plant pathogens need to interfere with plant sulphur metabolism? The answer is that (hemi)biotrophic pathogens (including oomycetes species) have lost the capacity of some stages of sulphur assimilation during their evolution and adaptation to their host(s) (Wang *et al.*, 2022a). As such, in their initial phase of interaction, these pathogens must rely heavily on the host metabolism to get access to and synthesise the vital sulphur-containing compounds (e.g., xanthan gum and other exopolymers) required for their virulence. Therefore, pathogens and hosts can really compete for sulphur sources and in fact sulphur deprivation is indicative of pathogen attack for plants (Criollo-Arteaga *et al.*, 2021; Kies and Hammer, 2022). This notion is also supported by the fact that the lack of sulphur led to SA-mediated defence responses such as induction of *PR1* and similar genes as well as to resistance against (hemi)biotrophic bacteria but not to necrotrophic fungi (Criollo-Arteaga *et al.*, 2021).
It is conceivable that inactivation or mutagenesis of these target genes participating in sulphur assimilation and/or metabolism will render the host more resistant to the pathogen. The proof of this concept was already delivered by Xu, *et al*. (2021): the targeted modification of the TAL effector-binding element in the rice *SULTR3;6* gene promoter effectively abolished binding the effector and the lower *SULTR3;6* expression was associated with *Xanthomonas* resistance as demonstrated with significantly diminished symptoms and bacterial accumulation.

Since *Rs* bacteria are known to produce active TAL effectors (de Lange *et al.*, 2013; Li *et al.*, 2013; Schandry *et al.*, 2016), we propose that some of these effectors may affect the expression of at least some of the 30 potato *SULTR* genes that can be identified in the genome (data not shown). This can be verified by testing if potato SULTR gene promoters contain characterised TAL effector-binding elements (including a canonical 17-bp sequence: (Wu *et al.*, 2019*;* Gallas *et al.*, 2023) or their homologues. If yes, this may fund the basis for their use as susceptibility genes. Alternatively, other types of effectors may also target and induce these *SULTR* genes.

Studies have also highlighted the pivotal role of the corky biosynthetic pathway in the resistance response of peanut plants to late leaf spot (Mahatma *et al.*, 2021). Similarly, in tomato plants, the induced formation of a lignin-corky vascular coating impeded the colonisation of *Rs* in resistant roots, showing its effectiveness in preventing pathogen invasion (Kashyap *et al.*, 2021). While the evidence supporting the direct involvement of suberin in defence against *Rs* in other plant interactions is currently limited (Shi *et al.*, 2023), its importance as a critical component of the cell wall has been emphasised. Recognising the multifaceted nature of plant defence mechanisms, the interplay of various components, including corky biosynthesis and the induction of specific coatings, contributes to the overall resilience of plants against pathogenic challenges.

6. CONCLUSIONS AND RECOMMENDATIONS

Having recognised the critical need for resistance in potatoes against economically significant diseases, our research focused on unravelling molecular mechanisms through genome editing. Our successful exploration has not only deepened our understanding but has also contributed to fortifying defence responses. Our investigation centered on two formidable pathogens, *Ralstonia solanacearum* (*Rs*) and *Phytophthora infestans* (*Pi*), both known for wreaking havoc on crops, particularly potato.

Initially, we assessed the resistance response of 'Calalo Gaspar' (CG) and 'Cruza 148' (CR), along with other cultivars resilient to *Rs* infections. Transcriptome analysis identified differently regulated genes and pathways associated with resistance/susceptibility in CG, CR, and the susceptible 'Désirée' (DES). CG downregulated genes linked to oxidative stress, while CR and DES induced cell wall and chitin metabolic processes. Metabolomic analysis revealed higher chlorogenic acid levels in resistant cultivars, particularly CG, with unique concentrations of chlorogenic acid and quercetin derivatives. Significant phenolic concentration changes occurred in CG and DES roots, while CR showed minor influence. DES exhibited the least lignification before infection, with varied lignification post-infection in CG and CR. Despite increased lignification in DES, *Rs* bacteria persisted, indicating insufficient basal lignification. Overall, our analysis unveiled significant metabolic and transcriptomic differences among potato cultivars to *Rs* infection, highlighting the roles of the phenylpropanoid pathway, glutathione metabolism, and lignification in resistance. This data not only characterises cultivars but also offers insights for future research, contributing to identifying susceptibility genes for targeted interventions.

Based on strong supportive literature of polyphenol oxidases (PPO) and microRNA396 (miR396) being involved in *Rs* and *Pi* resistance, we knocked out the tuber and root-specific *PPO* gene (in DES and ´Balatoni Rózsa' (BR) genotype) and *miR396* gene (in ´Botond' (Bt) and DES genotype) using modular CRISPR/Cas9 systems.

In our study of the *PPO* gene, analysis was done on CRISPR edited two lines per genotype, confirming successful varying edits with varied outcomes: reduced enzyme activity and browning of tubers and roots in edited lines compared to controls. BR12 exhibited the least impact on enzyme activity reduction and browning which aligned with it only targeting one allele of the *Pot32* gene contrary to other lines. To understand metabolic changes from *PPO* knockout, we conducted comprehensive metabolomic analysis on roots and leaves. The goal was to enhance antimicrobial compound production for resistance. UPLC-MS/MS analysis revealed variations in 26 phenolic compounds between control and *PPO*-edited potatoes. Principal component analysis showed distinct differences in root metabolome, notably in D14, D17, and BR25. Noteworthy metabolites

like phaseic acid and indole-3-acetic acid varied across mutants. *PPO*-knockout plants displayed higher levels of jasmonic acid in DES and various GA forms in BR mutants. Flavanols' role in *Rs* resistance was strengthened by their increase in three of four *PPO* mutants, contributing to basal *Rs* infection responses. The degree of metabolic regulation in mutants corresponded to alleles mutated in the *Pot32* gene.

When subjected to *Rs*, *PPO* mutants surprisingly exhibited increased susceptibility despite increased antimicrobial metabolites. D14 and D17 showed wilting percentages of 87% and 60%, respectively, surpassing the control's 40%. BR25 also displayed increased susceptibility with a wilting percentage of 73% compared to the control's 13%, highlighting PPOs' crucial role in resistance. PPOs, involved in lignification and cell wall formation, significantly contribute to *Rs* resistance, aligning with previous research. However, against *Pi*, *PPO*-knockout plants did not differ significantly from original cultivars at any time point, challenging previous reports suggesting *PPO* silencing enhances *Pi* resistance and emphasizing PPOs' complex roles in plant defence mechanisms.

For our studies with miR396, we generated mutant lines in Bt (6,7,8, and 9) and DES (3,6, and 10) genotypes, confirming mutations at the CRISPR target site through sequencing. The expression of *miR396* and its predicted targets was assessed in the edited lines compared to controls by RT-qPCRs. In all of the edited lines, there was reduced expression of both *miR396* leader and passenger strands. Surprisingly, despite the anticipated increase in the accumulation of target genes' mRNA due to the lack of miR396 inhibition, most targets were downregulated in the mutant lines. Noteworthy, exceptions included the upregulation of the NBS-LRR disease resistance gene and multicystatin in the edited lines. Given the established literature suggesting miR396's potential negative regulation of resistance to *Rs* and *Pi*, we conducted infection assays with both pathogens on the mutant lines. Bt Line 8, 9 and DES Lines 3 and 10 exhibited delayed symptom development when subjected to *Rs*. Conversely, when Bt edited lines were exposed to *Pi*, these lines showed increased susceptibility relative to control. The differential regulation of miR396 target genes, specifically the NBS-LRR disease resistance gene and multicystatin, may have influenced the distinct resistance responses observed against *Rs* and *Pi* in these mutant lines which is a novel finding and an interesting aspect to explore further. Here, we also noticed that the degree of mutations was proportional to the susceptibility of the line to *Pi.*

In our ongoing search for susceptibility gene candidates, the transcriptomic analysis of resistant potato lines highlighted sulphate transporters as a promising avenue. To investigate further, we obtained *Arabidopsis thaliana* mutant lines for the sulphate transporter genes *AtSULTR3;1* and *AtSULTR1;2*. Subsequently, one-month-old plants were subjected to *Rs* infection alongside the Columbia (Col-0) control. Remarkably, the *AtSultr3;1* and *Atsultr1*;2 mutant lines

exhibited a pronounced increase in resistance to *Rs* compared to the Col-0 control. In-depth analysis, including the study of suberisation levels, revealed a significant increase of suberin along the cortex of the mutants, suggesting a potential contributing factor to the observed resistance. To unravel the basis of the heightened resistance in the *AtSultr3;1* mutant line, we conducted cell wall staining with calcofluor and fast green staining on infected and uninfected root and shoot sections. The analysis revealed thicker cell walls in the mutant relative to the Col-0 control. Although *Rs* managed to degrade cell walls, progressing into the phloem in the roots of both genotypes, the shoot sections of *Atsultr3;1* exhibited visibly thicker cell walls even closer to the pith, providing a stronger obstruction to *Rs* invasion than the control. It became evident that in the *Atsultr3;1* mutant, the spread of bacteria was significantly impeded. Having such a strong backbone for resistance with the sulphate transporters, further exploration into the total sulphur content, glutathione and cysteine amounts along with other sulphur containing compounds will throw light on the resistance mechanisms. We are also generating these mutants in potato to harness the benefit of resistance offered by edited sulphate transporters.

Our study has uncovered some novel findings, elucidated vital concepts regarding *Rs* and *Pi* resistance and requires further research to come forth with ultimate resistance utilising CRISPR/Cas knockout of susceptibility genes.

7. NEW SCIENTIFIC RESULTS

- We characterised and identified the common and different defence responses between the two known *Rs* resistant varieties CG and CR including glutathione metabolism, phenylpropanoid pathways and lignification.
- We have generated several transgenic lines, namely, *PPO* knockout lines of ´Désirée' and 'Balatoni Rózsa', and *miR396* knockout lines of ´Désirée' and ´Botond'.
- We established that PPO is positively related to resistance to *Rs* and that the loss of PPO activity increases the susceptibility of plants despite increase of beneficial metabolites. We could not support the claim of *PPO* mutants being more resistant to *Pi*.
- We established that *miR396* mutant plants are delayed in symptom development to *Rs* and increased in susceptibility to *Pi* and that miR396 targets, the *MULTICYSTATIN147* and an *NBS-LRR* gene, have a role in these responses.
- We found *Rs* resistance in *SULTR* mutants in *Arabidopsis* making them good susceptibility gene candidates with increased suberisation along the root cortex and thick cell walls.

8. SUMMARY

In our study, we focused on understanding and inducing resistance mechanisms against the pathogens *Ralstonia solanacearum* (*Rs*) and *Phytophthora infestans* (*Pi*) in potato. Our approach was to uncover plant susceptibility genes and edit these genes via CRISPR/Cas9 approach. Firstly, we worked with *Rs* resistant genotypes 'CG', 'CR', and susceptible 'Désirée' (DES). Through transcriptome and metabolomics data analysis, we identified differences in resistance response mechanisms among these genotypes. Metabolites like chlorogenic acid were found to positively regulate resistance, and hormonal regulation, particularly an increase in salicylic acid, was observed in all genotypes. Basal lignification, phenylpropanoid pathway, and glutathione metabolism were highlighted as important in resistant genotypes. Transcriptome data helped identify differentially regulated genes in resistant genotypes compared to susceptible DES, which could be targeted for editing using CRISPR/Cas system.

Based on literature and transcriptome data, we selected candidate genes for editing, including polyphenol oxidase (*PPO*), MicroRNA *miR396*, and sulphate transporters *AtSultr3;1* and *AtSultr1;2*. Mutant lines with edited *PPO* genes showed reduced browning in tubers and roots but increased susceptibility to *Rs*. Even though several basal response remained intact and an increase of antimicrobial compounds was observed, the loss of PPO could not be compensated.

The *miR396* edited lines generated via targeted mutagenesis employing two target strategies exhibited delayed symptom development against *Rs* but increased susceptibility to *Pi*. Interestingly, in edited lines of DES, 3,6 and 10, the delayed symptom development was observed in Line 3 and Line 10 both of which had larger deletions at the first target site, contrary to line 6 which had smaller deletions and only at the second target site indicative of the effect of mutations on certain targets. In order to explain the effect of miR396, we evaluated the expression of its predicted targets in the edited lines and interestingly, found an *LRR RECEPTOR-LIKE SERINE/THREONINE PROTEIN KINASE* to increase in expression in all the edited lines and *MULTICYSTATIN 147* also showed an increase in expression especially in the edited ´Botond' lines compared to the control. The transcriptional increase of these genes is probably not effective enough against *Pi* but overall, we demonstrated that miR396 is involved in regulating defence response towards *Rs* and *Pi*. It's a promising aspect and a deeper look into the transcriptomic differences in the future will certainly highlight the mechanism involved in the observed differing response to *Rs* and *Pi*.

From transcriptomic data, we identified *SULTR3;1* as a potential susceptibility gene candidate. From literature, sulphate transporters (SULTRs) appeared promising towards contributing to resistance. Although very little is known about the group 3 sulphate transporters.

Tests on Arabidopsis mutants showed increased resistance to *Rs* in SULTR mutants, suggesting a promising approach for developing resistance. Further analysis revealed increased suberisation of cell walls in mutants. We also carried out sectioning and staining for the roots and stems of *AtSULTR3;1* mutant plants and observed that the stem offers more resistance to *Rs* with lesser *Rs* found in the pith of the mutant. The mutant also had thick cell walls. This opens up new discussion on *SULTR3;1* being a susceptibility gene, which may have a wide application in the future.

Overall, our research provided insights into resistance mechanisms employed against *Rs* and *Pi* in potato, identified candidate susceptibility genes for editing, and opened up avenues to induce resistance via targeted mutagenesis.

9. APPENDICES

A1: REFERENCES

Abdul Malik, N.A., Kumar, I.S. and Nadarajah, K. (2020) 'Elicitor and receptor molecules: orchestrators of plant defense and immunity', *International Journal of Molecular Sciences*, 21(3), p. 963.

Abudayyeh, O.O., Gootenberg, J.S., Konermann, S., Joung, J., Slaymaker, I.M., Cox, D.B.T., Shmakov, S., Makarova, K.S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E.S., Koonin, E. V. and Zhang, F. (2016) 'C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector', *Science*, 353(6299), p. aaf5573. Available at: https://doi.org/10.1126/science.aaf5573.

Acevedo-Garcia, J., Kusch, S. and Panstruga, R. (2014) 'Magical mystery tour: MLO proteins in plant immunity and beyond', *New Phytologist*, pp. 273–281. Available at: https://doi.org/10.1111/nph.12889.

Adolf, B., Andrade-Piedra, J., Bittara Molina, F., Przetakiewicz, J., Hausladen, H., Kromann, P., Lees, A., Lindqvist-Kreuze, H., Perez, W. and Secor, G.A. (2019) 'Fungal, oomycete, and plasmodiophorid diseases of potato', in *The Potato Crop: Its Agricultural, Nutritional and Social Contribution to Humankind*, pp. 307–350. Available at: https://doi.org/10.1007/978-3-030-28683-5_9.

Ah-Fong, A.M.V. and Judelson, H.S. (2003) 'Cell cycle regulator Cdc14 is expressed during sporulation but not hyphal growth in the fungus-like oomycete Phytophthora infestans', *Molecular Microbiology*, 50(2), pp. 487–494. Available at: https://doi.org/10.1046/j.1365-2958.2003.03735.x.

Ahn, I.P. (2007) 'Disturbance of the Ca2+/calmodulin-dependent signalling pathway is responsible for the resistance of Arabidopsis dnd1 against Pectobacterium carotovorum infection', *Molecular Plant Pathology*, 8(6), pp. 747–759. Available at: https://doi.org/10.1111/j.1364-3703.2007.00428.x.

Alariqi, M., Wei, H., Cheng, J., Sun, Y., Zhu, H., Wen, T., Li, Y., Wu, C., Jin, S. and Cao, J. (2022) 'Large-scale comparative transcriptome analysis of Nicotiana tabacum response to Ralstonia solanacearum infection', *Plant Biotechnology Reports*, 16(6), pp. 757–775. Available at: https://doi.org/10.1007/s11816-022-00765-x.

Allefs, S.J.H.M., De Jong, E.R., Florack, D.E.A., Hoogendoorn, C. and Stiekema, W.J. (1996) 'Erwinia soft rot resistance of potato cultivars expressing antimicrobial peptide tachyplesin I', *Molecular Breeding*, 2, pp. 97–105. Available at: https://doi.org/10.1007/BF00441425.

Álvarez, B., Biosca, E.G. and López, M.M. (2010) 'On the life of Ralstonia solanacearum, a

destructive bacterial plant pathogen', *Technology and education topics in applied microbiology and microbial biotechnology*, 1, pp. 267–279.

Álvarez, C., Ángeles Bermúdez, M., Romero, L.C., Gotor, C. and García, I. (2012) 'Cysteine homeostasis plays an essential role in plant immunity', *New Phytologist*, 193(1), pp. 165–177. Available at: https://doi.org/10.1111/j.1469-8137.2011.03889.x.

Andino, M., Gaiero, P., González-Barrios, P., Galván, G., Vilaró, F. and Speranza, P. (2022) 'Potato Introgressive Hybridisation Breeding for Bacterial Wilt Resistance Using Solanum commersonii Dun. as Donor: Genetic and Agronomic Characterisation of a Backcross 3 Progeny', *Potato Research*, 65(1), pp. 119-136. Available at: https://doi.org/10.1007/s11540-021-09512-1.

Antony, G., Zhou, J., Huang, S., Li, T., Liu, B., White, F. and Yang, B. (2010) 'Rice xa13 recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene Os-11N3', *Plant Cell*, 22(11), pp. 3864–3876. Available at: https://doi.org/10.1105/tpc.110.078964.

Araji, S., Grammer, T.A., Gertzen, R., Anderson, S.D., Mikulic-Petkovsek, M., Veberic, R., Phu, M.L., Solar, A., Leslie, C.A. and Dandekar, A.M. (2014) 'Novel roles for the polyphenol oxidase enzyme in secondary metabolism and the regulation of cell death in walnut', *Plant physiology*, 164(3), pp. 1191–1203. Available at: https://doi.org/10.1104/pp.113.228593.

Armstrong, M.R., Vossen, J., Lim, T.Y., Hutten, R.C.B., Xu, J., Strachan, S.M., Harrower, B., Champouret, N., Gilroy, E.M. and Hein, I. (2019) 'Tracking disease resistance deployment in potato breeding by enrichment sequencing', *Plant Biotechnology Journal*, 17(2), pp. 540-549. Available at: https://doi.org/10.1111/pbi.12997.

Aversano, R., Contaldi, F., Ercolano, M.R., Grosso, V., Iorizzo, M., Tatino, F., Xumerle, L., Molin, A.D., Avanzato, C., Ferrarini, A., Delledonne, M., Sanseverino, W., Cigliano, R.A., Capella-Gutierrez, S., Gabaldón, T., Frusciante, L., Bradeen, J.M. and Carputo, D. (2015) 'The Solanum commersonii genome sequence provides insights into adaptation to stress conditions and genome evolution of wild potato relatives', *Plant Cell*, 27(4), pp. 954–968. Available at: https://doi.org/10.1105/tpc.114.135954.

Avrova, A.O., Stewart, H.E., De Jong, W., Heilbronn, J., Lyon, G.D. and Birch, P.R.J. (1999) 'A cysteine protease gene is expressed early in resistant potato interactions with Phytophthora infestans', *Molecular Plant-Microbe Interactions*, 12(12). Available at: https://doi.org/10.1094/MPMI.1999.12.12.1114.

Aylor, D.E., Fry, W.E., Mayton, H. and Andrade-Piedra, J.L. (2001) 'Quantifying the rate of release and escape of Phytophthora infestans sporangia from a potato canopy', *Phytopathology*, 91(12), pp. 1189–1196. Available at:

https://doi.org/10.1094/PHYTO.2001.91.12.1189.

Babu, B.N.H., Wilfred, A. and Venkatesh, Y.P. (2017) 'Emerging food allergens: Identification of polyphenol oxidase as an important allergen in eggplant (Solanum melongena L.)', *Immunobiology*, 222(2), pp. 155–163. Available at: https://doi.org/10.1016/j.imbio.2016.10.009.

Bachem, C.W.B., Speckmann, G.J., Van der Linde Piet, C.G., Verheggen, F.T.M., Hunt, M.D., Steffens, J.C. and Zabeau, M. (1994) 'Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers', *Bio/Technology*, 12(11), pp. 1101–1105. Available at: https://doi.org/10.1038/nbt1194-1101.

Bai, Y., Ali, S., Liu, S., Zhou, J. and Tang, Y. (2023) 'Characterization of plant laccase genes and their functions', *Gene*, 852, p. 147060. Available at: https://doi.org/10.1016/j.gene.2022.147060.

Bai, Y., Pavan, S., Zheng, Z., Zappel, N.F., Reinstädler, A., Lotti, C., De Giovanni, C., Ricciardi, L., Lindhout, P., Visser, R., Theres, K. and Panstruga, R. (2008) 'Naturally occurring broad-spectrum powdery mildew resistance in a Central American tomato accession is caused by loss of Mlo function', *Molecular Plant-Microbe Interactions*, 21(1), pp. 30–39. Available at: https://doi.org/10.1094/MPMI-21-1-0030.

Baichoo, Z. and Jaufeerally-Fakim, Y. (2017) 'Ralstonia solanacearum upregulates marker genes of the salicylic acid and ethylene signaling pathways but not those of the jasmonic acid pathway in leaflets of Solanum lines during early stage of infection', *European Journal of Plant Pathology*, 147, pp. 615–625. Available at: https://doi.org/10.1007/s10658-016-1030-7.

Barberon, M., Vermeer, J.E.M., De Bellis, D., Wang, P., Naseer, S., Andersen, T.G., Humbel, B.M., Nawrath, C., Takano, J. and Salt, D.E. (2016) 'Adaptation of root function by nutrient-induced plasticity of endodermal differentiation', *Cell*, 164(3), pp. 447–459.

Batool, S., Uslu, V.V., Rajab, H., Ahmad, N., Waadt, R., Geiger, D., Malagoli, M., Xiang, C.-B., Hedrich, R. and Rennenberg, H. (2018) 'Sulfate is incorporated into cysteine to trigger ABA production and stomatal closure', *The Plant Cell*, 30(12), pp. 2973–2987.

Becktell, M.C., Smart, C.D., Haney, C.H. and Fry, W.E. (2006) 'Host-pathogen interactions between Phytophthora infestans and the solanaceous hosts Calibrachoa x hybridus, Petunia x hybrida, and Nicotiana benthamiana', *Plant Disease*, 90(1), pp. 24–32. Available at: https://doi.org/10.1094/PD-90-0024.

Bellincampi, D., Cervone, F. and Lionetti, V. (2014) 'Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions', *Frontiers in Plant Science*, p. 228. Available at: https://doi.org/10.3389/fpls.2014.00228.

Benjamini, Y. and Hochberg, Y. (1995) 'Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing', *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1), pp. 289–300. Available at: https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Berrocal-Lobo, M., Segura, A., Moreno, M., López, G., Garcıa-Olmedo, F. and Molina, A. (2002) 'Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection', *Plant physiology*, 128(3), pp. 951–961. Available at: https://doi.org/10.1104/pp.010685.

Bhonwong, A., Stout, M.J., Attajarusit, J. and Tantasawat, P. (2009) 'Defensive role of tomato polyphenol oxidases against cotton bollworm helicoverpa armigera and beet armyworm spodoptera exigua', *Journal of Chemical Ecology*, 35(1), pp. 28–38. Available at: https://doi.org/10.1007/s10886-008-9571-7.

Bi, W., Liu, J., Li, Y., He, Z., Chen, Y., Zhao, T., Liang, X., Wang, X., Meng, X., Dou, D. and Xu, G. (2023) 'CRISPR/Cas9-guided editing of a novel susceptibility gene in potato improves Phytophthora resistance without growth penalty', *Plant Biotechnology Journal*, 22(1), p. 4. Available at: https://doi.org/10.1111/pbi.14175.

Birch, P.R.J., Bryan, G., Fenton, B., Gilroy, E.M., Hein, I., Jones, J.T., Prashar, A., Taylor, M.A., Torrance, L. and Toth, I.K. (2012) 'Crops that feed the world 8: Potato: Are the trends of increased global production sustainable?', *Food Security*, 4(4), pp. 477–508. Available at: https://doi.org/10.1007/s12571-012-0220-1.

Bjor, T. (1987) 'Testing the resistance of potato genotypes to tuber late blight', *Potato Research*, 30, pp. 525–532. Available at: https://doi.org/10.1007/BF02361932.

Block, A., Li, G., Fu, Z.Q. and Alfano, J.R. (2008) 'Phytopathogen type III effector weaponry and their plant targets', *Current Opinion in Plant Biology*, pp. 396–403. Available at: https://doi.org/10.1016/j.pbi.2008.06.007.

Blodgett, F.M. and Stevenson, F.J. (1946) 'The new scab-resistant potatoes, Ontario, Seneca and Cayuga', *American Potato Journal*, 23(9). Available at: https://doi.org/10.1007/BF02888645.

Blount, J.W., Dixon, R.A. and Paiva, N.L. (1992) 'Stress responses in alfalfa (Medicago sativa L.) XVI. Antifungal activity of medicarpin and its biosynthetic precursors; implications for the genetic manipulation of stress metabolites', *Physiological and Molecular Plant Pathology*, 41(5), pp. 333–349. Available at: https://doi.org/10.1016/0885-5765(92)90020-V.

Boccara, M., Sarazin, A., Thiebeauld, O., Jay, F., Voinnet, O., Navarro, L. and Colot, V. (2014) 'The Arabidopsis miR472-RDR6 silencing pathway modulates PAMP-and effector-triggered immunity through the post-transcriptional control of disease resistance genes',

PLoS pathogens, 10(1), p. e1003883. Available at: https://doi.org/10.1371/journal.ppat.1003883.

Boch, J. and Bonas, U. (2010) 'Xanthomonas AvrBs3 family-type III effectors: Discovery and function', *Annual Review of Phytopathology*, 48, pp. 419–436. Available at: https://doi.org/10.1146/annurev-phyto-080508-081936.

Boller, T. and He, S.Y. (2009) 'Innate immunity in plants: An arms race between pattern recognition receptors in plants and effectors in microbial pathogens', *Science*, pp. 742–744. Available at: https://doi.org/10.1126/science.1171647.

Bologna, N.G. and Voinnet, O. (2014) 'The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis', *Annual Review of Plant Biology*, pp. 473–503. Available at: https://doi.org/10.1146/annurev-arplant-050213-035728.

Bonar, N., Liney, M., Zhang, R., Austin, C., Dessoly, J., Davidson, D., Stephens, J., McDougall, G., Taylor, M., Bryan, G.J. and Hornyik, C. (2018) 'Potato miR828 is associated with purple tuber skin and flesh color', *Frontiers in Plant Science*, 9, p. 1742. Available at: https://doi.org/10.3389/fpls.2018.01742.

Boschi, F., Schvartzman, C., Murchio, S., Ferreira, V., Siri, M.I., Galván, G.A., Smoker, M., Stransfeld, L., Zipfel, C., Vilaró, F.L. and Dalla-Rizza, M. (2017) 'Enhanced bacterial wilt resistance in potato through expression of arabidopsis efr and introgression of quantitative resistance from solanum commersonii', *Frontiers in Plant Science*, 8, p. 1642. Available at: https://doi.org/10.3389/fpls.2017.01642.

Brewer, H.C., Hawkins, N.D. and Hammond-Kosack, K.E. (2014) 'Mutations in the Arabidopsis homoserine kinase gene DMR1 confer enhanced resistance to Fusarium culmorum and F. graminearum', *BMC Plant Biology*, 14(1), pp. 1–15. Available at: https://doi.org/10.1186/s12870-014-0317-0.

Brodersen, P., Sakvarelidze-Achard, L., Bruun-Rasmussen, M., Dunoyer, P., Yamamoto, Y.Y., Sieburth, L. and Voinnet, O. (2008) 'Widespread translational inhibition by plant miRNAs and siRNAs', *Science*, 320(5880), pp. 1185–1190. Available at: https://doi.org/10.1126/science.1159151.

Brylińska, M. and Śliwka, J. (2018) 'Laboratory Assessment of Potato Resistance to Phytophthora Infestans', *Plant Breeding and Seed Science*, 76(1). Available at: https://doi.org/10.1515/plass-2017-0016.

Buddenhagen, I. and Kelman, A. (1964) 'Biological and Physiological Aspects of Bacterial Wilt Caused by Pseudomonas Solanacearum', *Annual Review of Phytopathology*, 2(1), pp. 203–230. Available at: https://doi.org/10.1146/annurev.py.02.090164.001223.

Buddenhagen, I., Sequeira, L. and Kelman, A. (1962) 'Designation of races in Pseudomonas

solanacearum', *Phytopathology*, 52, pp. 726-abstract.

Burke, R.M. and Cairney, J.W.G. (2002) 'Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi', *Mycorrhiza*, 12, pp. 105–116. Available at: https://doi.org/10.1007/s00572-002-0162-0.

Busch, J.M. (1999) 'Enzymic browning in potatoes: A simple assay for a polyphenol oxidase catalysed reaction', *Biochemical Education* [Preprint]. Available at: https://doi.org/10.1016/S0307-4412(99)00033-3.

Caldwell, D., Kim, B.S. and Iyer-Pascuzzi, A.S. (2017) 'Ralstonia solanacearum differentially colonizes roots of resistant and susceptible tomato plants', *Phytopathology*, 107(5). Available at: https://doi.org/10.1094/PHYTO-09-16-0353-R.

Cao, M., Wang, Z., Wirtz, M., Hell, R., Oliver, D.J. and Xiang, C. (2013) 'SULTR 3; 1 is a chloroplast-localized sulfate transporter in Arabidopsis thaliana', *The Plant Journal*, 73(4), pp. 607–616. Available at: https://doi.org/10.1111/tpj.12059.

Cao, M.J., Wang, Z., Zhao, Q., Mao, J.L., Speiser, A., Wirtz, M., Hell, R., Zhu, J.K. and Xiang, C. Bin (2014) 'Sulfate availability affects ABA levels and germination response to ABA and salt stress in Arabidopsis thaliana', *Plant Journal*, 77(4), pp. 604–615. Available at: https://doi.org/10.1111/tpj.12407.

Cao, W., Gan, L., Shang, K., Wang, C., Song, Y., Liu, H., Zhou, S. and Zhu, C. (2020) 'Global transcriptome analyses reveal the molecular signatures in the early response of potato (Solanum tuberosum L.) to Phytophthora infestans, Ralstonia solanacearum, and Potato virus Y infection', *Planta*, 252, pp. 1–13. Available at: https://doi.org/10.1007/s00425-020-03471-6.

Cao, Y., Ding, X., Cai, M., Zhao, J., Lin, Y., Li, X., Xu, C. and Wang, S. (2007) 'The expression pattern of a rice disease resistance gene Xa3/Xa26 is differentially regulated by the genetic backgrounds and developmental stages that influence its function', *Genetics*, 177(1), pp. 523–533. Available at: https://doi.org/10.1534/genetics.107.075176.

Carputo, D., Aversano, R., Barone, A., Di Matteo, A., Iorizzo, M., Sigillo, L., Zoina, A. and Frusciante, L. (2009) 'Resistance to ralstonia solanacearum of sexual hybrids between solanum commersonii and S. tuberosum', *American Journal of Potato Research*, 86, pp. 196–202. Available at: https://doi.org/10.1007/s12230-009-9072-4.

Cernadas, R.A., Doyle, E.L., Niño-Liu, D.O., Wilkins, K.E., Bancroft, T., Wang, L., Schmidt, C.L., Caldo, R., Yang, B. and White, F.F. (2014) 'Code-assisted discovery of TAL effector targets in bacterial leaf streak of rice reveals contrast with bacterial blight and a novel susceptibility gene', *PLoS Pathogens*, 10(2), p. e1003972. Available at: https://doi.org/10.1371/journal.ppat.1003972.

Cesarino, I. (2019) ‘Structural features and regulation of lignin deposited upon biotic and abiotic stresses’, *Current Opinion in Biotechnology*, pp. 209-214. Available at: https://doi.org/10.1016/j.copbio.2018.12.012.

Chai, Q., Shang, X., Wu, S., Zhu, G., Cheng, C., Cai, C., Wang, X. and Guo, W. (2017) ‘5-aminolevulinic acid dehydratase gene dosage affects programmed cell death and immunity’, *Plant Physiology*, 175(1), pp. 511–528. Available at: https://doi.org/10.1104/pp.17.00816.

Chakrabarti S.K., P.N.K.. T.J.K.. K.R.. S.S.. S.J.. B.T.. K.M. (2019) *Annual Report-2019, ICAR-Central Potato research Institute, Shimla, India*. Shimla, India.

Char, S.N., Neelakandan, A.K., Nahampun, H., Frame, B., Main, M., Spalding, M.H., Becraft, P.W., Meyers, B.C., Walbot, V., Wang, K. and Yang, B. (2017) ‘An Agrobacterium-delivered CRISPR/Cas9 system for high-frequency targeted mutagenesis in maize’, *Plant Biotechnology Journal*, 15(2), pp. 257–268. Available at: https://doi.org/10.1111/pbi.12611.

Charkowski, A., Sharma, K., Parker, M.L., Secor, G.A., Elphinstone, J. (2020) 'Bacterial diseases of potato', Campos, H., Ortiz, O. (eds), *The Potato Crop*. *Springer International Publishing*, Cham, pp. 351–388. Available at: https://doi.org/10.1007/978-3-030-28683-5_10

Chen, L., Guo, X., Xie, C., He, L., Cai, X., Tian, L., Song, B. and Liu, J. (2013) ‘Nuclear and cytoplasmic genome components of Solanum tuberosum + S. chacoense somatic hybrids and three SSR alleles related to bacterial wilt resistance’, *Theoretical and Applied Genetics*, 126, pp. 1861–1872. Available at: https://doi.org/10.1007/s00122-013-2098-5.

Chen, L., Luan, Y. and Zhai, J. (2015) ‘Sp-miR396a-5p acts as a stress-responsive genes regulator by conferring tolerance to abiotic stresses and susceptibility to Phytophthora nicotianae infection in transgenic tobacco’, *Plant cell reports*, 34, pp. 2013–2025. Available at: https://doi.org/10.1007/s00299-015-1847-0.

Chen, L., Meng, J., He, X.L., Zhang, M. and Luan, Y.S. (2019a) ‘Solanum lycopersicum microRNA1916 targets multiple target genes and negatively regulates the immune response in tomato’, *Plant Cell and Environment*, 42(4), pp. 1393–1407. Available at: https://doi.org/10.1111/pce.13468.

Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R. and Frommer, W.B. (2012a) ‘Sucrose efflux mediated by SWEET proteins as a key step for phloem transport’, *Science*, 335(6065), pp. 207–211. Available at: https://doi.org/10.1126/science.1213351.

Chen, N., Shao, Q., Lu, Q., Li, X. and Gao, Y. (2022) ‘Transcriptome analysis reveals differential transcription in tomato (Solanum lycopersicum) following inoculation with Ralstonia

solanacearum', *Scientific Reports*, 12(1), p. 22137. Available at: https://doi.org/10.1038/s41598-022-26693-y.

Chen, S., Zhou, Y., Chen, Y. and Gu, J. (2018) 'Fastp: An ultra-fast all-in-one FASTQ preprocessor', in *Bioinformatics*, pp. i884–i890. Available at: https://doi.org/10.1093/bioinformatics/bty560.

Chen, X. (2009) 'Small RNAs and their roles in plant development', *Annual Review of Cell and Developmental Biology*, 25, pp. 21–44. Available at: https://doi.org/10.1146/annurev.cellbio.042308.113417.

Chen, X. (2012) 'Small RNAs in development - insights from plants', *Current Opinion in Genetics and Development*, pp. 361–367. Available at: https://doi.org/10.1016/j.gde.2012.04.004.

Chen, X., Liang, H., Zhang, J., Zen, K. and Zhang, C.Y. (2012b) 'Secreted microRNAs: A new form of intercellular communication', *Trends in Cell Biology*, pp. 125–132. Available at: https://doi.org/10.1016/j.tcb.2011.12.001.

Chen, Z., Zhao, P.-X., Miao, Z.-Q., Qi, G.-F., Wang, Z., Yuan, Y., Ahmad, N., Cao, M.-J., Hell, R. and Wirtz, M. (2019b) 'SULTR3s function in chloroplast sulfate uptake and affect ABA biosynthesis and the stress response', *Plant physiology*, 180(1), pp. 593–604. Available at: https://doi.org/10.1104/pp.18.01439.

Chi, M., Bhagwat, B., Lane, W.D., Tang, G., Su, Y., Sun, R., Oomah, B.D., Wiersma, P.A. and Xiang, Y. (2014) 'Reduced polyphenol oxidase gene expression and enzymatic browning in potato (Solanum tuberosum L.) with artificial microRNAs', *BMC Plant Biology*, 14(1), pp. 1–18. Available at: https://doi.org/10.1186/1471-2229-14-62.

Chintamanani, S., Multani, D.S., Ruess, H. and Johal, G.S. (2008) 'Distinct mechanisms govern the dosage-dependent and developmentally regulated resistance conferred by the maize Hm2 gene', *Molecular Plant-Microbe Interactions*, 21(1), pp. 79–86. Available at: https://doi.org/10.1094/MPMI-21-1-0079.

Chisholm, S.T., Coaker, G., Day, B. and Staskawicz, B.J. (2006) 'Host-microbe interactions: Shaping the evolution of the plant immune response', *Cell*, pp. 803–814. Available at: https://doi.org/10.1016/j.cell.2006.02.008.

CIP, (1979) *Control of important bacterial diseases of potatoes*. Lima, 21–27. Available at: https://hdl.handle.net/10568/109496.

Clough, S.J., Fengler, K.A., Yu, I.C., Lippok, B., Smith, R.K. and Bent, A.F. (2000) 'The Arabidopsis dnd1 "defense, no death" gene encodes a mutated cyclic nucleotide-gated ion channel', *Proceedings of the National Academy of Sciences of the United States of America*, 97(16), pp. 9323–9328. Available at: https://doi.org/10.1073/pnas.150005697.

Cohen, Y., Gisi, U. and Mosinger, E. (1991) 'Systemic resistance of potato plants against

Phytophthora infestans induced by unsaturated fatty acids', *Physiological and Molecular Plant Pathology*, 38(4), pp. 255–263. Available at: https://doi.org/10.1016/S0885-5765(05)80117-1.

Coll, N.S. and Valls, M. (2013) 'Current knowledge on the Ralstonia solanacearum type III secretion system', *Microbial Biotechnology*, 6(6), pp. 614–620. Available at: https://doi.org/10.1111/1751-7915.12056.

Colon, L., Nielsen, B.J. and D.U. (2004) 'Eucablight protocol - Tuber slice test for tuber blight resistance'.

Constabel, C.P. and Ryan, C.A. (1998) 'A survey of wound- and methyl jasmonate-induced leaf polyphenol oxidase in crop plants', *Phytochemistry* [Preprint]. Available at: https://doi.org/10.1016/S0031-9422(97)00539-6.

Coppo, E. and Marchese, A. (2014) 'Antibacterial Activity of Polyphenols', *Current Pharmaceutical Biotechnology*, 15(4), pp. 380–390. Available at: https://doi.org/10.2174/1389201015041408251211 42.

Couto, D. and Zipfel, C. (2016) 'Regulation of pattern recognition receptor signalling in plants', *Nature Reviews Immunology*, 16(9), pp. 537–552. Available at: https://doi.org/10.1038/nri.2016.77.

Criollo-Arteaga, S., Moya-Jimenez, S., Jimenez-Meza, M., Gonzalez-Vera, V., Gordon-Nunez, J., Llerena-Llerena, S., Ramirez-Villacis, D.X., van 't Hof, P. and Leon-Reyes, A. (2021) 'Sulfur deprivation modulates salicylic acid responses via nonexpressor of pathogenesis-related gene 1 in arabidopsis thaliana', *Plants*, 10(6), p. 1065. Available at: https://doi.org/10.3390/plants10061065.

Cseh, A., Lenykó-Thegze, A., Makai, D., Szabados, F., Hamow, K.Á., Gulyás, Z., Kiss, T., Karsai, I., Moncsek, B., Mihók, E. and Sepsi, A. (2024) 'Meiotic instability and irregular chromosome pairing underpin heat-induced infertility in bread wheat carrying the Rht-B1b or Rht-D1b Green Revolution genes', *New Phytologist*, 241(1), pp. 180–196. Available at: https://doi.org/10.1111/nph.19256.

Cui, H., Tsuda, K. and Parker, J.E. (2015) 'Effector-triggered immunity: from pathogen perception to robust defense', *Annual review of plant biology*, 66, pp. 487–511. Available at: https://doi.org/10.1146/annurev-arplant-050213-040012.

Cvitanich, C. and Judelson, H.S. (2003) 'A gene expressed during sexual and asexual sporulation in Phytophthora infestans is a member of the Puf family of translational regulators', *Eukaryotic Cell*, 2(3), pp. 465–473. Available at: https://doi.org/10.1128/EC.2.3.465-473.2003.

Dai, G.H., Nicole, M., Andary, C., Martinez, C., Bresson, E., Boher, B., Daniel, J.F. and Geiger,

J.P. (1996) ‘Flavonoids accumulate in cell walls, middle lamellae and callose-rich papillae during an incompatible interaction between Xanthomonas campestris pv. malvacearum and cotton’, *Physiological and Molecular Plant Pathology*, 49(5), pp. 285–306. Available at: https://doi.org/10.1006/pmpp.1996.0055.

Dangl, J.L. and McDowell, J.M. (2006) ‘Two modes of pathogen recognition by plants’, *Proceedings of the National Academy of Sciences*, 103(23), pp. 8575–8576. Available at: https://doi.org/10.1073/pnas.0603183103.

De Lange, O., Schreiber, T., Schandry, N., Radeck, J., Braun, K.H., Koszinowski, J., Heuer, H., Strauß, A. and Lahaye, T. (2013) ‘Breaking the DNA-binding code of Ralstonia solanacearum TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease’, *New Phytologist*, 199(3), pp. 773–786. Available at: https://doi.org/10.1111/nph.12324.

De Toledo Thomazella, D.P., Brail, Q., Dahlbeck, D. and Staskawicz, B.J. (2016) 'CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance' , *BioRxiv*. p.064824. Available at: https://doi.org/10.1101/064824.

De Vries, S., Kukuk, A., von Dahlen, J.K., Schnake, A., Kloesges, T. and Rose, L.E. (2018) ‘Expression profiling across wild and cultivated tomatoes supports the relevance of early miR482/2118 suppression for Phytophthora resistance’, *Proceedings of the Royal Society B: Biological Sciences*, 285(1873), p. 20172560. Available at: https://doi.org/10.1098/rspb.2017.2560.

Denancé, N., Ranocha, P., Oria, N., Barlet, X., Rivière, M.P., Yadeta, K.A., Hoffmann, L., Perreau, F., Clément, G., Maia-Grondard, A., Van Den Berg, G.C.M., Savelli, B., Fournier, S., Aubert, Y., Pelletier, S., Thomma, B.P.H.J., Molina, A., Jouanin, L., Marco, Y. and Goffner, D. (2013) ‘Arabidopsis wat1 (walls are thin1)-mediated resistance to the bacterial vascular pathogen, Ralstonia solanacearum, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism’, *Plant Journal*, 73(2), pp. 225–239. Available at: https://doi.org/10.1111/tpj.12027.

Deng, Y., Wang, J., Tung, J., Liu, D., Zhou, Y., He, S., Du, Y., Baker, B. and Li, F. (2018) ‘A role for small RNA in regulating innate immunity during plant growth’, *PLoS Pathogens*, 14(1), p. e1006756. Available at: https://doi.org/10.1371/journal.ppat.1006756.

Denny, T.P. (2001) ‘Gram-negative bacteria: Ralstonia’, *Laboratory guide for identification of plant pathogenic bacteria* [Preprint].

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S. and Marco, Y. (2003) ‘Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant

nucleus', *Proceedings of the National Academy of Sciences of the United States of America*, 100(13), pp. 8024–8029. Available at: https://doi.org/10.1073/pnas.1230660100.

Deslandes, L. and Rivas, S. (2012) 'Catch me if you can: Bacterial effectors and plant targets', *Trends in Plant Science*, pp. 644–655. Available at: https://doi.org/10.1016/j.tplants.2012.06.011.

Devaux, A., Kromann, P. and Ortiz, O. (2014) 'Potatoes for Sustainable Global Food Security', *Potato Research*, 57(3–4), pp. 185-199. Available at: https://doi.org/10.1007/s11540-014-9265-1.

Dhingra, O.D. and Sinclair, J.B. (1985) *Basic plant pathology methods*. CRC Press, Inc.

Dietze, J. (1995) 'Agrobacteria-mediated transfor-mation of potato (Solanum tuberosum)', *Gene transfer to plants*, pp. 24–29.

Dievart, A. and Clark, S.E. (2004) 'LRR-containing receptors regulating plant development and defense', *Development*. Available at: https://doi.org/10.1242/dev.00998.

Ding, G., Lei, G.J., Yamaji, N., Yokosho, K., Mitani-Ueno, N., Huang, S. and Ma, J.F. (2020) 'Vascular cambium-localized AtSPDT mediates xylem-to-phloem transfer of phosphorus for its preferential distribution in Arabidopsis', *Molecular plant*, 13(1), pp. 99–111. Available at: https://doi.org/10.1016/j.molp.2019.10.002.

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Yaxi, Li, X. and Zhang, Yuelin (2018) 'Opposite roles of salicylic acid receptors NPR1 and NPR3/NPR4 in transcriptional regulation of plant immunity', *Cell*, 173(6), pp. 1454–1467. Available at: https://doi.org/10.1016/j.cell.2018.03.044.

Dodds, P.N. and Rathjen, J.P. (2010) 'Plant immunity: towards an integrated view of plant–pathogen interactions', *Nature Reviews Genetics*, 11(8), pp. 539–548. Available at: https://doi.org/10.1038/nrg2812.

Dong, S. meng and Zhou, S. qun (2022) 'Potato late blight caused by Phytophthora infestans: From molecular interactions to integrated management strategies', *Journal of Integrative Agriculture*, pp. 3456–3466. Available at: https://doi.org/10.1016/j.jia.2022.08.060.

Dorion, S., Ouellet, J.C. and Rivoal, J. (2021) 'Glutathione metabolism in plants under stress: Beyond reactive oxygen species detoxification', *Metabolites*, 11(9). Available at: https://doi.org/10.3390/metabo11090641.

Dorrance, A.E. and Inglis, D.A. (1997) 'Assessment of greenhouse and laboratory screening methods for evaluating potato foliage for resistance to late blight', *Plant Disease*, 81(10), pp. 1206–1213. Available at: https://doi.org/10.1094/PDIS.1997.81.10.1206.

Dorrance, A.E. and Inglis, D.A. (1998) 'Assessment of laboratory methods for evaluating potato tubers for resistance to late blight', *Plant Disease*, 82(4), pp. 442–446. Available at:

https://doi.org/10.1094/PDIS.1998.82.4.442.

Doyle, E.L., Stoddard, B.L., Voytas, D.F. and Bogdanove, A.J. (2013) 'TAL effectors: Highly adaptable phytobacterial virulence factors and readily engineered DNA-targeting proteins', *Trends in Cell Biology*, pp. 390–398. Available at: https://doi.org/10.1016/j.tcb.2013.04.003.

Du, J., Verzaux, E., Chaparro-Garcia, A., Bijsterbosch, G., Keizer, L.C., Zhou, J.I., Liebrand, T.W.H., Xie, C., Govers, F. and Robatzek, S. (2015) 'Elicitin recognition confers enhanced resistance to Phytophthora infestans in potato', *Nature plants*, 1(4), pp. 1–5. Available at: https://doi.org/10.1038/nplants.2015.34.

Du, M., Zhai, Q., Deng, L., Li, S., Li, H., Yan, L., Huang, Z., Wang, B., Jiang, H. and Huang, T. (2014) 'Closely related NAC transcription factors of tomato differentially regulate stomatal closure and reopening during pathogen attack', *The Plant Cell*, 26(7), pp. 3167–3184. Available at: https://doi.org/10.1105/tpc.114.128272.

Duffey, S.S. and Felton, G.W. (1991) 'Enzymatic Antinutritive Defenses of the Tomato Plant Against Insects', in. Available at: https://doi.org/10.1021/bk-1991-0449.ch012.

Eckardt, N.A. (2012) 'A MicroRNA cascade in plant defense', *Plant Cell*, 24(3), p. 840. Available at: https://doi.org/10.1105/tpc.112.240311.

ECPD (2011) '"Monona," in The European cultivated potato database (Science and Advice for Scottish Agriculture;)'. Available at: https://www.europotato.org/varieties/view/Monona.

Elnahal, A.S.M., Li, J., Wang, X., Zhou, C., Wen, G., Wang, J., Lindqvist-Kreuze, H., Meng, Y. and Shan, W. (2020) 'Identification of Natural Resistance Mediated by Recognition of Phytophthora infestans Effector Gene Avr3aEM in Potato', *Frontiers in Plant Science*, 11, p. 919. Available at: https://doi.org/10.3389/fpls.2020.00919.

Elphinstone, J.G. (2005) 'The current bacterial wilt situation: a global overview. In Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex', *American Phytopathological Society Press*, 8(10), pp. 9–28.

Eschen-Lippold, L., Landgraf, R., Smolka, U., Schulze, S., Heilmann, M., Heilmann, I., Hause, G. and Rosahl, S. (2012) 'Activation of defense against Phytophthora infestans in potato by down-regulation of syntaxin gene expression', *New Phytologist*, 193(4), pp. 985–996. Available at: https://doi.org/10.1111/j.1469-8137.2011.04024.x.

Fahlgren, N., Howell, M.D., Kasschau, K.D., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., Givan, S.A., Law, T.F., Grant, S.R., Dangl, J.L. and Carrington, J.C. (2007) 'High-throughput sequencing of Arabidopsis microRNAs: Evidence for frequent birth and death of MIRNA genes', *PLoS ONE*, 2(2), p. e219. Available at: https://doi.org/10.1371/journal.pone.0000219.

FAO (2006) *FAOSTAT F. FAO statistical databases.* Rome.

Fedoroff, N. V, Battisti, D.S., Beachy, R.N., Cooper, P.J., Fischhoff, D.A., Hodges, C.N. and Zhu, J. (2010) 'Radically Rethinking Agriculture for the 21st Century. Retrieved February 28, 2017', 327(5967), pp. 833–834. Available at: https://doi.org/10.1126/science.1186834.

Fegan, Mark and Prior, P. (2005) 'How complex is the "Ralstonia solanacearum species complex" Bacterial wilt disease and the Ralstonia solanacearum species complex', *APS Press*, 1(15), pp. 449–461.

Felton, G.W., Donato, K.K., Broadway, R.M. and Duffey, S.S. (1992) 'Impact of oxidized plant phenolics on the nutritional quality of dietar protein to a noctuid herbivore, Spodoptera exigua', *Journal of Insect Physiology*, 38(4), pp. 277–285. Available at: https://doi.org/10.1016/0022-1910(92)90128-Z.

Feng, J., Yuan, F., Gao, Y., Liang, C., Xu, J., Zhang, C. and He, L. (2003) 'A novel antimicrobial protein isolated from potato (Solanum tuberosum) shares homology with an acid phosphatase', *Biochemical Journal*, 376(2). Available at: https://doi.org/10.1042/BJ20030806.

Fernández, M.B., Daleo, G.R. and Guevara, M.G. (2012) 'DEVDase activity is induced in potato leaves during Phytophthora infestans infection', *Plant Physiology and Biochemistry*, 61. Available at: https://doi.org/10.1016/j.plaphy.2012.10.007.

Ferreira, V., Pianzzola, M.J., Vilaró, F.L., Galván, G.A., Tondo, M.L., Rodriguez, M. V, Orellano, E.G., Valls, M. and Siri, M.I. (2017) 'Interspecific potato breeding lines display differential colonization patterns and induced defense responses after Ralstonia solanacearum infection', *Frontiers in Plant Science*, 8, p. 1424. Available at: https://doi.org/10.3389/fpls.2017.01424.

Ferris, V.R. (1955) 'Histological study of pathogen-suscept relationships between Phytophthora infestans and derivatives of Solanum demissum', *Phytopathology*, 45(10), pp. 546–552.

Finnegan, E.J. and Matzke, M.A. (2003) 'The small RNA world', *Journal of cell science*, 116(23), pp. 4689–4693. Available at: https://doi.org/10.1242/jcs.00838.

Fitzpatrick, K.L., Tyerman, S.D. and Kaiser, B.N. (2008) 'Molybdate transport through the plant sulfate transporter SHST1', *FEBS letters*, 582(10), pp. 1508–1513. Available at: https://doi.org/10.1016/j.febslet.2008.03.045.

Flier, W.G., Van Den Bosch, G.B.M. and Turkensteen, L.J. (2003) 'Stability of partial resistance in potato cultivars exposed to aggressive strains of Phytophthora infestans', *Plant Pathology*, 52(3), pp. 326–337. Available at: https://doi.org/10.1046/j.1365-3059.2003.00862.x.

Fock, I., Collonnier, C., Luisetti, J., Purwito, A., Souvannavong, V., Vedel, F., Servaes, A.,

Ambroise, A., Kodja, H., Ducreux, G. and Sihachakr, D. (2001) 'Use of Solanum stenotomum for introduction of resistance to bacterial wilt in somatic hybrids of potato', *Plant Physiology and Biochemistry*, 39(10), pp. 899–908. Available at: https://doi.org/10.1016/S0981-9428(01)01307-9.

Fock, I., Collonnier, C., Purwito, A., Luisetti, J., Souvannavong, V., Vedel, F., Servaes, A., Ambroise, A., Kodja, H., Ducreux, G. and Sihachakr, D. (2000) 'Resistance to bacterial wilt in somatic hybrids between Solanum tuberosum and Solanum phureja', *Plant Science*, 160(1), pp. 165–176. Available at: https://doi.org/10.1016/S0168-9452(00)00375-7.

Fones, H.N., Bebber, D.P., Chaloner, T.M., Kay, W.T., Steinberg, G. and Gurr, S.J. (2020) 'Threats to global food security from emerging fungal and oomycete crop pathogens', *Nature Food*, 1(6), pp. 332–342. Available at: https://doi.org/10.1038/s43016-020-0075-0.

Freisleben, R. and Lein, A. (1942) 'Über die Auffindung einer mehltauresistenten Mutante nach Röntgenbestrahlung einer anfälligen reinen Linie von Sommergerste', *Die Naturwissenschaften*, 30(40), p. 608. Available at: https://doi.org/10.1007/BF01488231.

French, E., Kim, B.S., Rivera-Zuluaga, K. and Iyer-Pascuzzi, A.S. (2018) 'Whole root transcriptomic analysis suggests a role for auxin pathways in resistance to Ralstonia solanacearum in tomato', *Molecular Plant-Microbe Interactions*, 31(4), pp. 432–444. Available at: https://doi.org/10.1094/MPMI-08-17-0209-R.

French, E.R., Anguiz, R. and Aley, P. (1998) 'The Usefulness of Potato Resistance to Ralstonia solanacearum, for the Integrated Control of Bacterial Wilt', in *Bacterial Wilt Disease*. Berlin, Heidelberg: Springer Berlin Heidelberg., pp. 381–385. Available at: https://doi.org/10.1007/978-3-662-03592-4_58.

Friedman, M. (1996) 'Food browning and its prevention: An overview', *Journal of Agricultural and Food Chemistry*, 44(3), pp. 631–653. Available at: https://doi.org/10.1021/jf950394r.

Fry, W. (2008) 'Phytophthora infestans: The plant (and R gene) destroyer', *Molecular Plant Pathology*, 9(3), pp. 385–402. Available at: https://doi.org/10.1111/j.1364-3703.2008.00504.x.

Fry, W.E. (2016) 'Phytophthora infestans: New Tools (and Old Ones) Lead to New Understanding and Precision Management', *Annual Review of Phytopathology*, 54, pp. 529–547. Available at: https://doi.org/10.1146/annurev-phyto-080615-095951.

Fry, W.E., Birch, P.R.J., Judelson, H.S., Grünwald, N.J., Danies, G., Everts, K.L., Gevens, A.J., Gugino, B.K., Johnson, D.A., Johnson, S.B., McGrath, M.T., Myers, K.L., Ristaino, J.B., Roberts, P.D., Secor, G. and Smart, C.D. (2015) 'Five reasons to consider phytophthora infestans a reemerging pathogen', *Phytopathology*, 105(7), pp. 966–981. Available at:

https://doi.org/10.1094/PHYTO-01-15-0005-FI.

Fu, X., Li, C., Zhou, X., Liu, S. and Wu, F. (2016) 'Physiological response and sulfur metabolism of the V. dahliae-infected tomato plants in tomato/potato onion companion cropping', *Scientific Reports*, 6, p. 36445. Available at: https://doi.org/10.1038/srep36445.

Fu, Z.Q. and Dong, X. (2013) 'Systemic acquired resistance: turning local infection into global defense', *Annual review of plant biology*, 64, pp. 839–863. Available at: https://doi.org/10.1146/annurev-arplant-042811-105606.

Gallas, N., Li, X., von Roepenack-Lahaye, E., Schandry, N., Jiang, Y., Wu, D. and Lahaye, T. (2023) 'An ancient cis-element targeted by Ralstonia solanacearum TALE-like effectors facilitates the development of a promoter trap that could confer broad-spectrum wilt resistance', *Plant Biotechnology Journal* [Preprint]. Available at: https://doi.org/https://doi.org/10.1111/pbi.14208.

Gao, F., Wang, K., Liu, Y., Chen, Y., Chen, P., Shi, Z., Luo, J., Jiang, D., Fan, F. and Zhu, Y. (2015) 'Blocking miR396 increases rice yield by shaping inflorescence architecture', *Nature Plants*, 2(1), pp. 1–9. Available at: https://doi.org/10.1038/nplants.2015.196.

Gao, W., Chen, R., Pan, M., Tang, W., Lan, T., Huang, L., Chi, W. and Wu, W. (2019) 'Early transcriptional response of seedling roots to Ralstonia solanacearum in tobacco (Nicotiana tabacum L.)', *European Journal of Plant Pathology*, 155, pp. 527–536. Available at: https://doi.org/10.1007/s10658-019-01788-x.

Garcia-Ruiz, H., Szurek, B. and Van den Ackerveken, G. (2021) 'Stop helping pathogens: engineering plant susceptibility genes for durable resistance', *Current opinion in biotechnology*, 70, pp. 187–195. Available at: https://doi.org/https://doi.org/10.1016/j.copbio.2021.05.005.

Genger, R.K., Jurkowski, G.I., McDowell, J.M., Lu, H., Ho, W.J., Greenberg, J.T. and Bent, A.F. (2008) 'Signaling pathways that regulate the enhanced disease resistance of Arabidopsis "defense, no death" mutants', *Molecular Plant-Microbe Interactions*, 21(10), pp. 1285–1296. Available at: https://doi.org/10.1094/MPMI-21-10-1285.

Genin, S. and Denny, T.P. (2012) 'Pathogenomics of the ralstonia solanacearum species complex', *Annual Review of Phytopathology*, 50, pp. 67–89. Available at: https://doi.org/10.1146/annurev-phyto-081211-173000.

Giannakopoulou, A., Steele, J.F.C., Segretin, M.E., Bozkurt, T.O., Zhou, J., Robatzek, S., Banfield, M.J., Pais, M. and Kamoun, S. (2015) 'Tomato I2 immune receptor can be engineered to confer partial resistance to the oomycete phytophthora infestans in addition to the fungus fusarium oxysporum', *Molecular Plant-Microbe Interactions*, 28(12), pp. 1316–1329. Available at: https://doi.org/10.1094/MPMI-07-15-0147-R.

Gibbings, D.J., Ciaudo, C., Erhardt, M. and Voinnet, O. (2009) 'Multivesicular bodies associate with components of miRNA effector complexes and modulate miRNA activity', *Nature Cell Biology*, 11(9), pp. 1143–1149. Available at: https://doi.org/10.1038/ncb1929.

Gigot, J.A., Gundersen, B. and Inglis, D.A. (2009) 'Colonization and sporulation of Phytophthora infestans on volunteer potatoes under western Washington conditions', *American Journal of Potato Research*, 86, pp. 1–14. Available at: https://doi.org/10.1007/s12230-008-9054-y.

Glazebrook, J. (2005) 'Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens', *Annual Review of Phytopathology*. Available at: https://doi.org/10.1146/annurev.phyto.43.040204.135923.

Godiard, L., Sauviac, L., Torii, K.U., Grenon, O., Mangin, B., Grimsley, N.H. and Marco, Y. (2003) 'ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt', *Plant Journal*, 36(3), pp. 353–365. Available at: https://doi.org/10.1046/j.1365-313X.2003.01877.x.

Golan-Goldhirsh, A., Whitaker, J.R. and Kahn, V. (1984) 'Relation between structure of polyphenol oxidase and prevention of browning.', *Advances in experimental medicine and biology*, pp. 437–456. Available at: https://doi.org/10.1007/978-1-4684-4790-3_21.

González, M.N., Massa, G.A., Andersson, M., Turesson, H., Olsson, N., Fält, A.S., Storani, L., Décima Oneto, C.A., Hofvander, P. and Feingold, S.E. (2020) 'Reduced Enzymatic Browning in Potato Tubers by Specific Editing of a Polyphenol Oxidase Gene via Ribonucleoprotein Complexes Delivery of the CRISPR/Cas9 System', *Frontiers in Plant Science*, 10, p. 1649. Available at: https://doi.org/10.3389/fpls.2019.01649.

Gottschalk, W. (1984) 'The origin of the potato. An open problem', *Nucleus*, 27, pp. 37–44.

Gouveia, B.C., Calil, I.P., Machado, J.P.B., Santos, A.A. and Fontes, E.P.B. (2017) 'Immune receptors and co-receptors in antiviral innate immunity in plants', *Frontiers in microbiology*, 7, p. 2139. Available at: https://doi.org/10.3389/fmicb.2016.02139.

Govers, F. and Gijzen, M. (2006) 'Phytophthora genomics: The plant destroyers' genome decoded', *Molecular Plant-Microbe Interactions*, 19(12), pp. 1295–1301. Available at: https://doi.org/10.1094/MPMI-19-1295.

Govrin, E.M. and Levine, A. (2000) 'The hypersensitive response facilitates plant infection by the necrotrophic pathogen Botrytis cinerea', *Current biology*, 10(13), pp. 751–757. Available at: https://doi.org/10.1016/S0960-9822(00)00560-1.

Grant, M. and L.C. (2006) 'Systemic immunity', *Current opinion in plant biology*, 9(4), pp. 414–420.

Green, A.R., Nissen, M.S., Mohan Kumar, G.N., Knowles, N.R. and Kang, C.H. (2013)

‘Characterization of Solanum tuberosum multicystatin and the significance of core domains’, *Plant Cell*, 25(12). Available at: https://doi.org/10.1105/tpc.113.121004.

Grimault, V., Anais, G. and Prior, P. (1994) ‘Distribution of Pseudomonas solanacearum in the stem tissues of tomato plants with different levels of resistance to bacterial wilt’, *Plant Pathology*, 43(4), pp. 663–668. Available at: https://doi.org/10.1111/j.1365-3059.1994.tb01604.x.

Grimault, V. and Prior, P. (1993) ‘Bacterial wilt resistance in tomato associated with tolerance of vascular tissues to Pseudomonas solanacearum’, *Plant Pathology*, 42(4). Available at: https://doi.org/10.1111/j.1365-3059.1993.tb01539.x.

Guenthner, J.F., Michael, K.C. and Nolte, P. (2001) ‘The economic impact of potato late blight on US growers’, *Potato Research*, 44(2), pp. 121–125. Available at: https://doi.org/10.1007/BF02410098.

Habe, I., Miyatake, K., Nunome, T., Yamasaki, M. and Hayashi, T. (2019) ‘Qtl analysis of resistance to bacterial wilt caused by ralstonia solanacearum in potato’, *Breeding Science*, 69(4), pp. 592–600. Available at: https://doi.org/10.1270/jsbbs.19059.

Hakimi, S., Krohn, B. and Stark, D. (2002) ‘Method of imparting disease resistance to plants by reducing polyphenol oxidase activity’. Google Patents.

Hane, J.K., Paxman, J., Jones, D.A.B., Oliver, R.P. and de Wit, P. (2020) ‘“CATAStrophy,” a Genome-Informed Trophic Classification of Filamentous Plant Pathogens – How Many Different Types of Filamentous Plant Pathogens Are There?’, *Frontiers in Microbiology*, 10, p. 492799. Available at: https://doi.org/10.3389/fmicb.2019.03088.

Hartmann, M., Zeier, T., Bernsdorff, F., Reichel-Deland, V., Kim, D., Hohmann, M., Scholten, N., Schuck, S., Bräutigam, A. and Hölzel, T. (2018) ‘Flavin monooxygenase-generated N-hydroxypipecolic acid is a critical element of plant systemic immunity’, *Cell*, 173(2), pp. 456–469. Available at: https://doi.org/10.1016/j.cell.2018.02.049.

Haverkort, A.J., Struik, P.C., Visser, R.G.F. and Jacobsen, E. (2009) ‘Applied biotechnology to combat late blight in potato caused by phytophthora infestans’, *Potato Research*, 52(3), pp. 249–264. Available at: https://doi.org/10.1007/s11540-009-9136-3.

Hayward, A.C. (1964) ‘Characteristics of Pseudomonas solanacearum’, *Journal of Applied Bacteriology*, 27(2), pp. 265–277. Available at: https://doi.org/10.1111/j.1365-2672.1964.tb04912.x.

Hayward, A.C. (1991) ‘Biology and epidemiology of bacterial wilt caused by Pseudomonas solanacearum’, *Annual Review of Phytopathology*, 29(1), pp. 65–87. Available at: https://doi.org/10.1146/annurev.py.29.090191.000433.

He, Q., McLellan, H., Boevink, P.C. and Birch, P.R.J. (2020) ‘All Roads Lead to Susceptibility:

The Many Modes of Action of Fungal and Oomycete Intracellular Effectors', *Plant Communications*, 1(4). Available at: https://doi.org/10.1016/j.xplc.2020.100050.

He, Z., Larkin, R. and Honeycutt, W. (2012) *Sustainable potato production: Global case studies*, *Sustainable Potato Production: Global Case Studies*. Available at: https://doi.org/10.1007/978-94-007-4104-1.

Hernández-Blanco, C., Feng, D.X., Hu, J., Sánchez-Vallet, A., Deslandes, L., Llorente, F., Berrocal-Lobo, M., Keller, H., Barlet, X., Sánchez-Rodríguez, C., Anderson, L.K., Somerville, S., Marco, Y. and Molinaa, A. (2007) 'Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance', *Plant Cell*, 19(3), pp. 890–903. Available at: https://doi.org/10.1105/tpc.106.048058.

Hernández-Romero, D., Solano, F. and Sanchez-Amat, A. (2005) 'Polyphenol oxidase activity expression in Ralstonia solanacearum', *Applied and Environmental Microbiology*, 71(11), pp. 6808–6815. Available at: https://doi.org/10.1128/AEM.71.11.6808-6815.2005.

Hinderer, W., Petersen, M. and Seitz, H.U. (1984) 'Inhibition of flavonoid biosynthesis by gibberellic acid in cell suspension cultures of Daucus carota L.', *Planta*, 160(6), pp. 544–549. Available at: https://doi.org/10.1007/BF00411143.

Hirsch, J., Deslandes, L., Feng, D.X., Balagué, C. and Marco, Y. (2002) 'Delayed symptom development in ein2-1, an Arabidopsis ethylene-insensitive mutant, in response to bacterial wilt caused by Ralstonia solanacearum', *Phytopathology*, 92(10), pp. 1142–1148. Available at: https://doi.org/10.1094/PHYTO.2002.92.10.1142.

Holbein, J., Franke, R.B., Marhavý, P., Fujita, S., Górecka, M., Sobczak, M., Geldner, N., Schreiber, L., Grundler, F.M.W. and Siddique, S. (2019) 'Root endodermal barrier system contributes to defence against plant-parasitic cyst and root-knot nematodes', *The Plant Journal*, 100(2), pp. 221–236.

Holderbaum, D.F., Kon, T., Kudo, T. and Guerra, M.P. (2010) 'Enzymatic browning, polyphenol oxidase activity, and polyphenols in four apple cultivars: Dynamics during fruit development', *HortScience*, 45(8), pp. 1150–1154. Available at: https://doi.org/10.21273/hortsci.45.8.1150.

Hu, X., Persson Hodén, K., Liao, Z., Åsman, A. and Dixelius, C. (2022) 'Phytophthora infestans Ago1-associated miRNA promotes potato late blight disease', *New Phytologist*, 233(1), pp. 443–457. Available at: https://doi.org/10.1111/nph.17758.

Huang, S., Vleeshouwers, V.G.A.A., Visser, R.G.F. and Jacobsen, E. (2005) 'An accurate in vitro assay for high-throughput disease testing of Phytophthora infestans in potato', *Plant Disease*, 89(12), pp. 1263–1267. Available at: https://doi.org/10.1094/PD-89-1263.

Huet, G. (2014) 'Breeding for resistances to Ralstonia solanacearum', *Frontiers in Plant Science*,

5, p. 715. Available at: https://doi.org/10.3389/fpls.2014.00715.

Huibers, R.P., Loonen, A.E.H.M., Gao, D., Van den Ackerveken, G., Visser, R.G.F. and Bai, Y. (2013) 'Powdery Mildew Resistance in Tomato by Impairment of SlPMR4 and SlDMR1', *PLoS ONE*, 8(6), p. e67467. Available at: https://doi.org/10.1371/journal.pone.0067467.

Hwang, E.W., Shin, S.J., Yu, B.K., Byun, M.O. and Kwon, H. Bin (2011) 'miR171 Family Members are Involved in Drought Response in Solanum tuberosum', *Journal of Plant Biology*, 54(1), pp. 43–48. Available at: https://doi.org/10.1007/s12374-010-9141-8.

Ingram, D.S. and Williams, P.H. (1991) 'Phytophthora infestans, the cause of late blight of potato', *(No Title)* [Preprint].

Ishihama, N. and Yoshioka, H. (2012) 'Post-translational regulation of WRKY transcription factors in plant immunity', *Current opinion in plant biology*, 15(4), pp. 431–437. Available at: https://doi.org/https://doi.org/10.1016/j.pbi.2012.02.003.

Ishihara, T., Mitsuhara, I., Takahashi, H. and Nakaho, K. (2012) 'Transcriptome Analysis of Quantitative Resistance-Specific Response upon Ralstonia solanacearum Infection in Tomato', *PLoS ONE*, p. e46763. Available at: https://doi.org/10.1371/journal.pone.0046763.

Jackson M. T. (2012) *Cruza 148 . . . the serendipity of disease resistance*.

Jackson M. T., Gonzalez L.C. and Aguilar J .A. (1979) 'Avances en el combate de la marchitez bacteriana de papa en Costa Rica', *FITOPATOLOGIA* , 14(2), pp. 46–53.

Jackson, M.T., Rowe, P.R. and Hawkes, J.G. (1978) 'Crossability relationships of andean potato varieties of three ploidy levels', *Euphytica*, 27(2), pp. 541–551. Available at: https://doi.org/10.1007/BF00043180.

Jagadeeswaran, G., Zheng, Y., Li, Y., Shukla, L.I., Matts, J., Hoyt, P., Macmil, S.L., Wiley, G.B., Roe, B.A. and Zhang, W. (2009) 'Cloning and characterization of small RNAs from Medicago truncatula reveals four novel legume-specific microRNA families', *New Phytologist*, 184(1), pp. 85–98. Available at: https://doi.org/https://doi.org/10.1111/j.1469-8137.2009.02915.x.

Janse, J.D., Araluppan, F.A.X., Schans, J., Wenneker, M. and Westerhuis, W. (1998) 'Experiences with bacterial brown rot Ralstonia solanacearum biovar 2, race 3 in the Netherlands', in *Bacterial Wilt disease: Molecular and ecological aspects*. Springer, pp. 146–152.

Janse, J.D., Van den Beld, H.E., Elphinstone, J., Simpkins, S., Tjou-Tam-Sin, N.N.A. and Van Vaerenbergh, J. (2004) 'Introduction to Europe of Ralstonia solanacearum biovar 2, race 3 in Pelargonium zonale cuttings', *Journal of plant pathology*, 86(2), pp. 147–155.

Jaworski, C.A., Webb, R.E., Goth, R.W. and Phatak, S.C. (1980) 'Relative resistance of potato cultivars to bacterial wilt', *American Potato Journal*, 57, pp. 159–165. Available at:

https://doi.org/10.1007/BF02853867.

Jia, H., Zhao, P., Wang, B., Tariq, P., Zhao, F., Zhao, M., Wang, Q., Yang, T. and Fang, J. (2016) 'Overexpression of Polyphenol Oxidase Gene in Strawberry Fruit Delays the Fungus Infection Process', *Plant Molecular Biology Reporter*, 34(3), pp. 592–606. Available at: https://doi.org/10.1007/s11105-015-0946-y.

Jia, S.-R., Xic, Y., Tang, T., Feng, L.-X., Cao, D.-S., Zhao, Y.-L., Yuan, J., Bai, Y.-Y., Jiang, C.-X., Jayncs, J.M. and Dodds, J.D. (1993) 'Genetic Engineering of Chinese Potato Cultivars by Introducing Antibacterial Polypeptide Gene', in *Biotechnology in Agriculture: Proceedings of the First Asia-Pacific Conference on Agricultural Biotechnology, Beijing, China, 20–24 August 1992*. Springer Netherlands., pp. 208–212. Available at: https://doi.org/10.1007/978-94-011-1779-1_32.

Jiang, N., Fan, X., Lin, W., Wang, G. and Cai, K. (2019) 'Transcriptome analysis reveals new insights into the bacterial wilt resistance mechanism mediated by silicon in tomato', *International Journal of Molecular Sciences*, 20(3), p. 761. Available at: https://doi.org/10.3390/ijms20030761.

Jiang, N., Meng, J., Cui, J., Sun, G. and Luan, Y. (2018) 'Function identification of miR482b, a negative regulator during tomato resistance to Phytophthora infestans', *Horticulture Research*, 5(1). Available at: https://doi.org/10.1038/s41438-018-0017-2.

Jiang, N., Yan, J., Liang, Y., Shi, Y., He, Z., Wu, Y., Zeng, Q., Liu, X. and Peng, J. (2020) 'Resistance genes and their interactions with bacterial blight/leaf streak pathogens (Xanthomonas oryzae) in rice (Oryza sativa L.)—an updated review', *Rice*, 13(1), pp. 1–12.

Jiang, W., Bikard, D., Cox, D., Zhang, F. and Marraffini, L.A. (2013) 'RNA-guided editing of bacterial genomes using CRISPR-Cas systems', *Nature Biotechnology*, 31(3), pp. 233–239. Available at: https://doi.org/10.1038/nbt.2508.

Johnson, S.M., Doherty, S.J. and Croy, R.R.D. (2003) 'Biphasic superoxide generation in potato tubers. A self-amplifying response to stress', *Plant Physiology*, 131(3), pp. 1440–1449. Available at: https://doi.org/10.1104/pp.013300.

Jones-Rhoades, M.W. and Bartel, D.P. (2004) 'Computational identification of plant microRNAs and their targets, including a stress-induced miRNA', *Molecular cell*, 14(6), pp. 787–799. Available at: https://doi.org/10.1016/j.molcel.2004.05.027.

Jones, J.D.G. and Dangl, J.L. (2006) 'The plant immune system', *nature*, 444(7117), pp. 323–329. Available at: https://doi.org/10.1038/nature05286.

Jørgensen, I.H. (1992) 'Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley', *Euphytica*, 63, pp. 141–152. Available at:

https://doi.org/10.1007/BF00023919.

Jose, J., Éva, C., Bozsó, Z., Hamow, K.Á., Fekete, Z., Fábián, A., Bánfalvi, Z. and Sági, L. (2023) 'Global transcriptome and targeted metabolite analyses of roots reveal different defence mechanisms against Ralstonia solanacearum infection in two resistant potato cultivars', *Frontiers in Plant Science*, 13, p. 1065419. Available at: https://doi.org/doi.org/10.3389/fpls.2022.1065419.

Jurkowski, G.I., Smith Jr, R.K., Yu, I., Ham, J.H., Sharma, S.B., Klessig, D.F., Fengler, K.A. and Bent, A.F. (2004) 'Arabidopsis DND2, a second cyclic nucleotide-gated ion channel gene for which mutation causes the "defense, no death" phenotype', *Molecular Plant-Microbe Interactions*, 17(5), pp. 511–520. Available at: https://doi.org/10.1094/MPMI.2004.17.5.511.

Kabir, F., Katayama, S., Tanji, N. and Nakamura, S. (2014) 'Antimicrobial effects of chlorogenic acid and related compounds', *Journal of the Korean Society for Applied Biological Chemistry*, 57, pp. 359–365. Available at: https://doi.org/10.1007/s13765-014-4056-6.

Kanaar, R., Hoeijmakers, J.H.J. and Van Gent, D.C. (1998) 'Molecular mechanisms of DNA double-strand break repair', *Trends in Cell Biology*, 8(12), pp. 483–489. Available at: https://doi.org/10.1016/S0962-8924(98)01383-X.

Karim, Z., Hossain, M. and Begum, M. (2018) 'Ralstonia solanacearum: A Threat to Potato Production in Bangladesh', *Fundamental and Applied Agriculture*, 3(1), pp. 407–421. Available at: https://doi.org/10.5455/faa.280361.

Karlova, R., van Haarst, J.C., Maliepaard, C., van de Geest, H., Bovy, A.G., Lammers, M., Angenent, G.C. and de Maagd, R.A. (2013) 'Identification of microRNA targets in tomato fruit development using high-throughput sequencing and degradome analysis', *Journal of experimental botany*, 64(7), pp. 1863–1878.

Kaschani, F., Shabab, M., Bozkurt, T., Shindo, T., Schornack, S., Gu, C., Ilyas, M., Win, J., Kamoun, S. and van der Hoorn, R.A.L. (2010) 'An effector-targeted protease contributes to defense against Phytophthora infestans and is under diversifying selection in natural hosts', *Plant Physiology*, 154(4). Available at: https://doi.org/10.1104/pp.110.158030.

Kashyap, A., Planas-Marquès, M., Capellades, M., Valls, M. and Coll, N.S. (2021) 'Blocking intruders: inducible physico-chemical barriers against plant vascular wilt pathogens', *Journal of Experimental Botany*, 72(2), pp. 184–198. Available at: https://doi.org/10.1093/jxb/eraa444.

Kass, E.M. and Jasin, M. (2010) 'Collaboration and competition between DNA double-strand break repair pathways', *FEBS Letters*, 584(17), pp. 3703-3708. Available at: https://doi.org/10.1016/j.febslet.2010.07.057.

Kataoka, T., Hayashi, N., Yamaya, T. and Takahashi, H. (2004a) 'Root-to-shoot transport of sulfate in Arabidopsis. Evidence for the role of SULTR3; 5 as a component of low-affinity sulfate transport system in the root vasculature', *Plant physiology*, 136(4), pp. 4198–4204. Available at: https://doi.org/10.1104/pp.104.045625.

Kataoka, T., Watanabe-Takahashi, A., Hayashi, N., Ohnishi, M., Mimura, T., Buchner, P., Hawkesford, M.J., Yamaya, T. and Takahashi, H. (2004b) 'Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in Arabidopsis', *The Plant Cell*, 16(10), pp. 2693–2704. Available at: https://doi.org//10.1105/tpc.104.023960.

Katayama, K. and Kimura, S. (1987) 'Ecology and protection of bacterial wilt of potato 2. Some control methods and their integration.', *Bull Nagasaki Agric For Exp Stn*, 15, pp. 29-57.

Kawamura, Y., Hase, S., Takenaka, S., Kanayama, Y., Yoshioka, H., Kamoun, S. and Takahashi, H. (2009) 'INF1 elicitin activates jasmonic acid- and ethylene-mediated signalling pathways and induces resistance to bacterial wilt disease in tomato', *Journal of Phytopathology*, 157(5), pp. 287–297. Available at: https://doi.org/10.1111/j.1439-0434.2008.01489.x.

Kawashima, C.G., Yoshimoto, N., Maruyama-Nakashita, A., Tsuchiya, Y.N., Saito, K., Takahashi, H. and Dalmay, T. (2009) 'Sulphur starvation induces the expression of microRNA-395 and one of its target genes but in different cell types', *Plant Journal*, 57(2). Available at: https://doi.org/10.1111/j.1365-313X.2008.03690.x.

Kay, S. and Bonas, U. (2009) 'How Xanthomonas type III effectors manipulate the host plant', *Current opinion in microbiology*, 12(1), pp. 37–43. Available at: https://doi.org/https://doi.org/10.1016/j.mib.2008.12.006.

Kesten, C., Menna, A. and Sánchez-Rodríguez, C. (2017) 'Regulation of cellulose synthesis in response to stress', *Current Opinion in Plant Biology*, 40. Available at: https://doi.org/10.1016/j.pbi.2017.08.010.

Khraiwesh, B., Zhu, J.K. and Zhu, J. (2012) 'Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants', *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, 1819(2), pp. 137–148. Available at: https://doi.org/10.1016/j.bbagrm.2011.05.001.

Kies, P.J. and Hammer, N.D. (2022) 'A Resourceful Race: Bacterial Scavenging of Host Sulfur Metabolism during Colonization', *Infection and Immunity*, pp. e00579-21. Available at: https://doi.org/10.1128/iai.00579-21.

Kim-Lee, H., Moon, J.S., Hong, Y.J., Kim, M.S. and Cho, H.M. (2005) 'Bacterial wilt resistance in the progenies of the fusion hybrids between haploid of potato and Solanum commersonii', *American Journal of Potato Research*, 82, pp. 129–137. Available at: https://doi.org/10.1007/BF02853650.

Kim, D., Langmead, B. and Salzberg, S.L. (2015) 'HISAT: A fast spliced aligner with low memory requirements', *Nature Methods*, 12(4), pp. 357-360. Available at: https://doi.org/10.1038/nmeth.3317.

Kim, J.S. (2018) 'Precision genome engineering through adenine and cytosine base editing', *Nature Plants*, 4(3), pp. 148–151. Available at: https://doi.org/10.1038/s41477-018-0115-z.

Kim, K.S. and Judelson, H.S. (2003) 'Sporangium-Specific Gene Expression in the Oomycete Phytopathogen Phytophthora infestans', *Eukaryotic Cell*, 2(6), pp. 1376–1385. Available at: https://doi.org/10.1128/EC.2.6.1376-1385.2003.

Kinyua, Z.M., Miller, S.A., Ashlina, C. and Subedi, N. (2014) 'Bacterial wilt disease Ralstonia solanacearum: Standard operating procedure for use in diagnostic laboratories', *Version: EA-SOP-RS* [Preprint].

Kondhare, K.R., Malankar, N.N., Devani, R.S. and Banerjee, A.K. (2018) 'Genome-wide transcriptome analysis reveals small RNA profiles involved in early stages of stolon-to-tuber transitions in potato under photoperiodic conditions', *BMC Plant Biology*, 18, pp. 1–19. Available at: https://doi.org/10.1186/s12870-018-1501-4.

Kong, H.G., Bae, J.Y., Lee, H.J., Joo, H.J., Jung, E.J., Chung, E. and Lee, S.W. (2014) 'Induction of the viable but nonculturable state of ralstonia solanacearum by low temperature in the soil microcosm and its resuscitation by catalase', *PLoS ONE*, 9(10), p. e109792. Available at: https://doi.org/10.1371/journal.pone.0109792.

Kreuze, J.F., Souza-Dias, J.A.C., Jeevalatha, A., Figueira, A.R., Valkonen, J.P.T. and Jones, R.A.C. (2019) 'Viral diseases in potato', in *The Potato Crop: Its Agricultural, Nutritional and Social Contribution to Humankind*, pp. 389–430. Available at: https://doi.org/10.1007/978-3-030-28683-5_11.

Kriedemann, P., Loveys, B. and Downton, W. (1975) 'Internal Control of Stomatal Physiology and Photosynthesis. II. Photosynthetic Responses to Phaseic Acid', *Functional Plant Biology*, 2(4), pp. 553–567. Available at: https://doi.org/10.1071/pp9750553.

Kumar, M. and Chakrabarti, S.K. (2017) 'Expression of β-defensin gene in potato confers enhanced resistance to Ralstonia Solanacearum L.', *Defence Life Science Journal*, 3, pp. 15–23. Available at: https://doi.org/10.14429/dlsj.3.11416.

Kumar, R. (2014) 'Role of microRNAs in biotic and abiotic stress responses in crop plants', *Applied biochemistry and biotechnology*, 174(1). Available at: https://doi.org/10.1007/s12010-014-0914-2.

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T. and Felix, G. (2004) 'The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants', *Plant Cell*,

16(12), pp. 3496–3507. Available at: https://doi.org/10.1105/tpc.104.026765.

Kurabachew, H. and Ayana, G. (2017) 'Bacterial Wilt caused by Ralstonia solanacearum in Ethiopia: StatusaAnd Management Approaches: A Review', *International journal of Phytopathology*, 5(3), pp. 107–119. Available at: https://doi.org/10.33687/phytopath.005.03.1829.

Kuźnicki, D., Meller, B., Arasimowicz-Jelonek, M., Braszewska-Zalewska, A., Drozda, A. and Floryszak-Wieczorek, J. (2019) 'BABA-induced DNA methylome adjustment to intergenerational defense priming in potato to Phytophthora infestans', *Frontiers in Plant Science*, 10, p. 650. Available at: https://doi.org/10.3389/fpls.2019.00650.

Laferriere, L.T., Helgeson, J.P. and Allen, C. (1999) 'Fertile Solanum tuberosum + S. commersonii somatic hybrids as sources of resistance to bacterial wilt caused by Ralstonia solanacearum', *Theoretical and Applied Genetics*, 98(8), pp. 1272–1278. Available at: https://doi.org/10.1007/s001220051193.

Latijnhouwers, M., Ligterink, W., Vleeshouwers, V.G.A.A., Van West, P. and Govers, F. (2004) 'A Gα subunit controls zoospore motility and virulence in the potato late blight pathogen Phytophthora infestans', *Molecular Microbiology*, 51(4), pp. 925–936. Available at: https://doi.org/10.1046/j.1365-2958.2003.03893.x.

Lee, Y., Kim, M., Han, J., Yeom, K., Lee, S., Baek, S.H. and Kim, V.N. (2004) 'MicroRNA genes are transcribed by RNA polymerase II', *The EMBO journal*, 23(20), pp. 4051–4060. Available at: https://doi.org/10.1038/sj.emboj.7600385.

Leustek, T., Martin, M.N., Bick, J.-A. and Davies, J.P. (2000) 'Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies', *Annual review of plant biology*, 51(1), pp. 141–165. Available at: https://doi.org/10.1146/annurev.arplant.51.1.141.

Li, F., Pignatta, D., Bendix, C., Brunkard, J.O., Cohn, M.M., Tung, J., Sun, H., Kumar, P. and Baker, B. (2012) 'MicroRNA regulation of plant innate immune receptors', *Proceedings of the National Academy of Sciences of the United States of America*, 109(5), pp. 1790–1795. Available at: https://doi.org/10.1073/pnas.1118282109.

Li, L., Atef, A., Piatek, A., Ali, Z., Piatek, M., Aouida, M., Sharakuu, A., Mahjoub, A., Wang, G., Khan, S., Fedoroff, N. V., Zhu, J.K. and Mahfouz, M.M. (2013) 'Characterization and DNA-binding specificities of Ralstonia TAL-like effectors', *Molecular Plant*, 6(4), pp. 1318–133. Available at: https://doi.org/10.1093/mp/sst006.

Li, L. and Steffens, J.C. (2002) 'Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance', *Planta*, 215, pp. 239–247. Available at: https://doi.org/10.1007/s00425-002-0750-4.

Li, Y., Hu, X., Chen, J., Wang, W., Xiong, X. and He, C. (2017) 'Integrated mRNA and microRNA transcriptome analysis reveals miRNA regulation in response to PVA in potato', *Scientific Reports*, 7(1), p. 16925. Available at: https://doi.org/10.1038/s41598-017-17059-w.

Li, Y., Wang, L., Sun, G., Li, X., Chen, Z., Feng, J. and Yang, Y. (2021) 'Digital gene expression analysis of the response to Ralstonia solanacearum between resistant and susceptible tobacco varieties', *Scientific Reports*, 11(1), p. 3887. Available at: https://doi.org/10.1038/s41598-021-82576-8.

Liao, C.J., Hailemariam, S., Sharon, A. and Mengiste, T. (2022) 'Pathogenic strategies and immune mechanisms to necrotrophs: Differences and similarities to biotrophs and hemibiotrophs', *Current Opinion in Plant Biology*. Available at: https://doi.org/10.1016/j.pbi.2022.102291.

Liu, D., Song, Y., Chen, Z. and Yu, D. (2009) 'Ectopic expression of miR396 suppresses GRF target gene expression and alters leaf growth in Arabidopsis', *Physiologia plantarum*, 136(2), pp. 223–236. Available at: https://doi.org/10.1111/j.1399-3054.2009.01229.x.

Liu, H.H., Tian, X., Li, Y.J., Wu, C.A. and Zheng, C.C. (2008) 'Microarray-based analysis of stress-regulated microRNAs in Arabidopsis thaliana', *RNA*, 14(5), pp. 836–843. Available at: https://doi.org/10.1261/rna.895308.

Liu, J.J., Zhuang, Y., Huang, X.Z., Zhao, D.G. and Zhao, Y.C. (2020) 'Overexpression of cotton laccase gene LAC1 enhances resistance to botrytis cinereal in tomato plants', *International Journal of Agriculture and Biology*, 24(2), pp. 221–228. Available at: https://doi.org/10.17957/IJAB/15.1428.

Liu, T., Yu, Y., Cai, X., Tu, W., Xie, C. and Liu, J. (2016) 'Introgression of bacterial wilt resistance from Solanum melongena to S. tuberosum through asymmetric protoplast fusion', *Plant Cell, Tissue and Organ Culture*, 125(3), pp. 433–443. Available at: https://doi.org/10.1007/s11240-016-0958-9.

Livak, K.J. and Schmittgen, T.D. (2001) 'Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method', *Methods*, 25(4), pp. 402–408. Available at: https://doi.org/10.1006/meth.2001.1262.

Llave, C., Xie, Z., Kasschau, K.D. and Carrington, J.C. (2002) 'Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA', *Science*, 297(5589), pp. 2053–2056. Available at: https://doi.org/10.1126/science.1076311.

Llorente, B., Alonso, G.D., Bravo-Almonacid, F., Rodríguez, V., López, M.G., Carrari, F., Torres, H.N. and Flawiá, M.M. (2011) 'Safety assessment of nonbrowning potatoes: Opening the discussion about the relevance of substantial equivalence on next generation biotech crops', *Plant Biotechnology Journal*, 9(2), pp. 136–150. Available at:

https://doi.org/10.1111/j.1467-7652.2010.00534.x.

Llorente, B., López, M.G., Carrari, F., Asís, R., Di Paola Naranjo, R.D., Flawiá, M.M., Alonso, G.D. and Bravo-Almonacid, F. (2014) 'Downregulation of polyphenol oxidase in potato tubers redirects phenylpropanoid metabolism enhancing chlorogenate content and late blight resistance', *Molecular Breeding*, 34, pp. 2049–2063. Available at: https://doi.org/10.1007/s11032-014-0162-8.

Llorente, B., Rodríguez, V., Alonso, G.D., Torres, H.N., Flawiá, M.M. and Bravo-Almonacid, F.F. (2010) 'Improvement of aroma in transgenic potato as a consequence of impairing tuber browning', *PLoS ONE*, 5(11), p. e14030. Available at: https://doi.org/10.1371/journal.pone.0014030.

Lopez-Gomollon, S., Mohorianu, I., Szittya, G., Moulton, V. and Dalmay, T. (2012) 'Diverse correlation patterns between microRNAs and their targets during tomato fruit development indicates different modes of microRNA actions', *Planta*, 236(6). Available at: https://doi.org/10.1007/s00425-012-1734-7.

Love, M.I., Huber, W. and Anders, S. (2014) 'Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2', *Genome Biology*, 15(12), pp. 1–21. Available at: https://doi.org/10.1186/s13059-014-0550-8.

Lowe-Power, T.M., Jacobs, J.M., Ailloud, F., Fochs, B., Prior, P. and Allen, C. (2016) 'Degradation of the plant defense signal salicylic acid protects Ralstonia solanacearum from toxicity and enhances virulence on tobacco', *mBio*, 7(3), pp. 10–1128. Available at: https://doi.org/10.1128/mBio.00656-16.

Luan, Y., Cui, J., Zhai, J., Li, J., Han, L. and Meng, J. (2015) 'High-throughput sequencing reveals differential expression of miRNAs in tomato inoculated with Phytophthora infestans', *Planta*, 241(6), pp. 1405–1416. Available at: https://doi.org/10.1007/s00425-015-2267-7.

Lulai, E.C. and Corsini, D.L. (1998) 'Differential deposition of suberin phenolic and aliphatic domains and their roles in resistance to infection during potato tuber (Solanum tuberosumL.) wound-healing', *Physiological and Molecular Plant Pathology*, 53(4), pp. 209–222.

Luo, M., Sun, X., Xu, M. and Tian, Z. (2023) 'Identification of miRNAs Involving Potato-Phytophthora infestans Interaction', *Plants*, 12(3), p. 461. Available at: https://doi.org/10.3390/plants12030461.

Lux, A., Morita, S., Abe, J. and Ito, K. (2005) 'An improved method for clearing and staining free-hand sections and whole-mount samples', *Annals of Botany*, 96(6), pp. 989–996. Available at: https://doi.org/10.1093/aob/mci266.

Mahatma, M.K., Thawait, L.K., Jadon, K.S., Thirumalaisamy, P.P., Bishi, S.K., Rathod, K.J.,

Verma, A., Kumar, N. and Golakiya, B.A. (2021) ‘Metabolic profiling for dissection of late leaf spot disease resistance mechanism in groundnut’, *Physiology and Molecular Biology of Plants*, 27(5), pp. 1027–1041. Available at: https://doi.org/10.1007/s12298-021-00985-5.

Maisch, N.A., Bereswill, S. and Heimesaat, M.M. (2022) ‘Antibacterial effects of vanilla ingredients provide novel treatment options for infections with multidrug-resistant bacteria-A recent literature review’, *European Journal of Microbiology and Immunology*, 12(3), pp. 53–62. Available at: https://doi.org/10.1556/1886.2022.00015.

Malcolmson, J.F. (1969) ‘Races of Phytophthora infestans occurring in Great Britain’, *Transactions of the British Mycological Society*, 53(3), pp. 417-IN2. Available at: https://doi.org/10.1016/s0007-1536(69)80099-9.

Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K.A., Dangl, J.L. and Dietrich, R.A. (2000) ‘The transcriptome of Arabidopsis thaliana during systemic acquired resistance’, *Nature Genetics*, 26(4), pp. 403–410. Available at: https://doi.org/10.1038/82521.

Mallory, A.C., Elmayan, T. and Vaucheret, H. (2008) ‘MicroRNA maturation and action-the expanding roles of ARGONAUTEs’, *Current Opinion in Plant Biology*, pp. 560–566. Available at: https://doi.org/10.1016/j.pbi.2008.06.008.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M.A., Toth, I., Salmond, G. and Foster, G.D. (2012) ‘Top 10 plant pathogenic bacteria in molecular plant pathology’, *Molecular Plant Pathology*, 13(6), pp. 614–629. Available at: https://doi.org/10.1111/j.1364-3703.2012.00804.x.

Marin, J., Battistuzzi, F.U., Brown, A.C. and Hedges, S.B. (2017) ‘The Timetree of Prokaryotes: New Insights into Their Evolution and Speciation’, *Molecular biology and evolution*, 34(2), pp. 437–446. Available at: https://doi.org/10.1093/molbev/msw245.

Marraffini, L.A. and Sontheimer, E.J. (2010) ‘CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea’, *Nature Reviews Genetics*, pp. 181–190. Available at: https://doi.org/10.1038/nrg2749.

Martin, C. (1979). 'Sources of resistance to pseudomonas solanacearum,' *Developments in control of potato bacterial diseases,* Lima: International Potato Center, 49–54.

Martin, C. and French, E. (1985) ‘Bacterial wilt of potato: Pseudomonas solanacearum. Technical information Bulletin 13’, *Centro Internacional de la Papa (CIP), Lima, Perú* [Preprint].

Mayer, A.M. (2006) ‘Polyphenol oxidases in plants and fungi: Going places? A review’, *Phytochemistry*, 67(21), pp. 2318–2331. Available at: https://doi.org/10.1016/j.phytochem.2006.08.006.

Meister, G. (2013) ‘Argonaute proteins: Functional insights and emerging roles’, *Nature Reviews Genetics*, pp. 447–459. Available at: https://doi.org/10.1038/nrg3462.

Melhus, I.E. (1915) *Germination and infection with the fungus of the late blight of potato (Phytophthora infestans)*. Agricultural Experiment Station of the University of Wisconsin.

Melo, G.A., Shimizu, M.M. and Mazzafera, P. (2006) ‘Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust’, *Phytochemistry*, 67(3), pp. 277–285. Available at: https://doi.org/10.1016/j.phytochem.2005.11.003.

Miedes, E., Vanholme, R., Boerjan, W. and Molina, A. (2014) ‘The role of the secondary cell wall in plant resistance to pathogens’, *Frontiers in Plant Science*, p. 358. Available at: https://doi.org/10.3389/fpls.2014.00358.

Mierziak, J., Kostyn, K. and Kulma, A. (2014) ‘Flavonoids as important molecules of plant interactions with the environment’, *Molecules*, pp. 16240–16265. Available at: https://doi.org/10.3390/molecules191016240.

Miwa, H. and Okazaki, S. (2017) ‘How effectors promote beneficial interactions’, *Current Opinion in Plant Biology*, 38, pp. 148–154. Available at: https://doi.org/https://doi.org/10.1016/j.pbi.2017.05.011.

Montanelli, C., Stefanini, F.M., Chiari, A., Chiari, T. and Nascari, G. (1995) ‘Variability in the response to Pseudomonas solanacearum of transgenic lines of potato carrying a cecropin gene analogue’, *Potato Research*, 38(4). Available at: https://doi.org/10.1007/BF02357742.

Mullins, E., Milbourne, D., Petti, C., Doyle-Prestwich, B.M. and Meade, C. (2006) ‘Potato in the age of biotechnology’, *Trends in Plant Science*, 11(5), pp. 254–260. Available at: https://doi.org/10.1016/j.tplants.2006.03.002.

Murashige, T. and Skoog, F. (1962) ‘A revised medium for rapid growth and bio assays with tobacco tissue cultures’, *Physiologia plantarum*, 15(3), pp. 473–497. Available at: https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Muthoni, J., Shimelis, H. and Melis, R. (2020) ‘Conventional breeding of potatoes for resistance to bacterial wilt (Ralstonia solanacearum): Any light in the horizon?’, *Australian Journal of Crop Science*, 14(3), pp. 485–494. Available at: https://doi.org/10.21475/ajcs.20.14.03.p2144.

Nakaho, K., Hibino, H. and Miyagawa, H. (2000) ‘Possible mechanisms limiting movement of Ralstonia solanacearum in resistant tomato tissues’, *Journal of Phytopathology*, 148(3), pp. 181–190. Available at: https://doi.org/10.1046/j.1439-0434.2000.00476.x.

Nakaho, K., Inoue, H., Takayama, T. and Miyagawa, H. (2004) ‘Distribution and multiplication of Ralstonia solanacearum in tomato plants with resistance derived from different origins’,

Journal of General Plant Pathology, 70, pp. 115–119. Available at: https://doi.org/10.1007/s10327-003-0097-0.

Nakamura, K., Ishiyama, K., Sheng, H., Ikai, H., Kanno, T. and Niwano, Y. (2015) 'Bactericidal Activity and Mechanism of Photoirradiated Polyphenols against Gram-Positive and -Negative Bacteria', *Journal of Agricultural and Food Chemistry*, 63(35), pp. 7707–7713. Available at: https://doi.org/10.1021/jf5058588.

Natarajan, B., Kalsi, H.S., Godbole, P., Malankar, N., Thiagarayaselvam, A., Siddappa, S., Thulasiram, H. V., Chakrabarti, S.K. and Banerjee, A.K. (2018) 'MiRNA160 is associated with local defense and systemic acquired resistance against Phytophthora infestans infection in potato', *Journal of Experimental Botany*, 69(8), pp. 2023–2036. Available at: https://doi.org/10.1093/jxb/ery025.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. and Jones, J.D.G. (2006) 'A plant miRNA contributes to antibacterial resistance by repressing auxin signaling', *Science*, 312(5772), pp. 436–439. Available at: https://doi.org/10.1126/science.112608.

Nekrasov, V., Wang, C., Win, J., Lanz, C., Weigel, D. and Kamoun, S. (2017) 'Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion', *Scientific Reports*, 7(1), p. 482. Available at: https://doi.org/10.1038/s41598-017-00578-x.

Nicholson, R.L. and Hammerschmidt, R. (1992) 'Phenolic compounds and their role in disease resistance', *Annual review of phytopathology*, 30(1), pp. 369–389. Available at: https://doi.org/https://doi.org/10.1146/annurev.py.30.090192.002101.

Nielsen, L.W. and Haynes, F.L. (1960) 'Resistance in Solanum tuberosum to Pseudomonas solanacearum', *American Potato Journal*, 37(8), pp. 260–267. Available at: https://doi.org/10.1007/BF02855800.

Niu, D., Lii, Y.E., Chellappan, P., Lei, L., Peralta, K., Jiang, C., Guo, J., Coaker, G. and Jin, H. (2016b) 'miRNA863-3p sequentially targets negative immune regulator ARLPKs and positive regulator SERRATE upon bacterial infection', *Nature Communications*, 7(1), p. 11324. Available at: https://doi.org/10.1038/ncomms11324.

Niu, D., Xia, J., Jiang, C., Qi, B., Ling, X., Lin, S., Zhang, W., Guo, J., Jin, H. and Zhao, H. (2016a) 'Bacillus cereus AR156 primes induced systemic resistance by suppressing miR825/825* and activating defense-related genes in Arabidopsis', *Journal of Integrative Plant Biology*, 58(4), pp. 4256–439. Available at: https://doi.org/10.1111/jipb.12446.

O'Brien, J., Hayder, H., Zayed, Y. and Peng, C. (2018) 'Overview of microRNA biogenesis, mechanisms of actions, and circulation', *Frontiers in Endocrinology*, 9(AUG), p. 402. Available at: https://doi.org/10.3389/fendo.2018.00402.

Oerke, E.C. (2006) ‘Crop losses to pests’, *Journal of Agricultural Science*, 144(1), pp. 31–43. Available at: https://doi.org/10.1017/S0021859605005708.

Oliveira, C.M., Ferreira, A.C.S., De Freitas, V. and Silva, A.M.S. (2011) ‘Oxidation mechanisms occurring in wines’, *Food Research International*, 44(5), pp. 1115–1126. Available at: https://doi.org/10.1016/j.foodres.2011.03.050.

Padmanabhan, C., Zhang, X. and Jin, H. (2009) ‘Host small RNAs are big contributors to plant innate immunity’, *Current opinion in plant biology*, 12(4), pp. 465–472. Available at: https://doi.org/https://doi.org/10.1016/j.pbi.2009.06.005.

Pagliarulo, C., De Vito, V., Picariello, G., Colicchio, R., Pastore, G., Salvatore, P. and Volpe, M.G. (2016) ‘Inhibitory effect of pomegranate (Punica granatum L.) polyphenol extracts on the bacterial growth and survival of clinical isolates of pathogenic Staphylococcus aureus and Escherichia coli’, *Food Chemistry*, 190, pp. 824–831. Available at: https://doi.org/10.1016/j.foodchem.2015.06.028.

Pan, X., Chen, J., Yang, A., Yuan, Q., Zhao, W., Xu, T., Chen, B., Ren, M., Geng, R., Zong, Z., Ma, Z., Huang, Z. and Zhang, Z. (2021) ‘Comparative Transcriptome Profiling Reveals Defense-Related Genes Against Ralstonia solanacearum Infection in Tobacco’, *Frontiers in Plant Science*, 12, p. 767882. Available at: https://doi.org/10.3389/fpls.2021.767882.

Panis, F. and Rompel, A. (2020) ‘Identification of the amino acid position controlling the different enzymatic activities in walnut tyrosinase isoenzymes (jr PPO1 and jr PPO2)’, *Scientific Reports*, 10(1), p. 10813. Available at: https://doi.org/10.1038/s41598-020-67415-6.

Park, S., Gupta, R., Krishna, R., Kim, S.T., Lee, D.Y., Hwang, D.J., Bae, S.C. and Ahn, I.P. (2016) ‘Proteome analysis of disease resistance against Ralstonia solanacearum in potato cultivar CT206-10’, *Plant Pathology Journal*, 32(1), p. 25. Available at: https://doi.org/10.5423/PPJ.OA.05.2015.0076.

Patil, V.U., Gopal, J. and Singh, B.P. (2012) ‘Improvement for Bacterial Wilt Resistance in Potato By Conventional and Biotechnological Approaches’, *Agricultural Research*, pp. 299–316. Available at: https://doi.org/10.1007/s40003-012-0034-6.

Peeters, N., Carrère, S., Anisimova, M., Plener, L., Cazalé, A.C. and Genin, S. (2013) ‘Repertoire, unified nomenclature and evolution of the Type III effector gene set in the Ralstonia solanacearum species complex’, *BMC Genomics*, 14(1), pp. 1–19. Available at: https://doi.org/10.1186/1471-2164-14-859.

Peng, A., Chen, S., Lei, T., Xu, L., He, Y., Wu, L., Yao, L. and Zou, X. (2017) ‘Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus’, *Plant Biotechnology Journal*, 15(12), pp. 1509–1519. Available at: https://doi.org/10.1111/pbi.12733.

Peng, J., Wang, P., Fang, H., Zheng, J., Zhong, C., Yang, Y. and Yu, W. (2021) 'Weighted gene co-expression analysis network-based analysis on the candidate pathways and hub genes in eggplant bacterial wilt-resistance: a plant research study', *International Journal of Molecular Sciences*, 22(24), p. 13279. Available at: https://doi.org/10.3390/ijms222413279.

Pennisi, E. (2010) 'Armed and dangerous', *Science*, pp. 804–805. Available at: https://doi.org/10.1126/science.327.5967.804.

Pennisi, E. (2013) 'The CRISPR craze', *Science*, pp. 833–836. Available at: https://doi.org/10.1126/science.341.6148.833.

Pérez-Quintero, Á.L., Quintero, A., Urrego, O., Vanegas, P. and López, C. (2012) 'Bioinformatic identification of cassava miRNAs differentially expressed in response to infection by Xanthomonas axonopodis pv. manihotis', *BMC Plant Biology*, 12, pp. 1–11. Available at: https://doi.org/10.1186/1471-2229-12-29.

Pertea, M., Pertea, G.M., Antonescu, C.M., Chang, T.C., Mendell, J.T. and Salzberg, S.L. (2015) 'StringTie enables improved reconstruction of a transcriptome from RNA-seq reads', *Nature Biotechnology*, 33(3), pp. 290–295. Available at: https://doi.org/10.1038/nbt.3122.

Pessina, S., Pavan, S., Catalano, D., Gallotta, A., Visser, R.G.F., Bai, Y., Malnoy, M. and Schouten, H.J. (2014) 'Characterization of the MLO gene family in Rosaceae and gene expression analysis in Malus domestica', *BMC Genomics*, 15, pp. 1–12. Available at: https://doi.org/10.1186/1471-2164-15-618.

Petre, B., Morin, E., Tisserant, E., Hacquard, S., Da Silva, C., Poulain, J., Delaruelle, C., Martin, F., Rouhier, N., Kohler, A. and Duplessis, S. (2012) 'RNA-Seq of Early-Infected Poplar Leaves by the Rust Pathogen Melampsora larici-populina Uncovers PtSultr3;5, a Fungal-Induced Host Sulfate Transporter', *PLoS ONE*, 7(8), p. e44408. Available at: https://doi.org/10.1371/journal.pone.0044408.

Pfaffl, M.W. (2004) *A-Z of quantitative PCR: Quantification strategies in real-time PCR, A-Z of quantitative PCR*.

Pham, G.M., Hamilton, J.P., Wood, J.C., Burke, J.T., Zhao, H., Vaillancourt, B., Ou, S., Jiang, J. and Robin Buell, C. (2020) 'Construction of a chromosome-scale long-read reference genome assembly for potato', *GigaScience*, 9(9), p. giaa100. Available at: https://doi.org/10.1093/gigascience/giaa100.

Phiri, T.M., Bhattarai, G., Chiwina, K.E., Fan, Q., Xiong, H., Alatawi, I., Dickson, R., Joshi, N.K., Rojas, A., Ling, K.S. and Shi, A. (2024) 'An Evaluation of Bacterial Wilt (Ralstonia solanacearum) Resistance in a Set of Tomato Germplasm from the United States Department of Agriculture †', *Agronomy*, 14(2), p. 350. Available at:

https://doi.org/10.3390/agronomy14020350.

Pihet, M., Vandeputte, P., Tronchin, G., Renier, G., Saulnier, P., Georgeault, S., Mallet, R., Chabasse, D., Symoens, F. and Bouchara, J.P. (2009) 'Melanin is an essential component for the integrity of the cell wall of Aspergillus fumigatus conidia', *BMC Microbiology*, 9, pp. 1–11. Available at: https://doi.org/10.1186/1471-2180-9-177.

Priou, S., Salas, C., De Mendiburu, F., Aley, P. and Gutarra, L. (2001) 'Assessment of latent infection frequency in progeny tubers of advanced potato clones resistant to bacterial wilt: A new selection criterion', *Potato Research*, 44, pp. 359–373. Available at: https://doi.org/10.1007/BF02358596.

Qian, W., Yang, M., Wang, T., Sun, Z., Liu, M., Zhang, J., Zeng, Q., Cai, C. and Li, Y. (2020) 'Antibacterial Mechanism of Vanillic Acid on Physiological, Morphological, and Biofilm Properties of Carbapenem-Resistant Enterobacter hormaechei', *Journal of Food Protection*, 83(4), pp. 576–583. Available at: https://doi.org/10.4315/JFP-19-469.

Raboy, V., Cichy, K., Peterson, K., Reichman, S., Sompong, U., Srinives, P. and Saneoka, H. (2014) 'Barley (Hordeum vulgare L.) low phytic acid 1-1: an endosperm-specific, filial determinant of seed total phosphorus', *Journal of Heredity*, 105(5), pp. 656–665. Available at: https://doi.org/10.1093/jhered/esu044.

Radojičić, A., Li, X. and Zhang, Y. (2018) 'Salicylic acid: A double-edged sword for programed cell death in plants', *Frontiers in Plant Science*, 9, p. 1133. Available at: https://doi.org/10.3389/fpls.2018.01133.

Ranathunge, K., Thomas, R.H., Fang, X., Peterson, C.A., Gijzen, M. and Bernards, M.A. (2008) 'Soybean root suberin and partial resistance to root rot caused by Phytophthora sojae', *Phytopathology*, 98(11), pp. 1179–1189. Available at: https://doi.org/10.1094/PHYTO-98-11-1179.

Rauscher, G.M., Smart, C.D., Simko, I., Bonierbale, M., Mayton, H., Greenland, A. and Fry, W.E. (2006) 'Characterization and mapping of RPi-ber, a novel potato late blight resistance gene from Solanum berthaultii', *Theoretical and Applied Genetics*, 112, pp. 674–687. Available at: https://doi.org/10.1007/s00122-005-0171-4.

Reddick, D. (1934) 'Elimination of potato late blight from North America', *Phytopathology*, 24, pp. 555–557.

Reen, F.J., Gutiérrez-Barranquero, J.A., Parages, M.L. and O'Gara, F. (2018) 'Coumarin: a novel player in microbial quorum sensing and biofilm formation inhibition', *Applied Microbiology and Biotechnology*, 102, pp. 2063–2073. Available at: https://doi.org/10.1007/s00253-018-8787-x.

Remigi, P., Anisimova, M., Guidot, A., Genin, S. and Peeters, N. (2011) 'Functional

diversification of the GALA type III effector family contributes to Ralstonia solanacearum adaptation on different plant hosts', *New Phytologist*, 192(4), pp. 976–987. Available at: https://doi.org/10.1111/j.1469-8137.2011.03854.x.

Richter, C., Dirks, M.E., Gronover, C.S., Prüfer, D. and Moerschbacher, B.M. (2012) 'Silencing and heterologous expression of ppo-2 indicate a specific function of a single polyphenol oxidase isoform in resistance of dandelion (Taraxacum officinale) against Pseudomonas syringae pv. tomato', *Molecular Plant-Microbe Interactions*, 25(2), pp. 200–210. Available at: https://doi.org/10.1094/MPMI-04-11-0082.

Ristaino, J.B., Anderson, P.K., Bebber, D.P., Brauman, K.A., Cunniffe, N.J., Fedoroff, N. V, Finegold, C., Garrett, K.A., Gilligan, C.A. and Jones, C.M. (2021) 'The persistent threat of emerging plant disease pandemics to global food security', *Proceedings of the National Academy of Sciences*, 118(23), p. e2022239118. Available at: https://doi.org/10.1073/pnas.2022239118.

Rodewald, J. and Trognitz, B. (2013) 'Solanum resistance genes against Phytophthora infestans and their corresponding avirulence genes', *Molecular Plant Pathology*, pp. 740–757. Available at: https://doi.org/10.1111/mpp.12036.

Rouached, H., Berthomieu, P., El Kassis, E., Cathala, N., Catherinot, V., Labesse, G., Davidian, J.C. and Fourcroy, P. (2005) 'Structural and functional analysis of the C-terminal STAS (sulfate transporter and anti-sigma antagonist) domain of the Arabidopsis thaliana sulfate transporter SULTR1.2', *Journal of Biological Chemistry*, 280(16), pp. 15976–15983. Available at: https://doi.org/10.1074/jbc.M501635200.

Rupp, J. and Jacobsen, B. (2017) 'Bacterial and fungal diseases of potato and their management', *Extension Bulletin* [Preprint], (January).

Saijo, Y. and Loo, E.P. (2020) 'Plant immunity in signal integration between biotic and abiotic stress responses', *New Phytologist*, 225(1), pp. 87–104. Available at: https://doi.org/10.1111/nph.15989.

Saijo, Y., Loo, E.P. and Yasuda, S. (2018) 'Pattern recognition receptors and signaling in plant–microbe interactions', *The Plant Journal*, 93(4), pp. 592–613. Available at: https://doi.org/10.1111/tpj.13808.

Saito, K. (2000) 'Regulation of sulfate transport and synthesis of sulfur-containing amino acids', *Current Opinion in Plant Biology*, pp. 188–195. Available at: https://doi.org/10.1016/S1369-5266(00)00063-7.

Särkinen, T., Bohs, L., Olmstead, R.G. and Knapp, S. (2013) 'A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): A dated 1000-tip tree', *BMC Evolutionary Biology*, 13, pp. 1–15. Available at: https://doi.org/10.1186/1471-2148-13-

214.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N. and Nelson, A. (2019) 'The global burden of pathogens and pests on major food crops', *Nature Ecology and Evolution*, 3(3), pp. 430–439. Available at: https://doi.org/10.1038/s41559-018-0793-y.

Schaefer, D.G. (2001) 'Gene targeting in physcomitrella patens', *Current Opinion in Plant Biology*, 4(2), pp. 143–150. Available at: https://doi.org/10.1016/S1369-5266(00)00150-3.

Schandry, N., De Lange, O., Prior, P. and Lahaye, T. (2016) 'TALE-Like effectors are an ancestral feature of the ralstonia solanacearum species complex and converge in DNA targeting specificity', *Frontiers in Plant Science*, 7(AUG2016), p. 1225. Available at: https://doi.org/10.3389/fpls.2016.01225.

Schmiediche P. (1983) *Breeding bacterial wilt (Pseudomonas solanacearum) resistant germplasm*. Lima.

Scinto-Madonich, N.J., Baruah, S., Young, S., Vignona, K., Read, A.C., Carpenter, S.C.D., Wang, L., Shi, X., Chang, G. and Piñeros, M.A. (2023) 'Initial characterization of a bacterial leaf streak susceptibility gene suggests it encodes a membrane transporter that influences seed nutrition and germination', *Physiological and Molecular Plant Pathology*, 126, p. 102031. Available at: https://doi.org/https://doi.org/10.1016/j.pmpp.2023.102031.

Seo, J.K., Wu, J., Lii, Y., Li, Y. and Jin, H. (2013) 'Contribution of small RNA pathway components in plant immunity', *Molecular Plant-Microbe Interactions*, pp. 617–625. Available at: https://doi.org/10.1094/MPMI-10-12-0255-IA.

Sequeira, L. and Rowe, P.R. (1969) 'Selection and utilization of solanum phureja clones with high resistance to different strains of pseudomonas solanacearum', *American Potato Journal*, 46(12), pp. 451–462. Available at: https://doi.org/10.1007/BF02862028.

Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., Zhang, K., Liu, J., Xi, J.J., Qiu, J.L. and Gao, C. (2013) 'Targeted genome modification of crop plants using a CRISPR-Cas system', *Nature Biotechnology*, pp. 686–688. Available at: https://doi.org/10.1038/nbt.2650.

Shepherd, L.V.T., Alexander, C.J., Hackett, C.A., McRae, D., Sungurtas, J.A., Verrall, S.R., Morris, J.A., Hedley, P.E., Rockhold, D., Belknap, W. and Davies, H.V. (2015) 'Impacts on the metabolome of down-regulating polyphenol oxidase in potato tubers', *Transgenic Research*, 24, pp. 447–461. Available at: https://doi.org/10.1007/s11248-014-9850-8.

Shi, H., Liu, Y., Ding, A., Wang, W. and Sun, Y. (2023) 'Induced defense strategies of plants against Ralstonia solanacearum', *Frontiers in Microbiology*, p. 1059799. Available at: https://doi.org/10.3389/fmicb.2023.1059799.

Shibagaki, N., Rose, A., McDermott, J.P., Fujiwara, T., Hayashi, H., Yoneyama, T. and Davies,

J.P. (2002) ‘Selenate-resistant mutants of Arabidopsis thaliana identify Sultr1; 2, a sulfate transporter required for efficient transport of sulfate into roots’, *The Plant Journal*, 29(4), pp. 475–486. Available at: https://doi.org/https://doi.org/10.1046/j.0960-7412.2001.01232.x.

Shihan, M.H., Novo, S.G., Le Marchand, S.J., Wang, Y. and Duncan, M.K. (2021) ‘A simple method for quantitating confocal fluorescent images’, *Biochemistry and Biophysics Reports*, 25, p. 100916. Available at: https://doi.org/10.1016/j.bbrep.2021.100916.

Shivaprasad, P. V, Chen, H.-M., Patel, K., Bond, D.M., Santos, B.A.C.M. and Baulcombe, D.C. (2012) ‘A microRNA superfamily regulates nucleotide binding site–leucine-rich repeats and other mRNAs’, *The Plant Cell*, 24(3), pp. 859–874. Available at: https://doi.org/10.1105/tpc.111.095380.

Siri, M.I., Galván, G.A., Quirici, L., Silvera, E., Villanueva, P., Ferreira, F., Franco Fraguas, L. and Pianzzola, M.J. (2009) ‘Molecular marker diversity and bacterial wilt resistance in wild Solanum commersonii accessions from Uruguay’, *Euphytica*, 165, pp. 371–382. Available at: https://doi.org/10.1007/s10681-008-9800-8.

Smargon, A.A., Cox, D.B.T., Pyzocha, N.K., Zheng, K., Slaymaker, I.M., Gootenberg, J.S., Abudayyeh, O.A., Essletzbichler, P., Shmakov, S., Makarova, K.S., Koonin, E. V. and Zhang, F. (2017) ‘Cas13b Is a Type VI-B CRISPR-Associated RNA-Guided RNase Differentially Regulated by Accessory Proteins Csx27 and Csx28’, *Molecular Cell*, 65(4), pp. 618–630. Available at: https://doi.org/10.1016/j.molcel.2016.12.023.

Smith, F.W., Ealing, P.M., Hawkesford, M.J. and Clarkson, D.T. (1995) ‘Plant members of a family of sulfate transporters reveal functional subtypes.’, *Proceedings of the National Academy of Sciences*, 92(20), pp. 9373–9377. Available at: https://doi.org/10.1073/pnas.92.20.9373.

Song, J., Win, J., Tian, M., Schornack, S., Kaschani, F., Ilyas, M., Van Der Hoorn, R.A.L. and Kamoun, S. (2009) ‘Apoplastic effectors secreted by two unrelated eukaryotic plant pathogens target the tomato defense protease Rcr3’, *Proceedings of the National Academy of Sciences of the United States of America*, 106(5). Available at: https://doi.org/10.1073/pnas.0809201106.

Soto-Suárez, M., Baldrich, P., Weigel, D., Rubio-Somoza, I. and San Segundo, B. (2017) ‘The Arabidopsis miR396 mediates pathogen-associated molecular pattern-triggered immune responses against fungal pathogens’, *Scientific reports*, 7(1), p. 44898. Available at: https://doi.org/10.1038/srep44898.

Stevenson, F.J., Akeley, R. V., Newton, G. and Isleib, D. (1965) ‘Monona: A new variety of potato, distinctive for excellent chip color after numerous storage treatments’, *American Potato*

Journal, 42(9). Available at: https://doi.org/10.1007/BF02861153.

Stewart, R.J., Sawyer, B.J.B., Bucheli, C.S. and Robinson, S.P. (2001) 'Polyphenol oxidase is induced by chilling and wounding in pineapple', *Australian Journal of Plant Physiology*, 28(3), pp. 181–191. Available at: https://doi.org/10.1071/pp00094.

Stiekema, W.J., Heidekamp, F., Dirkse, W.G., van Beckum, J., de Haan, P., Bosch, C. ten and Louwerse, J.D. (1988) 'Molecular cloning and analysis of four potato tuber mRNAs', *Plant Molecular Biology*, 11, pp. 255–269. Available at: https://doi.org/10.1007/BF00027383.

Stirnweis, D., Milani, S.D., Jordan, T., Keller, B. and Brunne, S. (2014) 'Substitutions of two amino acids in the nucleotide-binding site domain of a resistance protein enhance the hypersensitive response and enlarge the PM3F resistance spectrum in wheat', *Molecular Plant-Microbe Interactions*, 27(3), pp. 265–276. Available at: https://doi.org/10.1094/MPMI-10-13-0297-FI.

Su'udi, M., Kim, M.G., Park, S.-R., Hwang, D.-J., Bae, S.-C. and Ahn, I.-P. (2011) 'Arabidopsis cell death in compatible and incompatible interactions with Alternaria brassicicola', *Molecules and cells*, 31, pp. 593–601. Available at: https://doi.org/10.1007/s10059-011-2203-z.

Subramanian, S., Fu, Y., Sunkar, R., Barbazuk, W.B., Zhu, J.-K. and Yu, O. (2008) 'Novel and nodulation-regulated microRNAs in soybean roots', *BMC genomics*, 9, pp. 1–14. Available at: https://doi.org/10.1186/1471-2164-9-160.

Sun, K., Schipper, D., Jacobsen, E., Visser, R.G.F., Govers, F., Bouwmeester, K. and Bai, Y. (2022) 'Silencing susceptibility genes in potato hinders primary infection with Phytophthora infestans at different stages', *Horticulture Research*, 9, p. uhab058. Available at: https://doi.org/10.1093/hr/uhab058.

Sun, K., Wolters, A.-M.A., Vossen, J.H., Rouwet, M.E., Loonen, A.E.H.M., Jacobsen, E., Visser, R.G.F. and Bai, Y. (2016a) 'Silencing of six susceptibility genes results in potato late blight resistance', *Transgenic research*, 25, pp. 731–742. Available at: https://doi.org/10.1007/s11248-016-9964-2.

Sun, K., Wolters, A.M.A., Loonen, A.E.H.M., Huibers, R.P., van der Vlugt, R., Goverse, A., Jacobsen, E., Visser, R.G.F. and Bai, Y. (2016b) 'Down-regulation of Arabidopsis DND1 orthologs in potato and tomato leads to broad-spectrum resistance to late blight and powdery mildew', *Transgenic Research*, 25, pp. 123–138. Available at: https://doi.org/10.1007/s11248-015-9921-5.

Sun, Y., Li, P., Deng, M., Shen, D., Dai, G., Yao, N. and Lu, Y. (2017) 'The Ralstonia solanacearum effector RipAK suppresses plant hypersensitive response by inhibiting the activity of host catalases', *Cellular Microbiology*, 19(8), p. e12736. Available at:

https://doi.org/10.1111/cmi.12736.

Sunkar, R., Li, Y.F. and Jagadeeswaran, G. (2012) 'Functions of microRNAs in plant stress responses', *Trends in Plant Science*, 17(4), pp. 196–203. Available at: https://doi.org/10.1016/j.tplants.2012.01.010.

Székely, A., Gulyás, Z., Balogh, E., Payet, R., Dalmay, T., Kocsy, G., & Kalapos, B. (2023) 'Identification of ascorbate-and salicylate-responsive miRNAs and verification of the spectral control of miR395 in Arabidopsis', *Physiologia plantarum*, 175(6), p. e14070.

Takahashi, H., Kopriva, S., Giordano, M., Saito, K. and Hell, R. (2011) 'Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes', *Annual review of plant biology*, 62, pp. 157–184. Available at: https://doi.org/10.1146/annurev-arplant-042110-103921.

Takahashi, H., Watanabe-Takahashi, A., Smith, F.W., Blake-Kalff, M., Hawkesford, M.J. and Saito, K. (2000) 'The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in Arabidopsis thaliana', *The Plant Journal*, 23(2), pp. 171–182. Available at: https://doi.org/10.1046/j.1365-313x.2000.00768.x.

Taneja, S.R. and Sachar, R.C. (1974) 'Stimulation of polyphenol oxidase (monophenolase) activity in wheat endosperm by gibberellic acid, cycloheximide and actinomycin D', *Planta*, 116, pp. 133–142. Available at: https://doi.org/10.1007/BF00380648.

Tang, J., Wang, J., Fang, Q., Wang, E., Yin, H. and Pan, X. (2018) 'Optimizing planting date and supplemental irrigation for potato across the agro-pastoral ecotone in North China', *European Journal of Agronomy*, 98, pp. 82–94. Available at: https://doi.org/10.1016/j.eja.2018.05.008.

Tani, S., Yatzkan, E. and Judelson, H.S. (2004) 'Multiple pathways regulate the induction of genes during zoosporogenesis in Phytophthora infestans', *Molecular Plant-Microbe Interactions*, 17(3), pp. 330–337. Available at: https://doi.org/10.1094/MPMI.2004.17.3.330.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H.-S., Han, B., Zhu, T., Zou, G. and Katagiri, F. (2003) 'Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen Pseudomonas syringae', *The plant cell*, 15(2), pp. 317–330. Available at: https://doi.org/10.1105/tpc.007591.

Taranto, F., Pasqualone, A., Mangini, G., Tripodi, P., Miazzi, M.M., Pavan, S. and Montemurro, C. (2017) 'Polyphenol oxidases in crops: Biochemical, physiological and genetic aspects', *International journal of molecular sciences*, 18(2), p. 377. Available at: https://doi.org/10.3390/ijms18020377.

Thurston, H.D. and Lozano T., J.C. (1968) 'Resistance to bacterial wilt of potatoes in Colombian clones of Solanum Phureja', *American Potato Journal*, 45, pp. 51–55. Available at:

https://doi.org/10.1007/BF02862862.

Thygesen, P.W., Dry, I.B. and Robinson, S.P. (1995) 'Polyphenol oxidase in potato: A multigene family that exhibits differential expression patterns', *Plant Physiology*, 109(2), pp. 525–531. Available at: https://doi.org/10.1104/pp.109.2.525.

Tian, M., Win, J., Song, J., Van Der Hoorn, R., Van Der Knaap, E. and Kamoun, S. (2007) 'A Phytophthora infestans cystatin-like protein targets a novel tomato papain-like apoplastic protease', *Plant Physiology*, 143(1). Available at: https://doi.org/10.1104/pp.106.090050.

Tolessa, E.S. (2018) 'Importance, Nutrient content and Factors Affecting Nutrient Content of Potato', *American Journal of Food, Nutrition and Health*, 3(3), pp. 37–41.

Tolia, N.H. and Joshua-Tor, L. (2007) 'Slicer and the argonautes', *Nature chemical biology*, 3(1), pp. 36–43. Available at: https://doi.org/10.1038/nchembio848.

Tomás-Barberán, F.A. and Espín, J.C. (2001) 'Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables', *Journal of the Science of Food and Agriculture*, 81(9), pp. 853–876. Available at: https://doi.org/10.1002/jsfa.885.

Toruño, T.Y., Stergiopoulos, I. and Coaker, G. (2016) 'Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners', *Annual review of phytopathology*, 54, pp. 419–441. Available at: https://doi.org/10.1146/annurev-phyto-080615-100204.

Toyota, M., Spencer, D., Sawai-Toyota, S., Jiaqi, W., Zhang, T., Koo, A.J., Howe, G.A. and Gilroy, S. (2018) 'Glutamate triggers long-distance, calcium-based plant defense signaling', *Science*, 361(6407), pp. 1112–1115. Available at: https://doi.org/10.1126/science.aat7744.

Tung, P.X., Rasco, E.T., Vander Zaag, P. and Schmiediche, P. (1990a) 'Resistance to Pseudomonas solanacearum in the potato: I. Effects of sources of resistance and adaptation', *Euphytica*, 45(3). Available at: https://doi.org/10.1007/BF00032987.

Tung, P.X., Rasco, E.T., Vander Zaag, P. and Schmiediche, P. (1990b) 'Resistance to Pseudomonas solanacearum in the potato: II. Aspects of host-pathogen-environment interaction', *Euphytica*, 45(3), pp. 212–215. Available at: https://doi.org/10.1007/BF00032988.

Turnbull, D., Wang, H., Breen, S., Malec, M., Naqvi, S., Yang, L., Welsh, L., Hemsley, P., Zhendong, T., Brunner, F., Gilroy, E.M. and Birch, P.R.J. (2019) 'AVR2 targets BSL family members, which act as susceptibility factors to suppress host immunity', *Plant Physiology*, 180(1), pp. 571–581. Available at: https://doi.org/10.1104/pp.18.01143.

Tyagi, B.R., Misra, P.C., Shekhawat, G.S. and Singh, R. (1980) 'Transfer of brown rot resistance

from Solanum microdontum to Solanum tuberosum', *JIPA, Journal of the Indian Potato Association* [Preprint].

Tyagi, S., Kumar, R., Kumar, V., Won, S.Y. and Shukla, P. (2021) 'Engineering disease resistant plants through CRISPR-Cas9 technology', *GM Crops and Food*, 12(1), pp. 125–144. Available at: https://doi.org/10.1080/21645698.2020.1831729.

Ursache, R., Fujita, S., Dénervaud Tendon, V. and Geldner, N. (2021) 'Combined fluorescent seed selection and multiplex CRISPR/Cas9 assembly for fast generation of multiple Arabidopsis mutants', *Plant Methods*, 17(1), pp. 1–14. Available at: https://doi.org/10.1186/s13007-021-00811-9.

Valiñas, M.A., Lanteri, M.L., Ten Have, A. and Andreu, A.B. (2015) 'Chlorogenic Acid Biosynthesis Appears Linked with Suberin Production in Potato Tuber (Solanum tuberosum)', *Journal of Agricultural and Food Chemistry*, 63(19), pp. 4902–4913. Available at: https://doi.org/10.1021/jf505777p.

Van Damme, M., Huibers, R.P., Elberse, J. and Van Den Ackerveken, G. (2008) 'Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew', *Plant Journal*, 54(5), pp. 785–793. Available at: https://doi.org/10.1111/j.1365-313X.2008.03427.x.

Van Elsas, J.D., Kastelein, P., Van Bekkum, P., Van der Wolf, J.M., De Vries, P.M. and Van Overbeek, L.S. (2000) 'Survival of Ralstonia solanacearum biovar 2, the causative agent of potato brown rot, in field and microcosm soils in temperate climates', *Phytopathology*, 90(12), pp. 1358–1366. Available at: https://doi.org/10.1094/PHYTO.2000.90.12.1358.

Van Gelder, C.W.G., Flurkey, W.H. and Wichers, H.J. (1997) 'Sequence and structural features of plant and fungal tyrosinases', *Phytochemistry*, 45(7), pp. 1309–1323. Available at: https://doi.org/10.1016/S0031-9422(97)00186-6.

Van Schie, C.C.N. and Takken, F.L.W. (2014) 'Susceptibility genes 101: How to be a good host', *Annual Review of Phytopathology*, 52, pp. 551–581. Available at: https://doi.org/10.1146/annurev-phyto-102313-045854.

Varkonyi-Gasic, E., Wu, R., Wood, M., Walton, E.F. and Hellens, R.P. (2007) 'Protocol: A highly sensitive RT-PCR method for detection and quantification of microRNAs', *Plant Methods*, 3(1), pp. 1–12. Available at: https://doi.org/10.1186/1746-4811-3-12.

Verma, V., Ravindran, P. and Kumar, P.P. (2016) 'Plant hormone-mediated regulation of stress responses', *BMC Plant Biology*, 16, pp. 1–10. Available at: https://doi.org/10.1186/s12870-016-0771-y.

Villarino, M., Sandín-España, P., Melgarejo, P. and De Cal, A. (2011) 'High chlorogenic and neochlorogenic acid levels in immature peaches reduce monilinia laxa infection by

interfering with fungal melanin biosynthesis', *Journal of Agricultural and Food Chemistry*, 59(7), pp. 3205–3213. Available at: https://doi.org/10.1021/jf104251z.

Vleeshouwers, V.G.A.A., Van Dooijeweert, W., Govers, F., Kamoun, S. and Colon, L.T. (2000) 'The hypersensitive response is associated with host and nonhost resistance to Phytophthora infestans', *Planta*, 210, pp. 853–864. Available at: https://doi.org/10.1007/s004250050690.

Vleeshouwers, V.G.A.A., Raffaele, S., Vossen, J.H., Champouret, N., Oliva, R., Segretin, M.E., Rietman, H., Cano, L.M., Lokossou, A., Kessel, G., Pel, M.A. and Kamoun, S. (2011) 'Understanding and exploiting late blight resistance in the age of effectors', *Annual Review of Phytopathology*, 49, pp. 507–531. Available at: https://doi.org/10.1146/annurev-phyto-072910-095326.

Voinnet, O. (2009) 'Origin, Biogenesis, and Activity of Plant MicroRNAs', *Cell*. Available at: https://doi.org/10.1016/j.cell.2009.01.046.

Vossen, J.H., van Arkel, G., Bergervoet, M., Jo, K.R., Jacobsen, E. and Visser, R.G.F. (2016) 'The Solanum demissum R8 late blight resistance gene is an Sw-5 homologue that has been deployed worldwide in late blight resistant varieties', *Theoretical and Applied Genetics*, 129(9), pp. 1785–1796. Available at: https://doi.org/10.1007/s00122-016-2740-0.

Voytas, D.F. and Gao, C. (2014) 'Precision Genome Engineering and Agriculture: Opportunities and Regulatory Challenges', *PLoS Biology*, 12(6), p. e1001877. Available at: https://doi.org/10.1371/journal.pbio.1001877.

Vurro, M., Bonciani, B. and Vannacci, G. (2010) 'Emerging infectious diseases of crop plants in developing countries: Impact on agriculture and socio-economic consequences', *Food Security*, pp. 113–132. Available at: https://doi.org/10.1007/s12571-010-0062-7.

Wang, G., Kong, J., Cui, D., Zhao, H., Niu, Y., Xu, M., Jiang, G., Zhao, Y. and Wang, W. (2019) 'Resistance against Ralstonia solanacearum in tomato depends on the methionine cycle and the γ-aminobutyric acid metabolic pathway', *Plant Journal*, 97(6), pp. 1032–1047. Available at: https://doi.org/10.1111/tpj.14175.

Wang, K., Kang, L., Anand, A., Lazarovits, G. and Mysore, K.S. (2007b) 'Monitoring in planta bacterial infection at both cellular and whole-plant levels using the green fluorescent protein variant GFPuv: Methods', *New Phytologist*, 174(1), pp. 212–223. Available at: https://doi.org/10.1111/j.1469-8137.2007.01999.x.

Wang, L., Chen, K. and Zhou, M. (2021) 'Structure and function of an Arabidopsis thaliana sulfate transporter', *Nature communications*, 12(1), p. 4455. Available at: https://doi.org/10.1038/s41467-021-24778-2.

Wang, L., Chen, M., Lam, P.Y., Dini-Andreote, F., Dai, L. and Wei, Z. (2022b) 'Multifaceted

roles of flavonoids mediating plant-microbe interactions', *Microbiome*, pp. 1–13. Available at: https://doi.org/10.1186/s40168-022-01420-x.

Wang, L., Gu, X., Xu, D., Wang, W., Wang, H., Zeng, M., Chang, Z., Huang, H. and Cui, X. (2011) 'miR396-targeted AtGRF transcription factors are required for coordination of cell division and differentiation during leaf development in Arabidopsis', *Journal of experimental botany*, 62(2), pp. 761–773. Available at: https://doi.org/10.1093/jxb/erq307.

Wang, W., Liu, J., Mishra, B., Mukhtar, M.S. and McDowell, J.M. (2022a) 'Sparking a sulfur war between plants and pathogens', *Trends in Plant Science*. Available at: https://doi.org/10.1016/j.tplants.2022.07.007.

Wang, Y., Chen, X. and Xiang, C. (2007a) 'Stomatal density and bio-water saving', *Journal of Integrative Plant Biology*, 49(10), pp. 1435–1444. Available at: https://doi.org/10.1111/j.1672-9072.2007.00554.x.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C. and Qiu, J.L. (2014) 'Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew', *Nature Biotechnology*, 32(9), pp. 947–951. Available at: https://doi.org/10.1038/nbt.2969.

Wang, Z., Luo, W., Cheng, S., Zhang, H., Zong, J. and Zhang, Z. (2023). *Ralstonia solanacearum*– a soil borne hidden enemy of plants: research development in management strategies, their action mechanism and challenges, *Frontiers in Plant Science*, 14, p.1141902. 10.3389/fpls.2023.1141902

Watanabe, J.A., Orrillo, M. and Watanabe, K.N. (1999) 'Frequency of potato genotypes with multiple quantitative pest resistance traits in 4x x 2x crosses', *Breeding Science*, 49(2), pp. 53–61. Available at: https://doi.org/10.1270/jsbbs.49.53.

Weigel, D. and Glazebrook, J. (2006) 'Transformation of Agrobacterium Using the Freeze-Thaw Method', *Cold Spring Harbor Protocols*, p. pdb-prot4666. Available at: https://doi.org/10.1101/pdb.prot4666.

Werij, J.S., Kloosterman, B., Celis-Gamboa, C., De Vos, C.H.R., America, T., Visser, R.G.F. and Bachem, C.W.B. (2007) 'Unravelling enzymatic discoloration in potato through a combined approach of candidate genes, QTL, and expression analysis', *Theoretical and Applied Genetics*, 115, pp. 245–252. Available at: https://doi.org/10.1007/s00122-007-0560-y.

Whitaker, J.R. and Lee, C.Y. (1995) 'Recent advances in chemistry of enzymatic browning: an overview', pp. 2–7.

Wolter, F., Klemm, J. and Puchta, H. (2018) 'Efficient in planta gene targeting in Arabidopsis using egg cell-specific expression of the Cas9 nuclease of Staphylococcus aureus', *Plant*

Journal, 94(4), pp. 735–746. Available at: https://doi.org/10.1111/tpj.13893.

Wright, A. V., Nuñez, J.K. and Doudna, J.A. (2016) 'Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering', *Cell*, pp. 29–44. Available at: https://doi.org/10.1016/j.cell.2015.12.035.

Wrighton, K.H. (2018) 'Genetic engineering: Expanding the reach of Cas9', *Nature Reviews Genetics*, pp. 250–251. Available at: https://doi.org/10.1038/nrg.2018.15.

Wu, D., von Roepenack-Lahaye, E., Buntru, M., de Lange, O., Schandry, N., Pérez-Quintero, A.L., Weinberg, Z., Lowe-Power, T.M., Szurek, B., Michael, A.J., Allen, C., Schillberg, S. and Lahaye, T. (2019) 'A Plant Pathogen Type III Effector Protein Subverts Translational Regulation to Boost Host Polyamine Levels', *Cell Host and Microbe*, 26(5), pp. 638–649. Available at: https://doi.org/10.1016/j.chom.2019.09.014.

Wu, F., Guo, Q., Zhang, W. and Jin, W. (2015) 'Identification and analysis of powdery mildew-responsive miRNAs in wheat', *Journal of Phytopathology*, 163(4), pp. 264–270. Available at: https://doi.org/10.1111/jph.12315.

Wu, T., Hu, E., Xu, S., Chen, M., Guo, P., Dai, Z., Feng, T., Zhou, L., Tang, W., Zhan, L., Fu, X., Liu, S., Bo, X. and Yu, G. (2021) 'clusterProfiler 4.0: A universal enrichment tool for interpreting omics data', *Innovation*, 2(3). Available at: https://doi.org/10.1016/j.xinn.2021.100141.

Xi'ou, X., Bihao, C., Guannan, L., Jianjun, L., Qinghua, C., Jin, J. and Yujing, C. (2015) 'Functional Characterization of a Putative Bacterial Wilt Resistance Gene (RE-bw) in Eggplant', *Plant Molecular Biology Reporter*, 33(4), pp. 1058–1073. Available at: https://doi.org/10.1007/s11105-014-0814-1.

Xiang, C., Werner, B.L., Christensen, E.M. and Oliver, D.J. (2001) 'The biological functions of glutathione revisited in Arabidopsis transgenic plants with altered glutathione levels', *Plant Physiology*, 126(2), pp. 564–574. Available at: https://doi.org/10.1104/pp.126.2.564.

Xiao, X.O., Lin, W., Feng, E. and Ou, X. (2023) 'Transcriptome and metabolome response of eggplant against Ralstonia solanacearum infection', *PeerJ*, 11, p. e14658. Available at: https://doi.org/10.7717/peerj.14658.

Xin, M., Wang, Y., Yao, Y., Xie, C., Peng, H., Ni, Z. and Sun, Q. (2010) 'Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (Triticum aestivum L.)', *BMC Plant Biology*, 10(1), pp. 1–11. Available at: https://doi.org/10.1186/1471-2229-10-123.

Xing, H.L., Dong, L., Wang, Z.P., Zhang, H.Y., Han, C.Y., Liu, B., Wang, X.C. and Chen, Q.J. (2014) 'A CRISPR/Cas9 toolkit for multiplex genome editing in plants', *BMC Plant Biology*, 14(1), pp. 1–12. Available at: https://doi.org/10.1186/s12870-014-0327-y.

Xu, X., Xu, Z., Li, Z., Zakria, M., Zou, L. and Chen, G. (2021) 'Increasing resistance to bacterial leaf streak in rice by editing the promoter of susceptibility gene OsSULRT3; 6', *Plant Biotechnology Journal*, 19(6), p. 1101. Available at: https://doi.org/10.1111/pbi.13602.

Xuping, L., Xuejian, L., Guangjin, W., Xianxing, L., Junxin, C. and Shaochang, X. (1996) 'The epidemic law of bacterial wilt in Eucalyptus', *Journal of Central-south Forestry College*, 16(3), pp. 49–55.

Yadav, V., Wang, Z., Wei, C., Amo, A., Ahmed, B., Yang, X. and Zhang, X. (2020) 'Phenylpropanoid pathway engineering: An emerging approach towards plant defense', *Pathogens*, p. 312. Available at: https://doi.org/10.3390/pathogens9040312.

Yamaji, N., Takemoto, Y., Miyaji, T., Mitani-Ueno, N., Yoshida, K.T. and Ma, J.F. (2017) 'Reducing phosphorus accumulation in rice grains with an impaired transporter in the node', *Nature*, 541(7635), pp. 92–95. Available at: https://doi.org/10.1038/nature20610.

Yan, W.X., Chong, S., Zhang, H., Makarova, K.S., Koonin, E. V., Cheng, D.R. and Scott, D.A. (2018) 'Cas13d Is a Compact RNA-Targeting Type VI CRISPR Effector Positively Modulated by a WYL-Domain-Containing Accessory Protein', *Molecular Cell*, 70(2), pp. 327–339. Available at: https://doi.org/10.1016/j.molcel.2018.02.028.

Yang, C., Zhou, Y., Zheng, Y., Li, C., Sheng, S., Wang, J. and Wu, F. (2016) 'Enzymatic modification of chitosan by cinnamic acids: Antibacterial activity against Ralstonia solanacearum', *International Journal of Biological Macromolecules*, 87, pp. 577–585. Available at: https://doi.org/10.1016/j.ijbiomac.2016.03.023.

Ye, H., Zhang, X.-Q., Broughton, S., Westcott, S., Wu, D., Lance, R. and Li, C. (2011) 'A nonsense mutation in a putative sulphate transporter gene results in low phytic acid in barley', *Functional & Integrative Genomics*, 11, pp. 103–110. Available at: https://doi.org/10.1007/s10142-011-0209-4.

Ye, N., Jia, L. and Zhang, J. (2012) 'ABA signal in rice under stress conditions', *Rice*. Available at: https://doi.org/10.1186/1939-8433-5-1.

Yin, K. and Qiu, J.L. (2019) 'Genome editing for plant disease resistance: Applications and perspectives', *Philosophical Transactions of the Royal Society B: Biological Sciences*, p. 20180322. Available at: https://doi.org/10.1098/rstb.2018.0322.

Yin, Z., Xie, F., Michalak, K., Zhang, B. and Zimnoch-Guzowska, E. (2021) 'Reference gene selection for miRNA and mRNA normalization in potato in response to potato virus Y', *Molecular and Cellular Probes*, 55, p. 101691. Available at: https://doi.org/10.1016/j.mcp.2020.101691.

Yogendra, K.N., Dhokane, D., Kushalappa, A.C., Sarmiento, F., Rodriguez, E. and Mosquera, T. (2017) 'StWRKY8 transcription factor regulates benzylisoquinoline alkaloid pathway in

potato conferring resistance to late blight', *Plant Science*, 256, pp. 208–216. Available at: https://doi.org/10.1016/j.plantsci.2016.12.014.

Yoshimoto, N., Takahashi, H., Smith, F.W., Yamaya, T. and Saito, K. (2002) 'Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in Arabidopsis roots', *The Plant Journal*, 29(4), pp. 465–473. Available at: https://doi.org/10.1046/j.0960-7412.2001.01231.x.

Yu, X., Feng, B., He, P. and Shan, L. (2017) 'From chaos to harmony: responses and signaling upon microbial pattern recognition', *Annual review of phytopathology*, 55, pp. 109–137. Available at: https://doi.org/10.1146/annurev-phyto-080516-035649.

Yu, Y., Ye, W., He, L., Cai, X., Liu, T. and Liu, J. (2013) 'Introgression of bacterial wilt resistance from eggplant to potato via protoplast fusion and genome components of the hybrids', *Plant Cell Reports*, 32, pp. 1687–1701. Available at: https://doi.org/10.1007/s00299-013-1480-8.

Yusibov, V.M., Steck, T.R., Gupta, V. and Gelvin, S.B. (1994) 'Association of single-stranded transferred DNA from Agrobacterium tumefaciens with tobacco cells', *Proceedings of the National Academy of Sciences of the United States of America*, 91(8), pp. 2994–2998. Available at: https://doi.org/10.1073/pnas.91.8.2994.

Zafar, K., Khan, M.Z., Amin, I., Mukhtar, Z., Yasmin, S., Arif, M., Ejaz, K. and Mansoor, S. (2020) 'Precise CRISPR-Cas9 Mediated Genome Editing in Super Basmati Rice for Resistance Against Bacterial Blight by Targeting the Major Susceptibility Gene', *Frontiers in Plant Science*, 11, p. 575. Available at: https://doi.org/10.3389/fpls.2020.00575.

Zaidi, S.S. e. A., Mukhtar, M.S. and Mansoor, S. (2018) 'Genome Editing: Targeting Susceptibility Genes for Plant Disease Resistance', *Trends in Biotechnology*, 36(9), pp. 898–906. Available at: https://doi.org/10.1016/j.tibtech.2018.04.005.

Zaidi, S.S. e. A., Tashkandi, M., Mansoor, S. and Mahfouz, M.M. (2016) 'Engineering plant immunity: Using CRISPR/Cas9 to generate virus resistance', *Frontiers in Plant Science*, 7, p. 1673. Available at: https://doi.org/10.3389/fpls.2016.01673.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M.H. and Bahadar, K. (2018) 'Role of secondary metabolites in plant defense against pathogens', *Microbial Pathogenesis*, 124, pp. 198–202. Available at: https://doi.org/10.1016/j.micpath.2018.08.034.

Zeilmaker, T., Ludwig, N.R., Elberse, J., Seidl, M.F., Berke, L., Van Doorn, A., Schuurink, R.C., Snel, B. and Van den Ackerveken, G. (2015) 'DOWNY MILDEW RESISTANT 6 and DMR 6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in Arabidopsis', *The Plant Journal*, 81(2), pp. 210–222. Available at:

https://doi.org/10.1111/tpj.12719.

Zhang, C., Chen, H., Cai, T., Deng, Y., Zhuang, R., Zhang, N., Zeng, Y., Zheng, Y., Tang, R., Pan, R. and Zhuang, W. (2017a) 'Overexpression of a novel peanut NBS-LRR gene AhRRS5 enhances disease resistance to Ralstonia solanacearum in tobacco', *Plant Biotechnology Journal*, 15(1), pp. 39–55. Available at: https://doi.org/10.1111/pbi.12589.

Zhang, J. and Sun, X. (2021) 'Recent advances in polyphenol oxidase-mediated plant stress responses', *Phytochemistry*, 181, p. 112588. Available at: https://doi.org/10.1016/j.phytochem.2020.112588.

Zhang, J., Yang, Y., Zheng, K., Xie, M., Feng, K., Jawdy, S.S., Gunter, L.E., Ranjan, P., Singan, V.R., Engle, N., Lindquist, E., Barry, K., Schmutz, J., Zhao, N., Tschaplinski, T.J., LeBoldus, J., Tuskan, G.A., Chen, J.G. and Muchero, W. (2018) 'Genome-wide association studies and expression-based quantitative trait loci analyses reveal roles of HCT2 in caffeoylquinic acid biosynthesis and its regulation by defense-responsive transcription factors in Populus', *New Phytologist*, 220(2), pp. 502–516. Available at: https://doi.org/10.1111/nph.15297.

Zhang, K., Shi, X., Zhao, X., Ding, D., Tang, J. and Niu, J. (2015) 'Investigation of miR396 and growth-regulating factor regulatory network in maize grain filling', *Acta physiologiae plantarum*, 37, pp. 1–12. Available at: https://doi.org/10.1007/s11738-014-1767-6.

Zhang, R., Marshall, D., Bryan, G.J. and Hornyik, C. (2013) 'Identification and Characterization of miRNA Transcriptome in Potato by High-Throughput Sequencing', *PLoS ONE*, 8(2). Available at: https://doi.org/10.1371/journal.pone.0057233.

Zhang, W., Gao, S., Zhou, Xiang, Chellappan, P., Chen, Z., Zhou, Xuefeng, Zhang, X., Fromuth, N., Coutino, G., Coffey, M. and Jin, H. (2011) 'Bacteria-responsive microRNAs regulate plant innate immunity by modulating plant hormone networks', *Plant Molecular Biology*, 75, pp. 93–105. Available at: https://doi.org/10.1007/s11103-010-9710-8.

Zhang, W., Luo, Y., Gong, X., Zeng, W. and Li, S. (2009) 'Computational identification of 48 potato microRNAs and their targets', *Computational Biology and Chemistry*, 33(1), pp. 84–93. Available at: https://doi.org/10.1016/j.compbiolchem.2008.07.006.

Zhang, Y., Bai, Y., Wu, G., Zou, S., Chen, Y., Gao, C. and Tang, D. (2017b) 'Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat', *Plant Journal*, 91(4), pp. 714–724. Available at: https://doi.org/10.1111/tpj.13599.

Zhang, Z., Wei, L., Zou, X., Tao, Y., Liu, Z. and Zheng, Y. (2008) 'Submergence-responsive microRNAs are potentially involved in the regulation of morphological and metabolic adaptations in maize root cells', *Annals of Botany*, 102(4), pp. 509–519. Available at:

https://doi.org/10.1093/aob/mcn129.

Zhao, C., Wang, H., Lu, Y., Hu, J., Qu, L., Li, Z., Wang, D., He, Y., Valls, M., Coll, N.S., Chen, Q. and Lu, H. (2019) 'Deep sequencing reveals early reprogramming of arabidopsis root transcriptomes upon ralstonia solanacearum infection', *Molecular Plant-Microbe Interactions*, 31(7), pp. 813–827. Available at: https://doi.org/10.1094/MPMI-10-18-0268-R.

Zhao, C., Xia, H., Cao, T., Yang, Y., Zhao, S., Hou, L., Zhang, Y., Li, C., Zhang, X. and Wang, X. (2015) 'Small RNA and Degradome Deep Sequencing Reveals Peanut MicroRNA Roles in Response to Pathogen Infection', *Plant Molecular Biology Reporter*, (33), pp. 1013–1029. Available at: https://doi.org/10.1007/s11105-014-0806-1.

Zhao, H., Frank, T., Tan, Y., Zhou, C., Jabnoune, M., Arpat, A.B., Cui, H., Huang, J., He, Z. and Poirier, Y. (2016b) 'Disruption of Os SULTR 3; 3 reduces phytate and phosphorus concentrations and alters the metabolite profile in rice grains', *New Phytologist*, 211(3), pp. 926–939. Available at: https://doi.org/10.1111/nph.13969.

Zhao, Y., Zhang, C., Liu, W., Gao, W., Liu, C., Song, G., Li, W.X., Mao, L., Chen, B., Xu, Y., Li, X. and Xie, C. (2016a) 'An alternative strategy for targeted gene replacement in plants using a dual-sgRNA/Cas9 design', *Scientific Reports*, 6(1), p. 23890. Available at: https://doi.org/10.1038/srep23890.

Zhou, J., Deng, K., Cheng, Y., Zhong, Z., Tian, L., Tang, X., Tang, A., Zheng, X., Zhang, T. and Qi, Y. (2017) 'CRISPR-Cas9 based genome editing reveals new insights into microRNA function and regulation in rice', *Frontiers in Plant Science*, 8, p. 1598. Available at: https://doi.org/10.3389/fpls.2017.01598.

Zhou, X., Wang, G., Sutoh, K., Zhu, J.-K. and Zhang, W. (2008) 'Identification of cold-inducible microRNAs in plants by transcriptome analysis', *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, 1779(11), pp. 780–788. Available at: https://doi.org/https://doi.org/10.1016/j.bbagrm.2008.04.005.

Zipfel C. (2014) 'Plant pattern-recognition receptors', *Trends in immunology.*, 35(7), pp. 345–351.

Zörb, C., Franzisky, B.L., Lehr, P.P., Kosch, R., Altenbuchinger, M. and Geilfus, C.-M. (2022) 'Impact of nutritional imbalance on guard cell metabolism and stomata regulation under saline hyperosmotic conditions', *Advances in Botanical Research*, 103, pp. 123–138. Available at: https://doi.org/https://doi.org/10.1016/bs.abr.2022.02.011.

Zoteyeva, N., Skrabule, I., Mežaka, I., Vilcāne, D., Usele, G. and Rostoks, N. (2016) 'The impact of R1and R3a genes on tuber resistance to late blight of the potato breeding clones', in *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences.*, pp. 58–63. Available at: https://doi.org/https://doi.org/10.1515/prolas-2016-

0010.

Zuber, H., Davidian, J.C., Aubert, G., Aimé, D., Belghazi, M., Lugan, R., Heintz, D., Wirtz, M., Hell, R., Thompson, R. and Gallardo, K. (2010) 'The seed composition of arabidopsis mutants for the group 3 sulfate transporters indicates a role in sulfate translocation within developing seeds', *Plant Physiology*, 154(2), pp. 913–926. Available at: https://doi.org/10.1104/pp.110.162123.

Zuluaga, A.P., Solé, M., Lu, H., Góngora-Castillo, E., Vaillancourt, B., Coll, N., Buell, C.R. and Valls, M. (2015) 'Transcriptome responses to Ralstonia solanacearum infection in the roots of the wild potato Solanum commersonii', *BMC Genomics*, 16, pp. 1–16. Available at: https://doi.org/10.1186/s12864-015-1460-1.

A2: SUPPLEMENTARY FIGURES AND TABLES

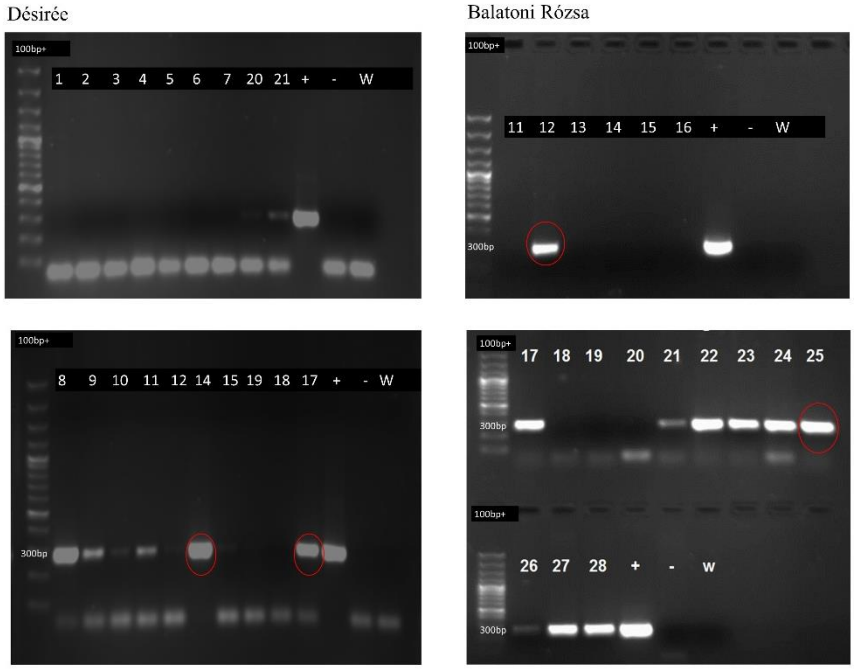


Figure 1. Evaluating the presence of sgRNA-coding transgene on regenerated *PPO* mutant lines (´Désirée' and ´Balatoni Rózsa' genotypes), checked with u6Forward and target lower strand giving a 300-bp product. '+' is positive control, '-' is negative template control and 'W' is water control. The red circles highlight the lines studied in detail from both the genotypes.

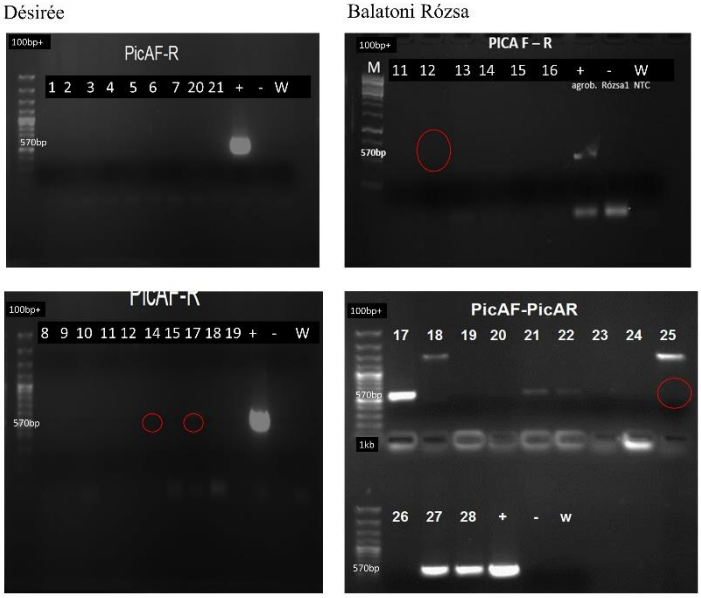


Figure 2. Checking the presence of *Agrobacterium* contamination in the regenerated *PPO* mutant lines (´Désirée' and ´Balatoni Rózsa' genotypes), checked with picaF-R primers giving a 570-bp product. . '+' is positive control, '-' is negative template control and 'W' is water control. The red circles highlight the lines studied in detail from both genotypes.

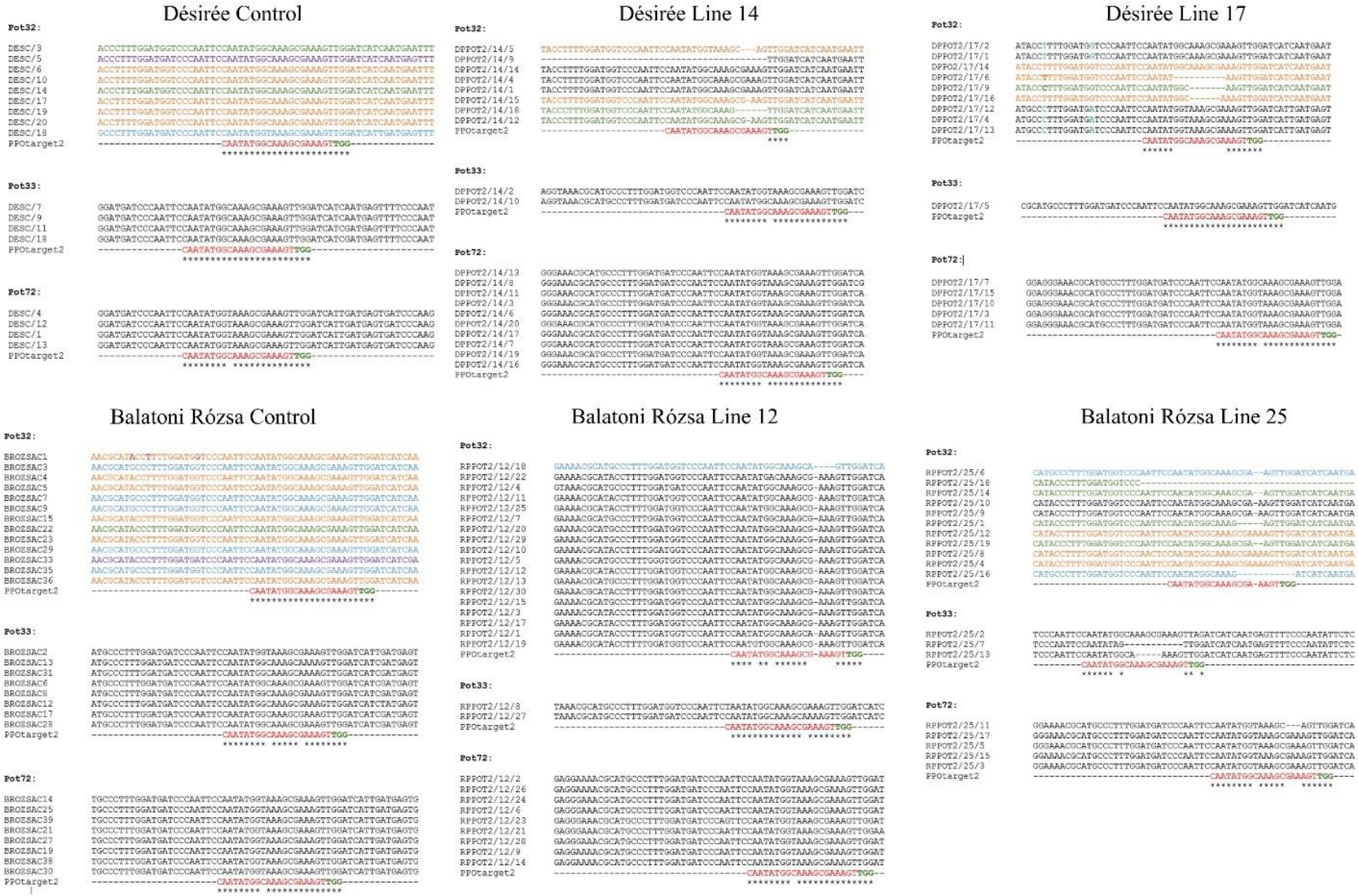
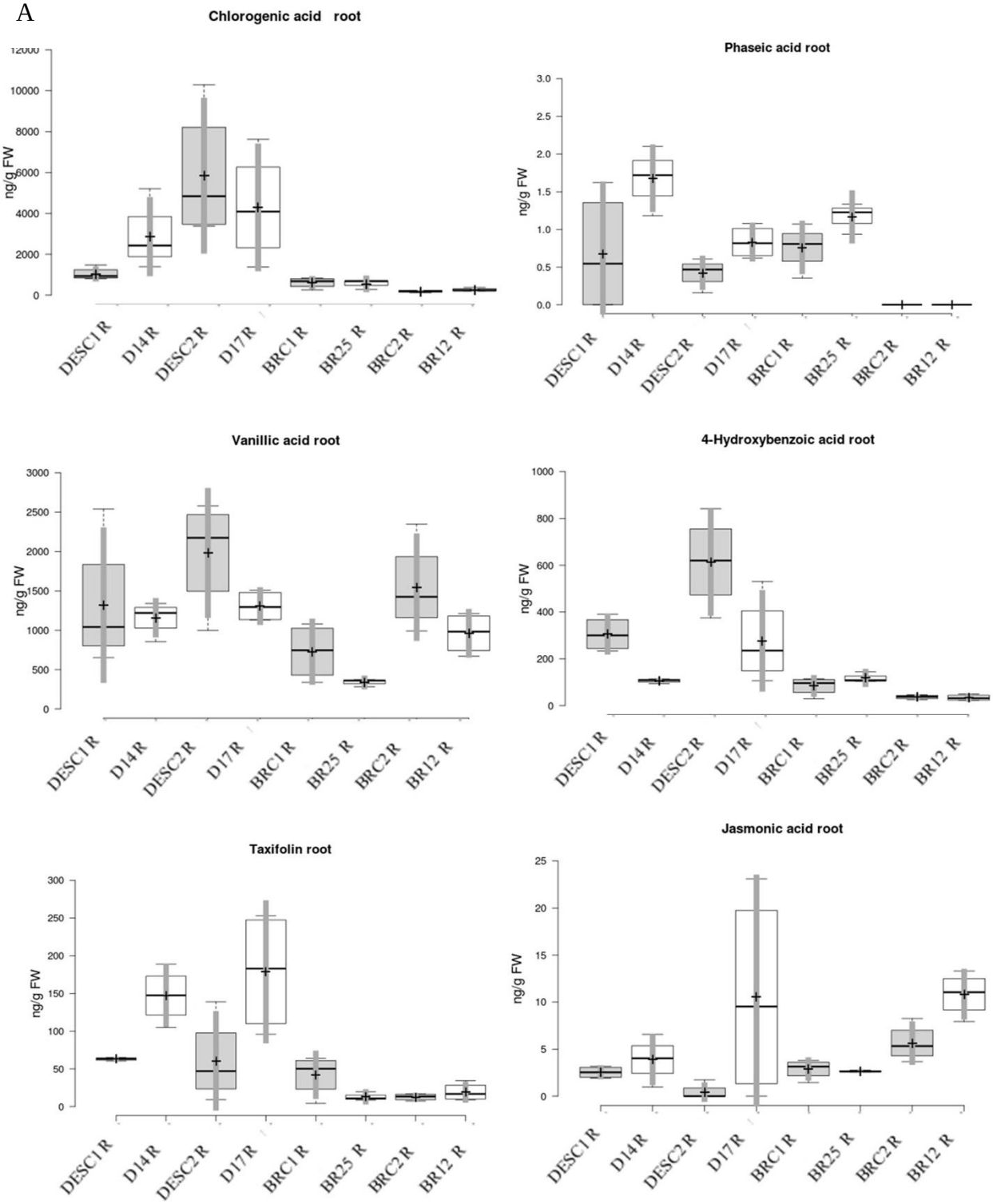
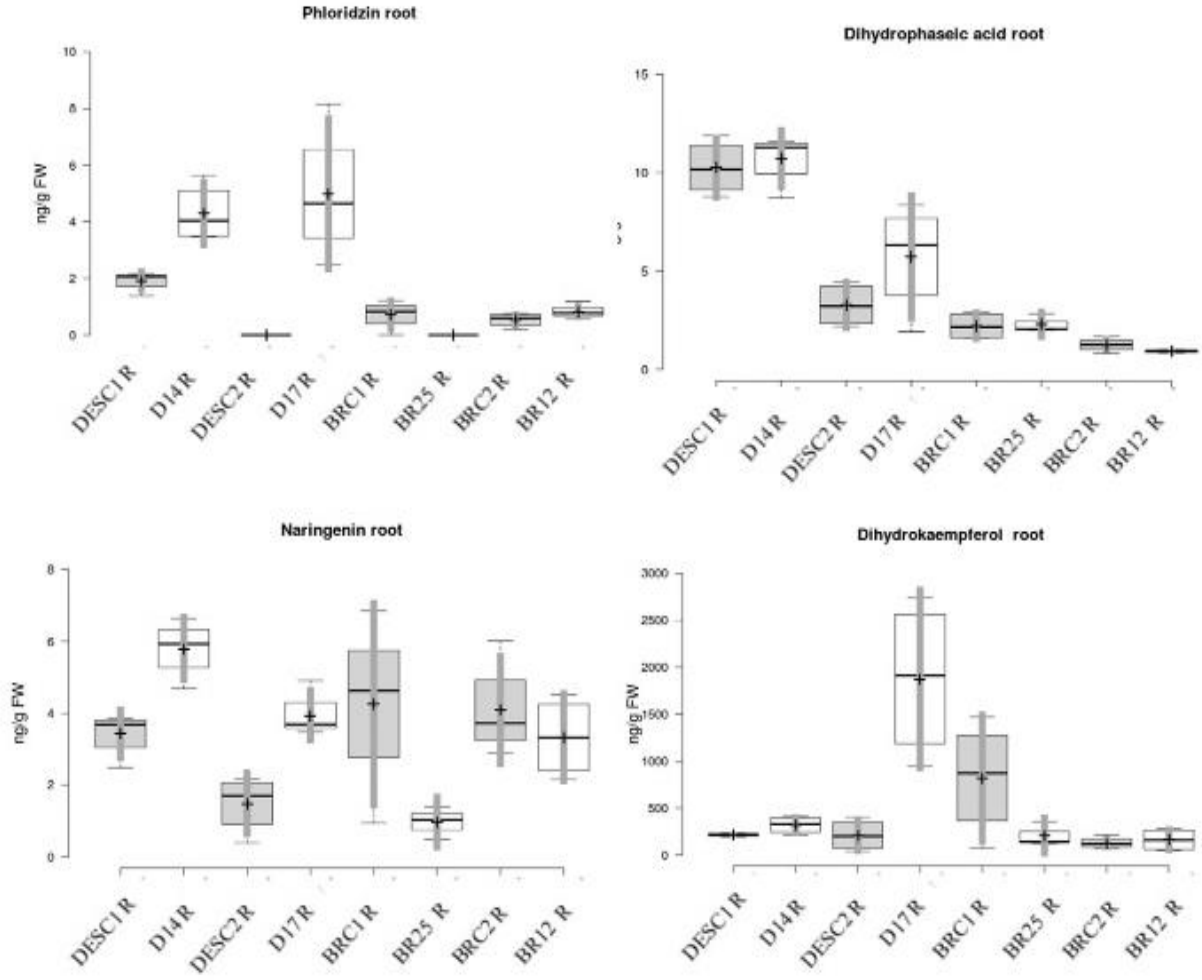


Figure 3. Sequences of *Pot 32*, *Pot33* and *Pot 72 PPO* genes using PPOAllF-R primers listed in Supplementary Table 1 with the highlighted (red) CRISPR target region showing mutations. ´Désirée' control and mutant lines 14 and 17 (top); ´Balatoni Rózsa control and mutant lines 12 and 25 (bottom). The different colours of sequences indicative the four alleles.

A


Chlorogenic acid root
Phaseic acid root
Vanillic acid root
4-Hydroxybenzoic acid root
Taxifolin root
Jasmonic acid root
ng/g FW
DESC1 R
D14 R
DESC2 R
D17 R
BRC1 R
BR25 R
BRC2 R
BR12 R

B


Phloridzin root
ng/g FW
DESC1 R
D14 R
DESC2 R
D17 R
BRC1 R
BR25 R
BRC2 R
BR12 R
Dihydrophaseic acid root
Naringenin root
Dihydrokaempferol root

C

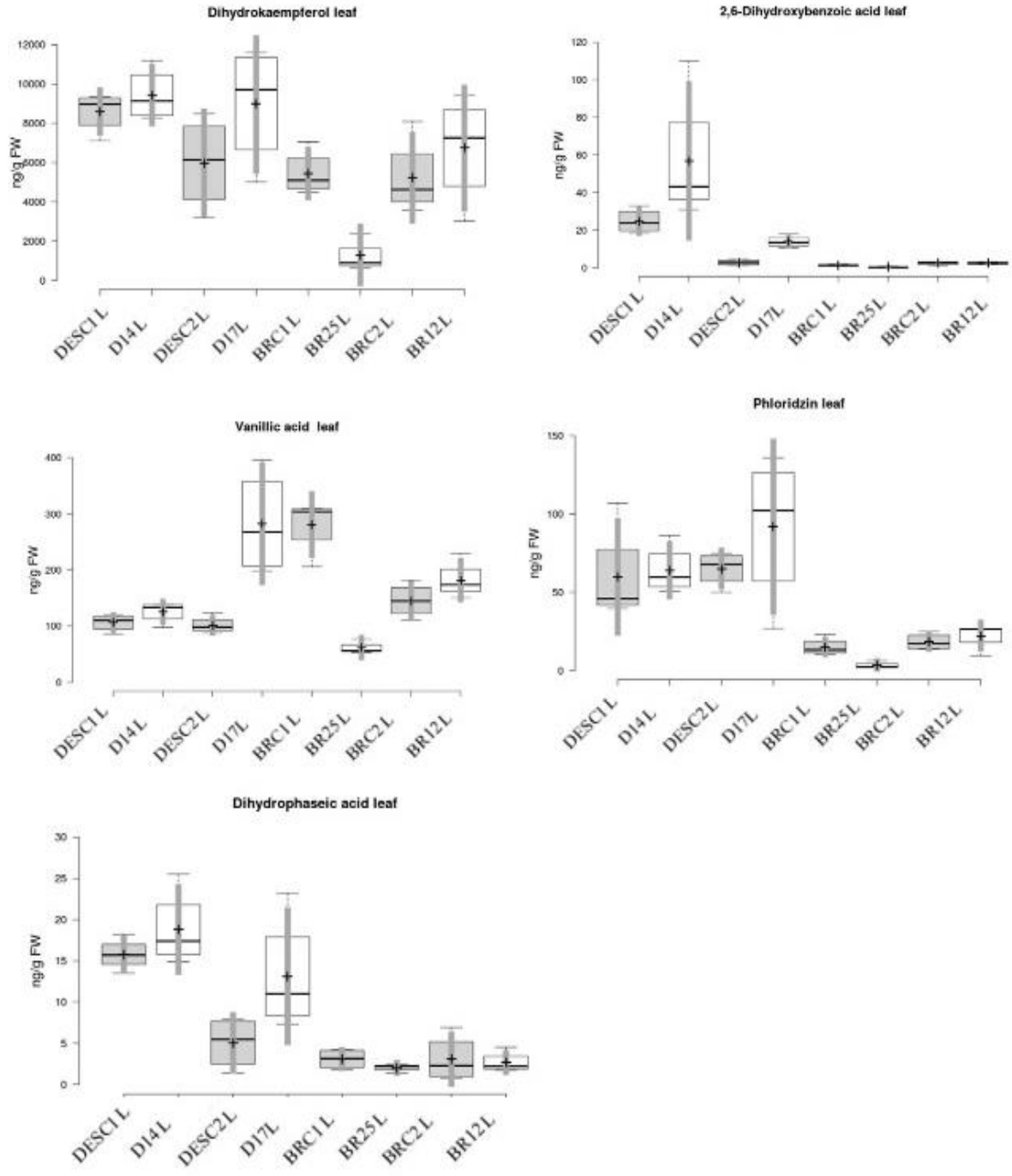


Figure 4. Boxplots of selected secondary metabolites showing concentration differences in the *PPO* mutants (D14 and D17) in comparison to ´Désirée' control (DES C) and *PPO* mutants (BR12 and BR25) along with the control BR C the root (R) metabolites (AB) and leaf (L) metabolites (C)

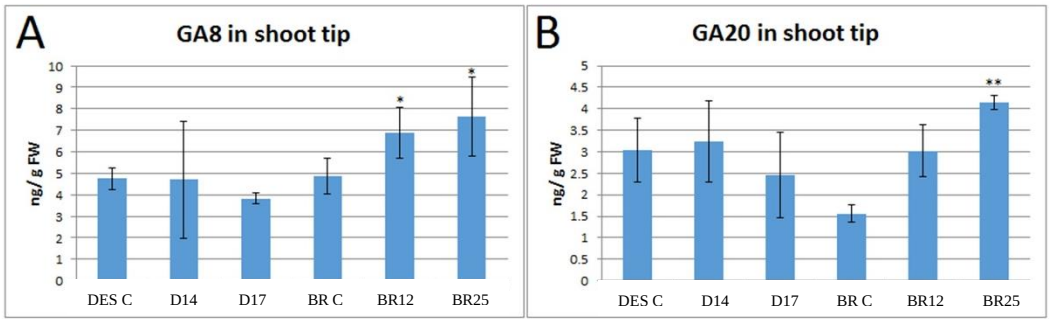


Figure 5. Gibberellic acid measurement in the shoot tips of the *PPO* mutant lines 'Désirée' (D14,D17), 'Balatoni Rózsa' (R12, R25) along with the respective controls (DES C and BR C)

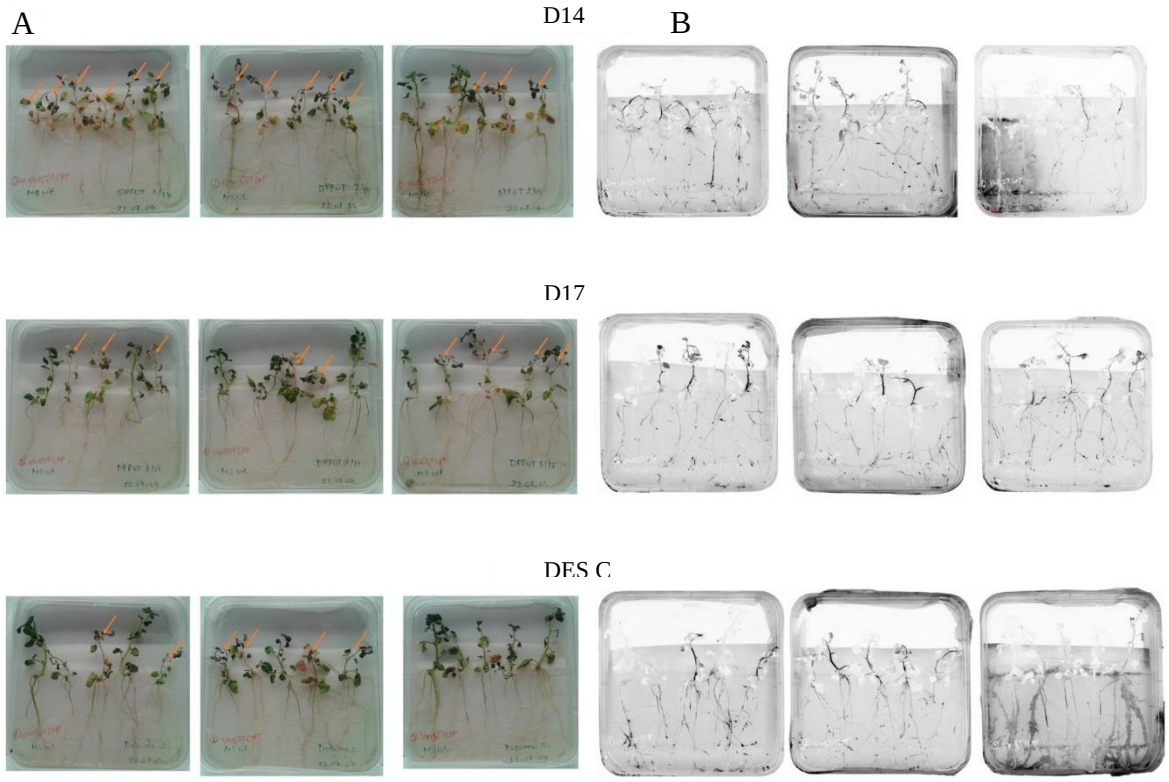


Figure 6. Visual evaluation of 'Désirée' (DES C) and its *PPO* mutants (D14 and D17) in response to *Rs* infection and bacterial spreading (19 dpi) in the *in vitro* inoculation bioassay under visible light for the observation of wilting (A) and UV light for the detection of *GFP*-expressing *Rs* bacteria (B). Arrows point to the wilted plants (Z. Bozsó, NÖVI).

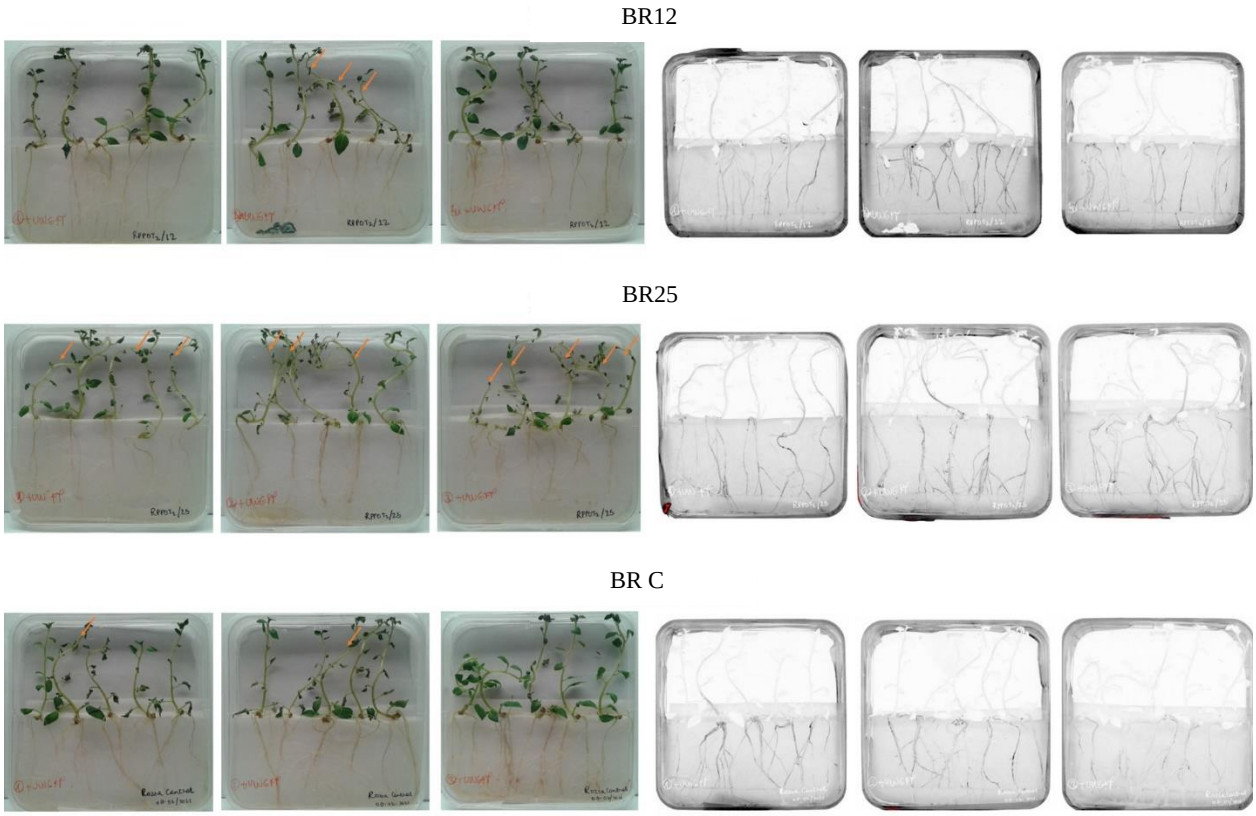


Figure 7. Visual evaluation of ´Balatoni Rózsa' (BR C) and its *PPO* mutants (BR12 and BR25) in response to *Rs* infection and bacterial spreading (13 dpi) in the *in vitro* inoculation bioassay under visible light for the observation of wilting (A) and UV light for the detection of *GFP*-expressing *Rs* bacteria (B). Arrows point to the wilted plants (Z. Bozsó, NÖVI).

A

B

Figure 8. Sequences of region around *miR396* target sites using Chr7mir396F-R primers listed in Supplementary Table 1 with the highlighted CRISPR target region. Red box highlights target 1 and blue box highlights target 2, for ´Botond' control and mutant lines miR396_line 6, 7, 8 and 9 (A) and for ´Désirée' control and mutant lines miR96_Line 3,6 and 10 (B)

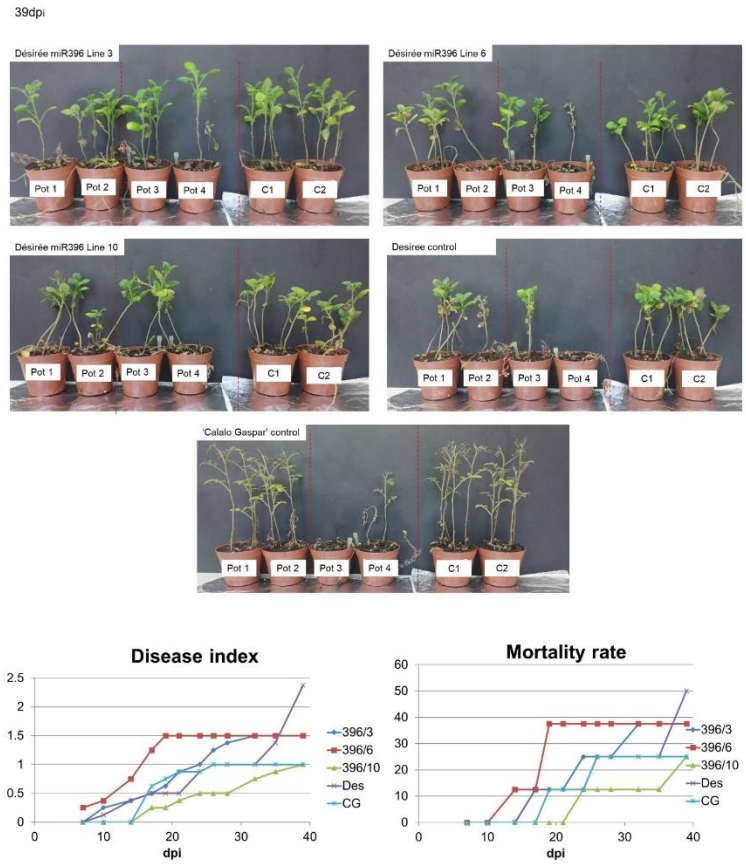


Figure 9. Disease symptoms of whole potato plants of 'Désirée' control and miR396 mutants in an *Rs* inoculation test (OD=0.8 10 ml/plant). Timing in the test: 39 dpi showing the delayed symptom development of Line 3 and Line 10 compared to the control. The plot of disease index and mortality rate of the progression of infection.

Supplementary Table 1. Compilation of different ways of conducting and evaluating *Phytophthora infestans* infection assay with potato explants

Reference	Potato genotype(s)	Potato explant(s)	Pi race/genotype	Inoculum preparation	Incoulation conditions	Evaluation
Huang *et al.* 2005	Differential set (12 clones) F1 seeds from SH83-92-488 (SH) × RH89-039-16 (RH)	Detached leaf: from 6-8-week GH plants	7 isolates (known races); GER8601 (race 1), 99018 (race 1.4), 91001 (race 7), 89148-27 (race 3a.3b.7), 89148-09 (race 0), H30P04 (race 3a.7), and IPO-0 (race 3b)	5x104 zoospores/mL	3rd-5th full leaf, 10 µl inoculum on the abaxial side. The trays were then covered and incubated at a 16-h/8-h day/night photoperiod at 16°C.	6-8 dpi, class 1-5, compatible interactions were scored as class 1 (spreading lesion with massive sporulation).The observation unit was compound leaf (with five leaflets) in vitro: 6-8 dpi, class 1-5, the unit of observation was a jar with five plantlets; disease index, t-test
		Plantlets with 3-4 fully developed leaves		2.5 × 104 spores/ mL	3 largest leaves, 10 µl of a zoospore suspension on adaxial side, kept at 16°C	
Bjor, 1987	cv. Troll and cv. Kerr's Pink, and two lines, CT-78-4-9 and N-80-31-34	Tubers	Mixture of races with different virulence genes	50000 sporangia/mL and held at 12°C for 2- 3 hs	The resultant mix was sprayed by a spray gun onto the tubers at a rate equivalent to 1 mL per tuber. Kept at about 15°C.	After 2 weeks score based on % of the surface with symptoms; score 5 for more than 50% of the surface with symptoms
Brylińska and Śliwka, 2018	Not specified	Tubers of susceptible and resistant standard cultivars		50 sporangia/µL	Two replicates of between three and ten double slices, each 10 mm thick. A drop of 30 µl of the inoculum between the double slices, are placed at 16°C in the dark and covered with glass	
		detached leaflet test: 3-5 fully-expanded lateral leaflets or 1-2 leaves are collected from the middle part of potato plants from 6-week-old plants			Leaflet is placed on wet paper the abaxial side up; Leaves were sprayed with the suspension. Placed at 16°C in the dark with high humidity >80RH. The next day leaves are turned over with constant light of about 1600 lx	Six days after incubation; 6 lateral leaflets are scored per leaf, each leaflet separately. Scoring is performed on a 1-9 scale where 9 means no disease symptoms. Scores 8-7: weak sporulation, 6-3: intense sporulation, 1-2: very intense sporulation.

Cohen, Gisi and Mosinger, 1991	Bintje	Whole plants	Isolate $49 (Sandoz collection)	500 sporangia/mL	Adaxial leaf surfaces: sprayed (about 10 mL per plant) kept in the dark at 100% RH for 24 h and then transferred to a 15 °C chamber (80-90% RH) illuminated with cool white fluorescent light (70 µEm^{-2}s $^{-1}$, 16 h light per day).	After 5 days, lesions on leaves 4-11 of each plant were recorded. The total leaf area blighted per plant was calculated
Dorrance and Inglis, 1997	Bzura Brador Brodick A84118-3 Alpha FR Elba COO83008-1 White Rose Kennebec Russet Burbank Ranger Russet Shepody Red LaSoda NorchipRusset Norkotah	GH whole plants	Isolate 95WWA with virulence genes 1,2,4,5,7	atomised with a suspension of 8,000 sporangia/mL	after spraying, placed in a mist chamber for approximately 40 h; ratings were taken periodically for 1 to 2 weeks	1-2 weeks until the most susceptible plant died estimate the percentage of the plant
		Leaf discs: (15-mm-diameter)		4,000 sporangia/mL for detached leaf	Inoculations with 40 µL at or near flowering; abaxial surface up in moist chambers, incubated for 7 days at 20°C under 12 h	blighted, where 9 = no blight and 1 =100% blighted
		detached leaves: two top fully expanded leaves				
Dorrance and Inglis, 1998	20 cultivars and adv selections	Tuber slice: seven-mm-thick sections from the middle of a tuber	One isolate, 95WWA	8×10^4/mL	The tuber slice on top of two moistened filter papers in a plastic Petri dish, and a 5-mm center of the tuber slice; incubated at 20°C in dark for 7 days colonised agar plug in the center of the tuber slice; incubated at 20°C in dark for 7 days; inoculum atomised onto each tuber; approximately 20 mL of the sporangial suspension was used for every 30 tubers; high RH; 7 days at 20°C	The percent colonisation of a tuber slice calculated which is the percent surface with symptoms of a whole tuber
		Whole tuber: three tubers per cultivar				
Flier, Van Den Bosch and Turkensteen, 2003	Bintje, Asarte and 8 others		Five isolates (IPO98014F95573, IPO655-2A and IPO428-2); IPO82001 serves as a reference isolate	$1{\cdot}0 \times 10^4$ sporangia/mL	When sporulating mycelium was present, small tufts of mycelium were placed in a 10-µL drop of water on the lower epidermis of leaflets; incubated for 7 days in a climate chamber at 15°C with a 16 h light period	Lesions were measured between 3-5 dpi. The length and width of each lesion were measured, and the average diameter, lesion area, and lesion growth rate were calculated. Sporulation was visually assessed using a 0–4 rating scale (0 = no sporulation to 4 = dense sporulation).
Gigot, Gundersen and Inglis, 2009	Russet Burbank	Tubers	'MV04-28', and 'MV110 B'	agar plugs (5 mm in diameter)	Rye agar plugs 5 mm diameter placed in each wound; moist towels for 6 days at 16°C then incubated in the dark in growth chambers set at 10°C for 15 days of incubation	Examined the number of lenticels and the number of eyes with *Pi* sporulation; the percentage of the whole tuber surface with late blight symptoms were assessed
				1×10^3 sporangia/mL		
Zoteyeva *et al.*, 2016		Cut tuber		15000 zoospores/ ml.	tuber surfaces of 7 mm diameter were inoculated with a 20 mL drop of inoculum	

Supplementary Table 2. List of primers used in the study

S no.	Primer name	Sequence (5'-3')	Tm °C	Product size (bp)	Gene name and ID
1	PPOallF	AAATGTTCTTCTTGGCTTAGG	54	1000bp	Polyphenol oxidase: Pot32 (U22921), Pot33 (U22922) andPOT72 (U22923)
2	PPOallR	ACTCGGAGTTCAACCAATC			
3	PPO US	**ATTG**CAATATGGCAAAGCGAAAGT			
4	PPO LS	**AAAC**ACTTTCGCTTTGCCATATTG			
5	U6-26p-F	TGTCCCAGGATTAGAATGATTAGGC	60		
6	U6-26t-R	CCCCAGAAATTGAACGCCGAAGAAC	60		
7	miR396T1-F0	TGATGAAAGTCCAAGAAAGCTGGTTTTAGAGCTAGAAATAGC			
8	miR396T2-R0	AACCAGCTTTCTTGAACTTCTTCCAATCTCTTAGTCGACTCTAC			
9	miR396T1-F1	ATATATGGTCTCGATTGATGAAAGTCCAAGAAAGCTGGTT			-
10	miR396T2-R1	ATTATTGGTCTCGAAACCAGCTTTCTTGAACTTCTTCAA			
11	miR396t1LS	AAACCAGCTTTCTTGGACTTTCAT	60	286	
12	miR396t2LS	AAACCAGCTTTCTTGAACTTCTTC	60	860	
13	PicAF	ATGCGCATGAGGCTCGTCTTCGAG	64	571	PicA (Yusibov et al., 1994)
14	PicAR	GACGCAACGCATCCTCGATCAGCT			
15	zCas9F	GACACGGCAGATCACTAAGC	66	300	
16	zCas9R	GCGATCATCTTCCTCACATC			
17	Stu396F:	TTCCACAGCTTTCTTGAACTTC	64	105	MicroRNA396 on Chromosome 7 (MI0025984)
18	Stu396R:	TTTCCCACAGCTTTCTTGGAC			
19	Chr7mir396F	TTTTCAGACGAAAGGGCATCT	61	459	

20	Chr7mir396R	GTTTTACGCTGGTGTTGATCG			
21	pJET1.2 forward sequencing primer	CGACTCACTATAGGGAGAGCGGC	60		
22	pJET1.2 reverse sequencing primer	AAGAACATCGATTTTCCATGGCAG	60		
23	SULTR3.1F	CTGGCTTTTGTGTCCTCTCTG	60	1197	Sulphate transporter 3.1 (AT3G51895.2)
24	SULTR3.1R	AATTCCAAGAGAGGCTTCGAG			
25	pROK LBb1.3F	ATTTTGCCGATTTCGGAAC	60		
26	SULTR1.2F	CACCTTCCAACTATCAATGGC	60	1168	Sulphate transporter 1.2 (AT1G78000)
27	SULTR1.2R	TTTTAATTGGCGTTGATCGAC			
28	AgH F	GGGCGAATAGGTGAAGATGA	60		Agmatine Hydroxycinnamoyltransferase 1 (Soltu.DM.11G024290.1)
29	AgH R	CGTGTGACTTGGACTTGGATT			
30	PR2 F	CGACCACAATTTCCCCTACA	60	162	Pathogenesis-Related Protein Sth-2 (Soltu.DM.09G027710.1)
31	PR2 R	TTGGACCACCTTCAACAAAG			
32	WIN F	ATCAACCCCAACTCCATCTG	60	170	Wound-Induced Protein Win1 (Soltu.DM.01G036460.1)
33	WIN R	CACAGAAAGCAGTCCAACCA			
34	ABs F	CGGTATGGGTATGGGTACTGG	60	153	Abscisic Acid And Environmental Stress-Inducible Protein Tas14 (Soltu.DM.02G024670.1)
35	ABs R	CCTCCTCCTGGCATCTTCTG			
36	AscP F	GGAGTAGTTGCTGTTGAAGTTACG	60	192	L-Ascorbate Peroxidase 2 (Soltu.DM.09G006560.1)
37	AscP R	CAGAGTGTGACCACCAGATAAAG			
38	Elf 1a F	AAACGGATATGCTCCAGTGC	60	87	Elongation factor 1 alpha (AB061263.1)
39	Elf 1a R	GAACGCCTGTCAATCTTGGT			
40	B- tub F	TTGGACAGTCTGGTGCTGG	60	108	Beta-tubulin (DQ228319.1)
41	B- tub R	CACAATTCTCCGCTTCTTTACG			
42	stu-miR396 LS	GCGGCGGTTCCACAGCTTTCTT	62	67	

43	stu-miR396 PS	GCGGCGGTCCAAGAAAGCTGT	62	66	
44	stu-miR R	ATCCAGTGCAGGGTCCGAGG		no product	
45	DNA meF	GCCCCTTTGGAACCTGATGA	62	69	DNA methyltransferase PGSC0003DMT400018502
46	DNA meR	ACCTCCTCCCCTTGTGTGAT			
47	emp70 F	TCTTTTCCCCTGTCCCTCCT	62	57	Endomembrane protein emp70 PGSC0003DMT400080455
48	emp70 R	TGGCGTACCACTCCAGTTTC			
49	GRIP F	ACCAAGGAATCTCCCCCTGA	62	98	GRIP and coiled-coil domain-containing protein PGSC0003DMT400046956
50	GRIP R	TTTCCAGCCTCTGTGTGGAC			
51	LRR F	TTACGGCTCAGTTTCTGCGT	60	68	LRR receptor-like serine/threonine-protein kinase PGSC0003DMT400054175
52	LRR R	CGGGAGAGATCCTGTCAAGG			
53	Multicys1 F	CGGCAACTGATGATGCTGGA	60	162	Multicystatin PGSC0003DMT400015250
54	Multicys1 R	AGTCCTCCCATTCTTTCACCC			
55	Multicys2 F	CCAAACCCAAACAGCCCC	60	101	Multicystatin PGSC0003DMG400026899 transcript PGSC0003DMT400069145
56	Multicys2 R	ACTCCAAATGAGCATTCTGAAAAG			
57	Multicys3 F	TTGTTCCATTCCCAAACAGTCCT	62	124	Multicystatin PGSC0003DMG400026899 transcript PGSC0003DMT400069147
58	Multicys3 R	ACAACCTGTTCCTTCAAATTCAAAAC			
59	Nbs F	GAAGGGCTTCCTTTGGCTCT	62	116	Nbs-lrr resistance protein PGSC0003DMG400007999
60	Nbs R	TGAGACTCGCAGACAGGTTG			
61	K trans F	AACTCTAGCTGCCGTGGTTG	60	91	Potassium transporter 17 PGSC0003DMT400006057
62	K trans R	TCGAGGGAAGCATCCAAGAG			
63	disul F	GGGTTCCCGACTTTGCTTCT	62	65	Protein disulfide isomerase PGSC0003DMT400006756
64	disul R	GCGGAGAATCCACCAGTGTAA			

Supplementary Table 3. Summary of of RNA integrity and RNA-seq quality in all samples

Sample	Integrity value (RIN)	Raw reads	Clean reads	Clean read rate %	Phred Q30(%)	Total mapped reads	Uniquely mapped reads	Total mapping rate %	Unique mapping rate %
CG_CTR1	9.6	46621895	45428436	97.4	93.35	82597562	80328822	90.91	88.41
CG_CTR2	9.4	54807337	53256280	97.2	93.19	96817646	94201598	90.9	88.44
CG_CTR3	9.3	42103939	40978890	97.3	92.93	74434996	72417793	91.82	88.36
CG_INF1	8.9	44143166	42861092	97.1	93.43	78327841	76105235	91.37	88.78
CG_INF2	9.2	41043870	39418633	96	93.63	71729042	69361458	90.98	87.98
CG_INF3	9.4	40899960	39827878	97.4	93.27	72592883	70606984	91.13	88.64
CR_CTR1	9.5	51584521	50347319	97.6	93.25	90237224	87707091	89.61	87.1
CR_CTR2	9.7	42264462	40850710	96.7	93.56	73253138	71188229	89.66	87.13
CR_INF1	9.2	43188342	42052798	97.4	93.1	75480149	73373886	89.74	87.24
CR_INF2	9.5	44806895	43539755	97.2	93.46	78614732	76220491	90.28	87.53
DES_CTR1	8.8	44413115	43106430	97.1	93.52	75830768	73785862	87.96	85.59
DES_CTR2	9.3	54297374	52042644	95.8	93.46	92377746	89861607	88.75	86.33
DES_CTR3	9.5	49726945	48539574	97.6	93.19	85604141	83220354	88.18	85.72
DES_INF1	9.1	49276161	47707909	96.8	93.43	83881741	81472455	87.91	85.39
DES_INF2	9.5	42712405	41694414	97.6	93.42	73499482	71478873	88.14	85.72
DES_INF3	9.7	40287178	39185699	97.3	93.46	69011905	67054493	88.06	85.56

Supplementary Table 4. Comparison (RNA-seq vs. qPCR) of the relative expression of five target genes used for validation

Gene name	CG (log2FoldChange)		CR (log2FoldChange)		DES (log2FoldChange)	
	CG RNA-seq	CG qPCR	CR RNA-seq	CR qPCR	DES RNA-seq	DES qPCR
AGMATINE HYDROXYCINNAMOYLTRANSFERASE 1	2.34	1.09	3.39	3.98	2.62	1.29
PATHOGENESIS-RELATED PROTEIN, STH-2	1.98	0.97	2.96	3.15	2.17	1.59
WOUND-INDUCED PROTEIN, WIN1	3.52	2.91	4.54	4.15	4.20	3.15
ABSCISIC ACID AND ENVIRONMENTAL STRESS-INDUCIBLE PROTEIN, TAS14	-1.99	-2.43	-2.03	-1.08	-1.79	-0.91
L-ASCORBATE PEROXIDASE 2	-1.30	-1.75	-1.29	-0.83	-1.02	-0.71

Supplementary Table 5. Targeted metabolite analysis of potato root samples of the resistant lines CG and CR together with the susceptible 'Désirée', both control (CTR) and 6dpi (INF) in 4 replicates

Sample	CR_CTR1	CR_CTR2	CR_CTR3	CR_CTR4	CR_INF1	CR_INF2	CR_INF3	CR_INF4	CG_CTR1	CG_CTR2	CG_CTR3	CG_CTR4	CG_INF1	CG_INF2	CG_INF3	CG_INF4	DES_CTR1	DES_CTR2	DES_CTR3	DES_CTR4	DES_INF1	DES_INF2	DES_INF3	DES_INF4
indole-3-acetic-a.	14.3	12.4	6.36	6.53	11.5	7.46	11.2	9.73	4.59	4.54	4.67	4.05	5.66	5.44	5.41	5.31	4.59	4.42	4.77	2.87	7.78	5.54	4.6	5.64
benzoic a.	219	181	137	143	158	229	111	191	877	729	709	520	419	284	584	548	219	450	321	330	274	237	260	292
salicylic a.	24.5	21.3	20.1	21.2	57.7	35	32.9	24	42.1	33	29.2	24	39.5	76.7	52.8	94	40.4	50.4	45.8	38.5	166	132	113	124
para-hydroxybenzoic a.	66	68.4	48.2	48.5	54.4	83.2	60	56.2	95.1	81.3	82.5	46.7	69	48.8	95.4	52.8	54.7	43.9	62.9	39.3	52.4	30.3	34.2	42.8
jasmonic a.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.495	0.663	0.379	0.571	1.34	1.84	0.401	1.19
abscisic a.	8.87	8.6	9.13	9.31	6.64	9.64	6.03	8.47	4.72	4.14	4.07	3.54	4.05	4.33	4.11	4.13	5.53	7.47	5.41	3.42	5.08	6.76	6.91	8.77
phaseic a.	0.193	0.166	0.216	0.257	0.149	0.214	0.182	0.163	0.258	0.323	0.304	0.272	0.281	0.344	0.442	0.407	0.415	0.413	0.32	0.282	1.03	2.28	0.97	1.03
dihydrophaseic a.	2.69	2.02	2.54	4.02	0.79	1.69	1.4	1.29	2.76	2.23	2.83	2.24	0.87	0.72	1.76	1.54	5.41	4.63	4.2	3.33	7.84	13.8	8.19	10.8
naringenin	0.328	0.216	0.36	0.657	0.165	0.537	0.331	0.07442	0.151	0.425	0.107	0.07447	0.052	0.1	0.34	0.105	0.197	0.398	0.327	0.343	1.23	0.41	0.781	1.03
taxifolin	12.3	8.49	43.2	47	12	24.1	28.6	3.74	12.1	25.6	54	18.2	14.5	20.7	88.8	49.1	206	428	398	303	481	366	768	618
dihydrokaempferol	6.38	3.74	7.77	11.1	3.94	12.7	10.2	2.44	4.05	14.1	27.9	5.73	2.55	2.98	4.58	8.22	230	662	902	562	445	280	804	1060
chlorogenic a.	854	1100	4060	2900	4280	2700	4920	3000	2790	3770	4500	6880	6460	9920	7840	12700	1060	718	266	298	4410	4340	2510	1090
rutin	0.858	0.909	8.43	6.09	2.2	4.71	8.97	1.56	23.5	15.4	15.4	18.1	97.3	293	289	411	17	6.25	8.4	6.17	130	102	73	103
jasmonic a.-leucin conjugate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
vanillin	173.7	182.2	105.5	105.4	195.8	234.4	141.7	297.5	667.5	583.2	633	706.5	546.1	393.5	911.7	433.2	111.9	160.8	106	93.3	144.7	169.1	136	174.5
vanillic a.	268	115	200	207	152	392	121	188	772	530	447	302	392	158	857	396	385	1143	639	799	299	249	332	476
caffeic a.	103.6	62.1	700.8	607.2	385.1	452.6	438.7	330.7	249.9	204.6	378.4	226	435.9	413.3	781.3	1335.2	231.3	249.8	198.8	147.7	404.8	405.9	722.6	408.3
syringaldehyde	12	9.88	6.15	9.02	38	12.1	9.51	18.8	62.9	40.4	67	71.5	84.9	58.8	162.7	95.5	7.01	12.1	7.74	5.1	14.5	19	13	18.5
scopoletin	0.98	0.0503	1.77	1.29	2.09	7.67	6.47	3.32	1.21	0.688	0.131	0.133	1.34	0.984	2.96	1.45	22.8	42.9	13.4	12.1	8.15	8.73	19	11.1
ferulic a.	237	204	163	159	224	459	181	280	692	492	553	264	343	117	866	147	224	417	314	498	177	143	308	234
esculin	1.83	1.99	4.31	3.72	4.72	6.25	3.31	5.94	1.38	2.6	1.09	4.57	10.26	7.29	5.95	5.84	5.17	8.69	3.05	5.6	8.7	6.73	8.14	5.1
quercetin-3-O-glucoside	<LOQ	0.183	0.33	<LOQ	<LOQ	0.64	0.61	<LOQ	12.05	18.66	30.21	68.58	40.51	52.96	246.48	115.37	6.23	5.31	2.61	2.52	13.86	11.41	25.08	15.17
quercetin-4'-O-glucoside	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

quercetin-3-O-galactoside	1.49	0.9	8.22	4.7	3.26	5.46	6.46	2.4	14.42	9.65	8.6	64.44	70.87	190.99	356.38	286.94	9.57	8.83	5.75	5.87	64.6	49.54	61.17	37.84
kaempferol-3-O-rutinoside	11.8	43.7	78.7	40.7	22.1	44.2	48.6	27.3	38.8	162	127	383	196	300	1694	1297	176	214	281	110	449	563	1803	1363
isorhamnetin-3-O-rutinoside	3.22	28.2	13.97	11.01	6.34	9.83	14.29	4.63	15.44	23.91	27.14	78.15	85.27	116.48	413.81	158.33	22.18	16.16	19.6	15.18	57.66	59.49	139.95	63.94
quercetin-3,4'-diglucoside	10.9	43.9	58.5	33.1	24.6	36.9	46.8	16.7	40.1	47.2	60.2	190	108.6	239.1	915.1	375.8	30.1	29.9	21.9	25.4	89.1	54.4	154.2	85.4
neochlorogenic a.	127	1295.67	2040	1720	1740	763	2210	419	90.2	84.3	145	257	670	1050	912	2120	34	15.2	9.72	0.833	165	87.9	143	29
cryptochlorogenic a.	612	410	5980	4650	4350	3030	3730	1860	1290	1140	1890	3790	4740	7320	8100	15200	89.9	89.2	21.4	31.9	758	346	387	93.5
phloridzin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Supplementary Table 6. Targeted metabolite analysis of potato leaf samples of the resistant lines CG and CR together with the susceptible 'Désirée', both control (CTR) and 6dpi (INF) in replicates

Sample	**CR_CTR1**	**CR_CTR2**	**CR_CTR3**	**CR_CTR4**	**CR_INF1**	**CR_INF2**	**CR_INF3**	**CR_INF4**	**CG_CTR1**	**CG_CTR2**	**CG_CTR3**	**CG_INF1**	**CG_INF2**	**CG_INF3**	**DES_CTR1**	**DES_CTR2**	**DES_CTR3**	**DES_CTR4**	**DES_INF1**	**DES_INF2**	**DES_INF3**	**DES_INF4**
indole-3-acetic-a.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
benzoic a.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
salicylic a.	70.5	37.9	89.6	91.7	78.3	53.9	131	106	37.7	45.7	41.5	22.6	25.2	60.1	110	115	148	135	79.5	110	85	204
para-hydroxybenzoic a.	2.25	3.52	7.55	5.57	6.6	4.98	6.73	6.32	26.8	69	57.9	81	76.2	108	41.8	42.8	22.1	36	13	38.7	17.6	36.4
jasmonic a.	3.09	0.507	6.57	0.422	1.54	1.07	0.372	1.44	2.06	1.59	5.27	0.812	0.0931	0.565	16	3.53	15.3	22.1	2.09	1.25	0.0303	1.12
abscisic a.	4.68	3.47	4.87	5.76	4.76	8.82	4.85	5.88	2.81	4.84	3.84	7.39	5.73	7.5	86.7	58.6	62.9	69.8	172	140	116	154
phaseic a.	2.37	2.14	3.98	3.16	6.75	6.21	4.88	8.22	1.97	2.99	4.08	6.39	4.57	7.04	10.3	12.7	11.7	10.7	31.6	24.3	14.3	27.8
dihydrophaseic a.	1.04	0.475	1.07	1.06	1.9	0.952	0.72	1.54	0.735	0.705	0.971	1.11	0.903	1.67	13	7.58	14.4	15.2	21.9	31.9	10.5	46.2
naringenin	3.93	3.24	3.08	3.72	2.87	5.49	4.78	4.69	0.439	0.526	0.415	1.56	0.955	0.604	1.09	0.948	1.13	0.867	2.41	1.99	1.85	1.83
taxifolin	81.8	84.8	67.3	149	74.7	195	163	169	10.6	24.5	18.5	18	22.4	19.3	2760	1620	3170	2390	1710	1270	938	1620
dihydrokaempferol	89.2	148	62.2	157	99.5	192	171	202	74.9	141	111	122	141	154	1980	1630	1970	1950	1700	1820	1320	1930
chlorogenic a.	14300	25000	23400	25100	23100	24900	24300	25500	30100	24000	21800	27900	26700	12500	4590	6820	5140	4210	14500	7620	5780	4140
rutin	73	39.1	66.4	89.9	65.5	77.9	65.1	76.1	113	123	144	115	179	143	599	250	741	517	297	303	288	370

jasmonic a.-leucin conjugate	2.23	0.744	4.73	0.418	1.06	1.05	0.452	1.13	1.85	1.15	4.79	0.666	0.113	0.208	7.16	1.14	5.48	9.78	1.45	0.359	0.0793	0.592
vanillin	27.40142	17.09751	19.41008	23.7135	59.43398	24.12538	32.11446	50.77227	59.06031	100.5545	115.6577	28.01 89	38.41068	136.0916	13.93792	12.86495	11.04083	6.794138	9.560098	7.244368	9.061427	16.8586
vanillic a.	248.287	424.9343	396.7804	291.9636	221.035	240.2275	224.3309	223.5139	233.8988	146.2557	175.9379	238.0872	164.7418	188.1367	45.29865	57.22993	49.75964	48.35203	41.89441	52.63766	30.4806	31.71939
caffeic a.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
syringaldehyde	103.2951	38.4654	57.33774	91.3663	232.9066	51.33498	80.23058	148.4773	168.114	271.4499	449.2085	106.0303	198.1653	468.7671	13.50167	14.97569	9.805613	18.86356	7.902411	28.72813	12.27001	39.93866
scopoletin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
ferulic a.	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
esculin	68.35255	119.5748	84.78154	70.43083	93.91459	69.99368	102.3292	77.3445	118.0873	69.02405	93.14792	68.31789	58.30247	56.20613	14.39457	21.23636	12.52449	17.27498	27.47676	18.42294	18.00633	18.82259
quercetin-3-O-glucoside	28.69707	69.15261	44.05384	43.37281	43.32237	28.64779	36.10943	47.77425	460.7636	415.222	349.3411	340.1264	333.1584	252.5066	52.96801	48.41122	50.46955	62.66266	129.3543	129.2799	92.3754	112.024
quercetin-4'-O-glucoside	5.995472	16.19492	9.849955	7.801819	9.949464	11.78182	11.27192	10.46593	27.75936	19.87113	13.56135	18.09939	15.96974	11.89677	4.313561	5.415462	3.87494	3.285185	16.27575	8.472209	7.769003	6.927124
quercetin-3-O-galactoside	1771.233	1926.016	1669.445	1979.354	1491.447	1197.72	1250.331	1355.134	5058.533	4779.075	4658.354	3594.905	4297.912	2894.271	541.5221	691.94	609.5723	569.4053	743.1174	904.9713	868.158	864.6692
kaempferol-3-O-rutinoside	5885.599	11748.15	5761.225	5770.631	6651.578	4123.591	3688.053	4819.839	84301.37	71335.45	68661.74	67785.86	59611.98	63955.96	1062.47	3750.674	1048.071	2595.162	2440.526	2328.912	1464.628	1998.551
isorhamnetin-3-O-rutinoside	378.9317	545.4731	413.3872	442.7022	461.1696	317.8073	300.1483	355.3836	2735.185	1450.127	1865.371	2039.334	1922.178	1914.224	100.8337	131.4215	78.90691	130.534	190.177	160.6998	139.6043	169.9694
quercetin-3,4'-diglucoside	4113.137	5029.427	4356.532	5112.895	4122.261	3844.089	2931.099	3851.273	3178.125	2819.86	2344.806	2872.7	2514.011	2118.608	1445.434	1796.835	1682.293	2220.518	2625.274	2461.897	2222.111	2667.658
neochlorogenic a.	35500	102000	65100	62000	57000	55700	56200	46200	107000	45800	28700	57100	65300	12700	1860	4570	1990	1700	9490	2890	4580	1540
cryptochlorogenic a.	38900	71500	57600	59100	56300	52700	51700	56400	68500	52500	38400	56900	48200	18200	6920	11600	8720	7290	23900	11800	9290	7170
phloridzin	85.5694	214.2857	117.4253	138.996	204.0919	155.1654	149.7654	160.7254	654.3052	685.9023	600.418	699.5603	606.9482	684.3086	115.348	176.5487	92.78021	148.098	316.8357	296.7335	126.7771	265.7372

Supplementary Table 7. Browning assay quantificantion of PPO mutants

Tubers							
Genotype	**Désirée Control**	**Désirée Line 14**	**Désirée Line 17**	**Balatoni Rózsa Control**	**Balatoni Rózsa Line 12**	**Balatoni Rózsa Line 25**	
Mean Intensity	62.956	76.274	132.643	76.872	80.813	91.43	
	74.13	83.618	122.292	94.928	111.384	112.488	
	51.125	89.384	129.052	69.046	107.409	107.942	
Average	62.737	83.092	127.9956667	80.282	99.86866667	103.9533333	
Genotype	**Mean**	**St Dev**	**% of reduction in browning**	**Genotype**	**Mean**	**St Dev**	**% of reduction in browning**
Désirée Control	62.737	11.5040635		**Balatoni Rózsa Control**	80.282	13.27367907	
Désirée Line 14	83.092	6.570809083		**Balatoni Rózsa Line 12**	99.86866667	16.62194274	
Désirée Line 17	127.9956667	5.255728335		**Balatoni Rózsa Line 25**	103.9533333	11.08115235	
Degree of browning (255-mean intensity)	Désirée Control	192.263		**Balatoni Rózsa Control**	174.718		
	Désirée Line 14	171.908	**10.9375**	**Balatoni Rózsa Line 12**	155.1313333		**10.91954023**
	Désirée Line 17	127.0043333	**33.85416667**	**Balatoni Rózsa Line 25**	151.0466667		**13.2183908**
Roots							
Genotype	**Désirée Control**	**Désirée Line 14**	**Désirée Line 17**	**Balatoni Rózsa Control**	**Balatoni Rózsa Line 12**	**Balatoni Rózsa Line 25**	
Mean Intensity	151	158	163	139	135	149	
Browning (255-mean intensity)	104	97	92	116	120	106	
	Genotype		**% of reduction in browning**				

Roots browning	**Balatoni Rózsa Control**	116					
	Balatoni Rózsa Line 12	120	3.005462428	*increase			
	Balatoni Rózsa Line 25	106	**8.620689655**				
Roots browning	**Désirée Control**	104					
	Désirée Line 14	97	**7.692307692**				
	Désirée Line 17	92	**11.53846154**				

Supplementary Table 8. Foldchange and p-value calculated for the 26 metabolites quantified for the 'Désirée' and 'Balatoni Rózsa' mutants with respect to their respective controls

Leaves									
Compound	D14Log2FoldChange	D17Log2FoldChange	R12FoldChange	R25FoldChange	**Group**	D14 pvalue	D17 pvalue	R12 pvalue	R25pvalue
Salicylic acid	0.61377977	0.915923812	0.691788713	-0.94134787	phenolic (aromatic) acids	0.3274105	0.0093742	0.0916424	0.0261332
4-Hydroxybenzoic acid	0.29380092	0.723376529	0.562760865	-1.84943054	phenolic (aromatic) acids	0.2733607	0.0265724	0.0169939	0.0154104
2,6-Dihydroxybenzoic acid	0.82939071	2.386296914	-0.003056558	-2.62636876	phenolic (aromatic) acids	0.0337265	0.0019666	0.9930167	0.0171005
Vanillin	0.3704045	0.658963082	-0.40275917	-0.52683845	phenolic (aromatic) aldehyde	0.1795563	0.0633466	0.4297341	0.0727301
Acetovanillone	0.11855892	1.61740481	0.010033687	-0.60802934	phenolic (aromatic) acids	0.6149108	0.0020245	0.9604561	0.0511454
Syringaldehyde	1.18879221	0.473601392	-0.265006263	-0.58634505	phenolic (aromatic) aldehyde	0.002262	0.1507557	0.1745799	0.0579145
Vanillic acid	0.25663663	1.476915929	0.080418682	-2.23054447	phenolic (aromatic) acids	0.021266	0.0281194	0.6076769	0.0022391
Caffeic acid	-0.23463398	#DIV/0!	0.098915026	#DIV/0!	phenolic (aromatic) acids	0.3800236	#DIV/0!	0.8246438	#DIV/0!
Chlorogenic acid	0.11141259	0.030317813	-1.850595097	0.704888888	phenolic (aromatic) acids	0.7440454	0.978687	0.1528631	0.2387453
Cryptochlorogenic acid	0.13922347	0.121284285	-1.355385683	0.786442236	phenolic (aromatic) acids	0.6162688	0.9141798	0.0836168	0.15366
Neochlorogenic acid	-0.35572871	-0.001913383	-0.646126363	0.946228744	phenolic (aromatic) acids	0.3098934	0.998734	0.5006903	0.1451975
Ferulic acid	0.30572513	0.139099008	-0.938029107	-1.15393183	phenolic (aromatic) acids	0.3144653	0.8074474	0.0791872	0.1632773
Ferulic acid isomer	0.26955139	#DIV/0!	-2.352106244	0.358475617	phenolic (aromatic) acids	0.5536372	#DIV/0!	0.1190271	0.4761462
Sinapinic acid	-0.38558065	-1.84375806	-0.069092523	#DIV/0!	phenolic (aromatic) acids	0.4874978	0.4223686	0.9228158	#DIV/0!
Sinapinic acid isomer	0.10591437	2.08054971	-0.046088507	1.363999198	phenolic (aromatic) acids	0.6279972	0.3692486	0.945869	0.0851014
Scopoletin	0.62269515	3.191927043	0.183837802	-3.38695664	coumarins	0.0518905	0.1171922	0.6615076	0.0551284

Fraxin	#DIV/0!	4.619095186	#DIV/0!	#NUM!	coumarins	#DIV/0!	0.2911698	#DIV/0!	0.1803901
Kaempferol-3-O-rutinoside	0.06222031	0.2410081	1.097124349	-1.37265765	flavonol	0.8131255	0.7106749	0.0556362	0.0123728
Isorhamnetin-3-O-rutinoside	1.0148514	0.278642417	0.41241203	-0.95020177	flavonol	0.0417533	0.6184083	0.1722689	0.0605215
Quercetin-3,4'-diglucoside	0.58267386	0.220557475	0.417238945	-2.56798873	flavonol	0.0532924	0.5574184	0.3828361	0.1851249
Dihydrokaempferol	0.13372298	0.579392243	0.902676587	-1.7856716	flavanonol	0.3645335	0.0750793	0.0303129	0.0069581
Taxifolin	-0.32881954	-0.584607026	1.178190616	-0.67755335	flavanonol	0.0409052	0.2545585	0.0345953	0.276127
Phloridzin	0.36682708	0.692203568	0.730929204	-2.14633606	chalcones (dihydrochalcone)	0.0466254	0.0848839	0.0363426	0.0046505
Indole-3-acetic acid	-0.18181514	-0.651320567	-0.260582332	0.057919976	NA	0.2659667	0.2036519	0.5075111	0.7754742
Jasmonic acid	1.05091697	3.527100271	2.464563875	-0.51126847	NA	0.319765	0.0258186	0.0201237	0.4746195
Dihydrophaseic acid	0.25309266	0.638128929	-0.91320437	-0.66110382	isoprenoid	0.2959763	0.1681157	0.3988269	0.1608064
Roots									
Compound	D14Log2FoldChange	D17Log2FoldChange	R12Log2Foldchange	R25Log2FoldChange	**Group**	**pvalue D14**	**pvalued17**	**pvalue R12**	**pvalue R25**
Salicylic acid	0.05069724	0.48566316	-0.554278329	0.267420241	phenolic (aromatic) acids	0.8232385	0.0976214	0.214386	0.5155529
4-Hydroxybenzoic acid	-1.52794802	-1.853829352	-0.117857429	0.024798028	phenolic (aromatic) acids	0.0117649	0.0093503	0.727828	0.8686127
2,6-Dihydroxybenzoic acid	0	1.109988038	0	0.250795756	phenolic (aromatic) acids	#DIV/0!	0.0559373	#DIV/0!	0.6999277
Acetovanillone	1.30854907	3.002947284	0.023660084	-0.83889162	phenolic (aromatic) acids	0.0005892	0.0375602	0.955797	0.3956025
Vanillic acid	0.4668454	-0.897552731	-0.275179836	-1.34940344	phenolic (aromatic) acids	0.1195541	0.0101551	0.3267777	0.0887038
p-Coumaric acid	-0.08766441	-0.719864229	-0.10664256	0.455647002	phenolic (aromatic) acids	0.5955307	0.0438533	0.6842326	0.0115055
Caffeic acid	1.47446613	#DIV/0!	0.002671658	#DIV/0!	phenolic (aromatic) acids	0.0118334	#DIV/0!	0.9899764	#DIV/0!
Chlorogenic acid	1.22686925	0.273856274	0.510405539	-0.40910183	phenolic (aromatic) acids	0.0714024	0.5924642	0.1410739	0.3225704
Cryptochlorogenic acid	2.57087204	0.712373606	0.198621729	-0.35282951	phenolic (aromatic) acids	0.0737996	0.3435568	0.7147628	0.44559
Neochlorogenic acid	2.42388516	1.354567651	-1.058574826	-0.30842059	phenolic (aromatic) acids	0.0863351	0.2343533	0.0053226	0.599059
Coniferyl alcohol	1.29014161	0.860837252	0.11522781	-0.32901119	phenolic (aromatic) alcohol	0.0041417	0.0754856	0.5497948	0.1739586
Sinapinic acid	0.45172195	1.70901098	-0.024825279	0.037303699	phenolic (aromatic) acids	0.252469	0.1036772	0.8489008	0.7819501
Sinapinic acid isomer	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	phenolic (aromatic) acids	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
Esculin	-0.41095632	-0.385371357	0.177738783	0.160323895	coumarins	0.0135493	0.2581282	0.3434316	0.789816
Scopoletin	0.18737587	0.504097498	-0.445770627	0.558513964	coumarins	0.3374712	0.0877134	0.0591181	0.3995069

Kaempferol-3-O-glucoside	0.52270419	1.497738664	-0.090602549	-2.78228211	flavonol	0.1226417	0.134964	0.8752083	0.0023758
Isorhamnetin-3-O-rutinoside	0.41869082	1.628470299	0.366127899	-3.01798575	flavonol	0.0801793	0.0375384	0.2513803	0.0001411
Quercetin-3-O-glucoside	0.5666755	0.340210913	0.20918067	-2.06218428	flavonol	0.0350176	0.3882403	0.5287653	0.1714723
Quercetin-3,4'-diglucoside	0.84357532	0.627783237	0.105810283	-2.40939094	flavonol	0.0034456	0.016397	0.6949642	0.2724203
Rutin	0.46016065	2.866392428	0.53352818	-3.57680837	flavonol	0.0574048	0.1043471	0.337294	0.0122748
Naringenin	0.75932693	0.964538255	-0.29501422	-2.25239243	flavanone	0.0042866	0.0091762	0.4207722	0.0261573
Dihydrokaempferol	0.39627944	3.03562391	-0.303886655	-2.90644109	flavanonol	0.2939689	0.0338505	0.7144111	0.057862
Taxifolin	1.22427152	1.409609455	-0.036355415	-1.76623739	flavanonol	0.0171113	0.0723505	0.9420203	0.0159469
Phloridzin	0.87905639	#DIV/0!	0.626213529	#NUM!	chalcones (dihydrochalcone)	0.038823	0.0239274	0.1492104	0.0672922
Jasmonic acid	1.06457895	#DIV/0!	0.94032075	0.016147423	NA	0.0821698	0.1445542	0.0137553	0.9577098
Phaseic acid	1.31016838	0.971786398	#DIV/0!	0.518811741	isoprenoid	0.0848571	0.0304324	#DIV/0!	0.0366448
Dihydrophaseic acid	0.23099545	1.28824866	-0.424390498	-0.0764507	isoprenoid	0.1091322	0.0161052	0.173861	0.8111994

Supplementary Table 9. Number of wilted plants among *Ralstonia-solanacearum*-infected 'Désirée' (at 19 DPI) and 'Balatoni Rózsa' (at 13 DPI) potato plants and Bacterial quantification

	Experiment 1						
Genotype	**Désirée Control**	**Désirée Line 14**	**Désirée Line 17**	**Balatoni Rózsa Control**	**Balatoni Rózsa Line 12**	**Balatoni Rózsa Line 25**	
Total plants	15	15	15	15	15	15	
Wilted plants	6	13	9	2	3	11	
Wilted plants (%)	40	87	60	13	20	73	
	Experiment 2						
Genotype	**Désirée Control**	**Désirée Line 14**	**Désirée Line 17**	**Balatoni Rózsa Control**	**Balatoni Rózsa Line 12**	**Balatoni Rózsa Line 25**	
Total plants	20	10	20	20	20	20	
Wilted plants	15	10	20	16	13	20	
Wilted plants (%)	75	100	100	80	65	100	
Bacterial quantification (mean gray value) values obtained from ImageJ							
		Ex-1			Ex-1		
Désirée Line 14	5dpi	9dpi	12dpi	**Balatoni Rózsa Line 12**	5dpi	9dpi	12dpi

1st plate	56.911	161.234	198.901	1st plate	46.818	81.296	53.998
2nd plate	53.529	114.287	192.179	2nd plate	44.332	101.541	98.644
3rd plate	49.389	57.798	138.109	3rd plate	37.651	74.333	39.625
	53.27633333	111.1063333	176.3963333	Avg	42.93366667	85.72333333	64.089
Désirée Line 17	5dpi	9dpi	12dpi	**Balatoni Rózsa Line 25**	5dpi	9dpi	12dpi
1st plate	62.109	106.601	131.163	1st plate	45.981	103.554	105.554
2nd plate	59.573	84.325	123.612	2nd plate	20.091	95.942	53.528
3rd plate	52.245	63.095	137.927	3rd plate	52.426	86.728	67.837
Avg	57.97566667	84.67366667	130.9006667	Avg	39.49933333	95.408	75.639667
Désirée Control	5dpi	9dpi	12dpi	**Balatoni Rózsa Control**	5dpi	9dpi	12dpi
1st plate	56.757	68.284	88.412	1st plate	39.401	62.593	67.573
2nd plate	26.2	70.301	112.521	2nd plate	14.087	27.704	35.269
3rd plate	24.203	35.524	88.117	3rd plate	46.388	83.398	100.066
Avg	35.72	58.03633333	96.35	Avg	76.61733333	91.732	100.066
Plotted values							
12dpi	DC	D14	D17	**9dpi**	BRC	BR12	BR25
1st plate	88.412	198.901	131.163	1st plate	62.593	81.296	103.554
2nd plate	112.521	192.179	123.612	2nd plate	27.704	101.541	95.942
3rd plate	88.117	138.109	137.927	3rd plate	83.398	74.333	86.728
pvalue		0.037926372	0.032304861	pvalue		0.224965574	0.1380737

Supplementary Table 10. Mycelial mat quantification on tubers of *PPO* mutant lines (intensity) by ImageJ

S. no.	**Genotype**	**Area of ROI**	**Intensity**	**st Deviation**	**Minimum**	**Maximum**
1	Désirée C	6497	123.197	16.137	72	193
2	Désirée C	6497	108.144	20.439	54	198
3	Désirée C	6497	122.202	23.384	58	220
4	Désirée Line 14	6497	169.334	25.032	88	219
5	Désirée Line 14	6497	161.993	24.216	0	207

6	Désirée Line 14	6497	153.738	23.905	67	200
7	Désirée Line 17	6497	145.429	23.638	78	232
8	Désirée Line 17	6497	119.396	21.214	55	197
9	Désirée Line 17	6497	104.734	15.508	60	178
10	Balotoni Rózsa C	6497	125.008	33.765	35	192
11	Balotoni Rózsa C	6497	132.629	23.072	0	197
12	Balotoni Rózsa Line12	6497	135.565	22.328	69	197
13	Balotoni Rózsa Line12	6497	135.514	28.188	0	188
14	Balotoni Rózsa Line12	6497	123.016	31.291	53	190
15	Balotoni Rózsa Line12	6497	122.016	19.23	63	178
16	Balotoni Rózsa Line25	6497	116.883	38.753	47	199
17	Balotoni Rózsa Line25	6497	122.788	41.289	31	202

Supplementary Table 11A. Predicted miR396 targets in potato

miRNA_Acc.	miRNA family	Target_Acc.	target gene function annotation	Expectation	UPE	miRNA_start	miRNA_end	Target_start	Target_end	miRNA_aligned_fragment	Target_aligned_fragment	Inhibition	Multiplicity	SPUD DB GeneID	Information from SPUD DB
PotatoMir10057795 50_x16205	miR396	PGSC0003DMT400080455	Endomembrane protein emp70	2.5	12.235	1	21	359	379	UUCCACAGCUUUCUUGAACUU	AAGUUUAAGAGAGAUGUGGAG	Cleavage	1	Soltu.DM.06G020520.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 19.5241 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 29.1261 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 18.3671 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 47.924
PotatoMir10057795 50_x16205	miR396	PGSC0003DMT400015250	Multicystatin	2	16.618	1	21	1496	1516	UUCCACAGCUUUCUUGAACUU	AACUUCAAGAAAGUUGUUGAA	Cleavage	1	Soltu.DM.06G028940	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 21.6115 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 9.74438 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 24.429 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 19.6996

PotatoMir100577950_x16205	miR396	PGSC0003DMT400006756	Protein disulfide isomerase	1.5	11.115	1	21	1122	1142	UUCCACAGCUUUCUUGAACUU	GACUUCAAGAAACUUGUGGAA	Translation	1	Soltu.DM.03G035420.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 15.3544 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 21.606 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 21.6509 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 32.316
PotatoMir100577950_x16205	miR396	PGSC0003DMT400046956	GRIP and coiled-coil domain-containing protein	1.5	12.827	1	21	581	601	UUCCACAGCUUUCUUGAACUU	GAGGUAAAGAAAGCUGUGGAG	Cleavage	1	Soltu.DM.01G041150.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 29.1422 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 37.0468 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 47.3839 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 55.9774
PotatoMir100577950_x16205	miR396	PGSC0003DMT400016105	Tospovirus resistance protein A	0	13.345	1	21	1876	1896	UUCCACAGCUUUCUUGAACUU	AUGAUCAAGAAAGCUGUGGAA	Cleavage	1	Soltu.DM.01G023270.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 0 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 0 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 0 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 0.1262
PotatoMir100577950_x16205	miR396	PGSC0003DMT400084792	DNA binding protein	0	15.266	1	17	236	252	UUCCACAGCUUUCUUGA	UCAAGAAAGCUGUGGAA	Cleavage	1	Soltu.DM.11G008570.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 0 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 0 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 0 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 0
PotatoMir100577950_x16205	miR396	PGSC0003DMT400069147	Multicystatin	2	17.724	1	21	400	420	UUCCACAGCUUUCUUGAACUU	AACUUCAAGAAAGUUGUUGAA	Cleavage	1	Soltu.DM.06G028910	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 4.21636 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 2.55397 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 4.34826 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 2.35919

PotatoMir100577950_x16205	miR396	PGSC0003DMT400069145	Multicystatin	2	17.736	1	21	416	436	UUCCACAGCUUUCUUGAACUU	AACUUCAAGAAAGUUGUUGAA	Cleavage	2	Soltu.DM.06G028940.5	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 0 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 0 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 2.52985e-08 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 1.21191e-06
PotatoMir100577950_x16205	miR396	PGSC0003DMT400049891	DNA binding protein	0.5	24.61	1	21	466	486	UUCCACAGCUUUCUUGAACUU	AGGGUCAAGGAAGCUGUGGAA	Cleavage	1	Soltu.DM.10G005950.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 1.11008 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 1.12396 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 1.09264 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 1.29944
PotatoMir100577950_x16205	miR396	PGSC0003DMT400006057	Potassium transporter 17	2.5	11.206	1	21	241	261	UUCCACAGCUUUCUUGAACUU	GAGUUUAAGAAAGUUGUGCAA	Cleavage	1	Soltu.DM.06G013620.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 3.85799 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 3.63155 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 5.22392 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 5.85298
PotatoMir100577950_x16205	miR396	PGSC0003DMT400054175	LRR receptor-like serine/threonine-protein kinase	3	13.706	1	21	199	219	UUCCACAGCUUUCUUGAACUU	GAGUUUAAGAAAGGUGUCGAA	Cleavage	1	Soltu.DM.02G002890.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 4.24479 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 1.57159 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 6.72537 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 3.22415
PotatoMir100577950_x16205	miR396	PGSC0003DMT400018502	DNA methyltransferase	2.5	16.016	1	21	1408	1428	UUCCACAGCUUUCUUGAACUU	AAGAUCAGGAAAGCUGUCGAG	Cleavage	1	Soltu.DM.10G030090.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 7.87775 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 9.61453 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 10.9964 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 13.0721
PotatoMir100577950_x16205	miR396	PGSC0003DMT400053146	Transcription factor	3	17.8	1	20	337	356	UUCCACAGCUUUCUUGAACU	AGUUUAAGAGAGUGGUGGAA	Cleavage	1	Soltu.DM.04G021690.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 0 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 0 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 0.144488 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 0

	miR396-3p	PGSC0003DMG400007999	Nbs-lrr resistance protein											Soltu.DM.04G034340.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 78.481 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 108.794 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 105.054 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 145.301
	miR396-3p	PGSC0003DMG400004054	TCP-protein											Soltu.DM.06G025210.1	SRR2442808 bulk of 8 heterozygous genotypes Resistant potato prior to Phytophthora infestans infection 0.706333 SRR2442809 bulk of 8 heterozygous genotypes Resistant potato one to two days after Phytophthora infestans infection 0.98221 SRR2442810 bulk of 8 heterozygous genotypes Susceptible potato prior to Phytophthora infestans infection 1.22073 SRR2442811 bulk of 8 heterozygous genotypes Susceptible potato one to two days after Phytophthora infestans infection 1.799

Supplementary Table 11B. miRNA and target-specific qPCR primers used for evaluating miR396 influenced gene expression

Gene	**Forward primer (5'-3')**	**Reverse primer (5'-3')**	**Product size (bp)**	**Tm (°C)**	**C (nM)**	**E**
stu-miR396 LS	GCGGCGGTTCCACAGCTTTCTT	ATCCAGTGCAGGGTCCGAGG	67	62	500	1.84
stu-miR396 PS	GCGGCGGTCCAAGAAAGCTGT		66	62	1000	2
stu-miR159a LS	GCGGCGGTTTGGATTGAAGGGA		67	60	2000	1.93
stu-miR159a PS	GCGGCGGTAGATCTTACTTCC		66	no product amplified		
DNA methyltransferase PGSC0003DMT400018502	GCCCCTTTGGAACCTGATGA	ACCTCCTCCCCTTGTGTGAT	69	62	500	1.98
Endomembrane protein emp70 PGSC0003DMT400080455	TCTTTTCCCCTGTCCCTCCT	TGGCGTACCACTCCAGTTTC	57	62	500	1.9
GRIP and coiled-coil domain-containing protein PGSC0003DMT400046956	ACCAAGGAATCTCCCCCTGA	TTTCCAGCCTCTGTGTGGAC	98	62	500	1.94
LRR receptor-like serine/threonine-protein kinase PGSC0003DMT400054175	TTACGGCTCAGTTTCTGCGT	CGGGAGAGATCCTGTCAAGG	68	60	500	2
Multicystatin PGSC0003DMT400015250	CGGCAACTGATGATGCTGGA	AGTCCTCCCATTCTTTCACCC	162	60	1000	1.96
Multicystatin PGSC0003DMG400026899 transcript PGSC0003DMT400069145	CCAAACCCAAACAGCCCC	ACTCCAAATGAGCATTCTGAAAAG	101	60	500	2

Multicystatin PGSC0003DMG400026899 transcript PGSC0003DMT400069147	TTGTTCCATTCCCAAACAGTCCT	ACAACCTGTTCCTTCAAATTCAAAAC	124	62?	500	2
Nbs-lrr resistance protein PGSC0003DMG400007999	GAAGGGCTTCCTTTGGCTCT	TGAGACTCGCAGACAGGTTG	116	62	500	2
Potassium transporter 17 PGSC0003DMT400006057	AACTCTAGCTGCCGTGGTTG	TCGAGGGAAGCATCCAAGAG	91	60	500	2
Protein disulfide isomerase PGSC0003DMT400006756	GGGTTCCCGACTTTGCTTCT	GCGGAGAATCCACCAGTGTAA	65	62	500	1.99

Supplementary Table 12A. Number of wilted plants among *Ralstonia-solanacearum*-infected 'Botond' miR396 potato plants at 15 DPI and Bacterial quantification

	Experiment 1			
Genotype	Botond C	Botond L7	Botond L8	Botond 9
Total plants	10	5	9	9
Wilted plants	8	5	2	2
Wilted plants (%)	80	100	22.22	22.22
	Experiment 2			
Genotype	Botond C	Botond L6	Botond L8	Botond 9
Total plants	18	15	13	21
Wilted plants	16	NA	1	6
Wilted plants (%)	88.88889		7.692308	28.57143
	Bacterial Quantification			
Mean flurorescence intensity				
Botond C	miR396/8	miR396/9		
13.482	14.467	12.64		
20.599	14.556	9.59		
23.234	9.584	8.114		

Supplementary Table 12B. Quantification of infected plants among *Ralstonia-solanacearum*-infected 'Désirée' miR396 potato plants until 40 DPI

miR396 Line 3 (8 plants)						**disease index**	**mortality rate**	**396/3 (8 plants) disturbed soil**						**disease index**	**mortality rate**
	no symtoms	0-25%	26-50%	51-75%	76-100%				no symtoms	0-25%	26-50%	51-75%	76-100%		
	0	**1**	**2**	**3**	**4**	DI	MR		**0**	**1**	**2**	**3**	**4**	DI	MR
7 dpi	8	0	0	0	0	0	0	7 dpi	7	0	1	0	0	0.25	0
10 dpi	7	0	1	0	0	0.25	0	10 dpi	6	0	0	2	0	0.75	0
14 dpi	7	0	0	1	0	0.375	0	14 dpi	6	0	0	0	2	1	25
17 dpi	7	0	0	0	1	0.5	12.5	17 dpi	6	0	0	0	2	1	25
19 dpi	6	1	0	0	1	0.625	12.5	19 dpi	6	0	0	0	2	1	25
21 dpi	6	0	0	1	1	0.875	12.5	21 dpi	5	1	0	0	2	1.125	25
24 dpi	6	0	0	0	2	1	25	24 dpi	5	0	1	0	2	1.25	25
26 dpi	5	0	1	0	2	1.25	25	26 dpi	5	0	0	1	2	1.375	25
28 dpi	5	0	0	1	2	1.375	25	28 dpi	5	0	0	1	2	1.375	25
32 dpi	5	0	0	0	3	1.5	37.5	32 dpi	4	0	0	1	3	1.875	37.5
35 dpi	5	0	0	0	3	1.5	37.5	35 dpi	4	0	0	1	3	1.875	37.5
39 dpi	5	0	0	0	3	1.5	37.5	39 dpi	4	0	0	0	4	2	50
miR396 Line 6 (8 plants)						**disease index**	**mortality rate**	**396/6 (8 plants) disturbed soil**						**disease index**	**mortality rate**
	no symtoms	0-25%	26-50%	51-75%	76-100%				no symtoms	0-25%	26-50%	51-75%	76-100%		
	0	**1**	**2**	**3**	**4**	DI	MR		**0**	**1**	**2**	**3**	**4**	DI	MR
7 dpi	7	0	1	0	0	0.25	0	7 dpi	7	1	0	0	0	0.125	0
10 dpi	7	0	0	1	0	0.375	0	10 dpi	4	0	2	2	0	1.25	0
14 dpi	5	2	0	0	1	0.75	12.5	14 dpi	4	0	0	2	2	1.75	25
17 dpi	5	0	0	2	1	1.25	12.5	17 dpi	4	0	0	0	4	2	50
19 dpi	5	0	0	0	3	1.5	37.5	19 dpi	4	0	0	0	4	2	50
21 dpi	5	0	0	0	3	1.5	37.5	21 dpi	4	0	0	0	4	2	50
24 dpi	5	0	0	0	3	1.5	37.5	24 dpi	4	0	0	0	4	2	50
26 dpi	5	0	0	0	3	1.5	37.5	26 dpi	4	0	0	0	4	2	50
28 dpi	5	0	0	0	3	1.5	37.5	28 dpi	4	0	0	0	4	2	50
32 dpi	5	0	0	0	3	1.5	37.5	32 dpi	3	0	0	1	4	2.375	50

35 dpi	5	0	0	0	3	1.5	37.5	35 dpi	2	0	1	0	5	2.75	62.5
39 dpi	5	0	0	0	3	1.5	37.5	39 dpi	2	0	0	1	5	2.875	62.5
miR396 Line10 (8 plants)						**disease index**	**mortality rate**	**396/10 (8 plants) disturbed soil**						**disease index**	**mortality rate**
	no symtoms	0-25%	26-50%	51-75%	76-100%				no symtoms	0-25%	26-50%	51-75%	76-100%		
	0	**1**	**2**	**3**	**4**	DI	MR		**0**	**1**	**2**	**3**	**4**	DI	MR
7 dpi	8	0	0	0	0	0	0	7 dpi	8	0	0	0	0	0	0
10 dpi	8	0	0	0	0	0	0	10 dpi	5	1	2	0	0	0.625	0
14 dpi	8	0	0	0	0	0	0	14 dpi	5	0	1	2	0	1	0
17 dpi	7	0	1	0	0	0.25	0	17 dpi	5	0	0	1	2	1.375	25
19 dpi	7	0	1	0	0	0.25	0	19 dpi	5	0	0	0	3	1.5	37.5
21 dpi	7	0	0	1	0	0.375	0	21 dpi	5	0	0	0	3	1.5	37.5
24 dpi	7	0	0	0	1	0.5	12.5	24 dpi	5	0	0	0	3	1.5	37.5
26 dpi	7	0	0	0	1	0.5	12.5	26 dpi	5	0	0	0	3	1.5	37.5
28 dpi	7	0	0	0	1	0.5	12.5	28 dpi	5	0	0	0	3	1.5	37.5
32 dpi	6	0	1	0	1	0.75	12.5	32 dpi	3	0	1	1	3	2.125	37.5
35 dpi	6	0	0	1	1	0.875	12.5	35 dpi	3	0	0	0	5	2.5	62.5
39 dpi	6	0	0	0	2	1	25	39 dpi	2	0	1	0	5	2.75	62.5
Desiree control (8 plants)						**disease index**	**mortality rate**	**Desiree (8 plants) disturbed soil**						**disease index**	**mortality rate**
	no symtoms	0-25%	26-50%	51-75%	76-100%				no symtoms	0-25%	26-50%	51-75%	76-100%		
	0	**1**	**2**	**3**	**4**	DI	MR		**0**	**1**	**2**	**3**	**4**	DI	MR
7 dpi	8	0	0	0	0	0	0	7 dpi	6	0	2	0	0	0.5	0
10 dpi	7	1	0	0	0	0.125	0	10 dpi	6	0	0	2	0	0.75	0
14 dpi	7	0	0	1	0	0.375	0	14 dpi	6	0	0	0	2	1	25
17 dpi	7	0	0	0	1	0.5	12.5	17 dpi	4	0	2	0	2	1.5	25
19 dpi	7	0	0	0	1	0.5	12.5	19 dpi	3	1	2	0	2	1.625	25
21 dpi	7	0	0	0	1	0.5	12.5	21 dpi	3	0	1	2	2	2	25
24 dpi	6	0	0	1	1	0.875	12.5	24 dpi	2	1	0	1	4	2.5	50
26 dpi	6	0	0	0	2	1	25	26 dpi	2	0	1	0	5	2.75	62.5

28 dpi	6	0	0	0	2	1	25	28 dpi	2	0	1	0	5	2.75	62.5
32 dpi	6	0	0	0	2	1	25	32 dpi	2	0	0	0	6	3	75
35 dpi	5	0	0	1	2	1.375	25	35 dpi	2	0	0	0	6	3	75
39 dpi	3	0	0	1	4	2.375	50	39 dpi	1	0	0	1	6	3.375	75
Calalo Gaspar (8 plants)						**disease index**	**mortality rate**	**CG (8 plants) disturbed soil**						**disease index**	**mortality rate**
	no symtoms	0-25%	26-50%	51-75%	76-100%				no symtoms	0-25%	26-50%	51-75%	76-100%		
	0	**1**	**2**	**3**	**4**	DI	MR		**0**	**1**	**2**	**3**	**4**	DI	MR
7 dpi	8	0	0	0	0	0	0	7 dpi	8	0	0	0	0	0	0
10 dpi	8	0	0	0	0	0	0	10 dpi	7	0	0	1	0	0.375	0
14 dpi	8	0	0	0	0	0	0	14 dpi	5	1	1	0	1	0.875	12.5
17 dpi	6	0	1	1	0	0.625	0	17 dpi	2	1	0	2	3	2.375	37.5
19 dpi	6	0	1	0	1	0.75	12.5	19 dpi	2	0	1	0	5	2.75	62.5
21 dpi	6	0	0	1	1	0.875	12.5	21 dpi	1	0	2	0	5	3	62.5
24 dpi	6	0	0	1	1	0.875	12.5	24 dpi	2	0	0	0	6	3	75
26 dpi	6	0	0	0	2	1	25	26 dpi	2	0	0	0	6	3	75
28 dpi	6	0	0	0	2	1	25	28 dpi	2	0	0	0	6	3	75
32 dpi	6	0	0	0	2	1	25	32 dpi	1	1	0	0	6	3.125	75
35 dpi	6	0	0	0	2	1	25	35 dpi	1	0	1	0	6	3.25	75
39 dpi	6	0	0	0	2	1	25	39 dpi	1	0	0	0	7	3.5	87.5

Supplementary Table 13. Quantification of infected plants among *Ralstonia-solanacearum*-infected *Arabidopsis thalina* control and sulphate transporter mutants (SULTR1;2 and SULTR3;1) plants until 19 DPI

							disease index	**mortality rate**
SULTR1;2 (10 plants)								
	no symtoms	0-25%	26-50%	51-75%	76-100%			
	0	**1**	**2**	**3**	**4**			
7 dpi	10					10	0	0
10 dpi	9	1				10	0.1	0
11 dpi	7	3				10	0.3	0

12 dpi	6	1	3			10	0.7	0
13 dpi	5	1	2	2	0	10	1.1	0
17 dpi	4		1	1	4	10	2.1	40
19 dpi	2		2		6	10	2.8	60
							disease index	**mortality rate**
SULTR3;1								
	no symtoms	0-25%	26-50%	51-75%	76-100%			
	0	**1**	**2**	**3**	**4**			
7 dpi	9			1		10	0.3	0
10 dpi	9				1	10	0.4	10
11 dpi	9				1	10	0.4	10
12 dpi	8	1			1	10	0.5	10
13 dpi	8	1			1	10	0.5	10
17 dpi	8		1		1	10	0.6	10
19 dpi	8			1	1	10	0.7	10
COL-0 (9 plants)								
	no symtoms	0-25%	26-50%	51-75%	76-100%		**disease index**	**mortality rate**
	0	**1**	**2**	**3**	**4**			
7 dpi	8			1		9	0.3	0.0
10 dpi	3	1	2	2	1	9	1.7	11.1
11 dpi	3		1	2	3	9	2.2	33.3
12 dpi	2		1	2	4	9	2.7	44.4
13 dpi	0		1	2	6	9	3.6	66.7
17 dpi	0			2	7	9	3.8	77.8
19 dpi				1	8	9	3.9	88.9

Supplementary Table 14. Measuring the degree of suberisation in *Arabidopsis thaliana* control and sulphate transporter mutants (SULTR1;2 and SULTR3;1) plants using Lasx software

COL control	Unit	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1
Number of Pixels in Area	pixel	131494	109438	143178	135864	119336	118258	122353	126552	120001	127782	130746	126307	118796	128676	124814	124814	85755	92058	119336	126307
ROI Area	m²	3.34E-08	2.78E-08	3.64E-08	3.45E-08	3.03E-08	3.01E-08	3.11E-08	3.22E-08	3.05E-08	3.25E-08	3.32E-08	3.21E-08	3.02E-08	3.27E-08	3.17E-08	3.17E-08	2.18E-08	2.34E-08	3.034E-08	3.21E-08
Mean Intensity		45.487566	32.282991	47.699046	39.775158	69.711328	42.472103	48.927137	60.47748	60.595028	55.74415	46.310036	48.364192	51.212238	50.032516	36.046357	42.428894	42.556609	31.90267	69.711328	48.364192
SULTR1;2	Unit	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1
Number of Pixels in Area	pixel	127181	135311	125378	130282	140794	110889	101699	102831	99021	93171	117981	110044	113808	98160	117088	116372	100366	96910	88846	97676
ROI Area	m²	3.23E-08	3.44E-08	3.19E-08	3.31E-08	3.58E-08	2.82E-08	2.59E-08	2.61E-08	2.52E-08	2.37E-08	3.00E-08	2.80E-08	2.89E-08	2.50E-08	2.98E-08	2.96E-08	2.55E-08	2.46E-08	2.26E-08	2.48E-08
Mean Intensity		94.719604	154.10873	102.05159	137.52647	122.5069	119.67522	131.02366	147.2814	129.94649	110.31612	163.77855	177.19749	162.27491	86.265831	79.595253	65.285558	110.31021	111.36373	152.09351	102.50264
SULTR3;1	Unit	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1	Ch1/ROI 1
Number of Pixels in Area	pixel	105817	123565	97852	90567	95133	112156	107922	110537	118463	124076	149290	147232	130427	127962	110907	110675	99848	116853	137820	135391
ROI Area	m²	2.691E-08	3.142E-08	2.488E-08	2.303E-08	2.419E-08	2.852E-08	2.744E-08	2.811E-08	3.012E-08	3.155E-08	3.796E-08	3.744E-08	3.316E-08	3.254E-08	2.82E-08	2.814E-08	2.539E-08	2.971E-08	3.504E-08	3.442E-08
Mean Intensity		111.11123	116.91619	128.18091	122.93169	71.058024	87.362513	110.06298	117.87358	92.553954	108.51778	148.45947	127.53975	129.47941	107.52216	137.83674	134.66924	152.02999	166.55548	151.76625	153.86112

A3: LIST OF TABLES AND FIGURES

A4: LIST OF SCIENTIFIC ACTIVITIES

Articles published in peer reviewed scientific journals

Jose, J., Bánfalvi, Z. (2019): The role of GIGANTEA in flowering and abiotic stress adaptation in plants. Columella – Journal of Agricultural and Environmental Sciences, 6(1), 7-18. https://doi.org/10.18380/SZIE.COLUM.2019.6.1.

Jose, J., Éva, C., Bozsó, Z., Hamow, K.Á., Fekete, Z., Fábián, A., Bánfalvi, Z. and Sági, L. (2023): Global transcriptome and targeted metabolite analyses of roots reveal different defence mechanisms against *Ralstonia solanacearum* infection in two resistant potato cultivars. Frontiers in Plant Science (IF:4.1), 13, 1065419. https://doi.org/10.3389/fpls.2022.1065419

Karsai-Rektenwald, F., Odgerel K., **Jose J**., Bánfalvi Z. (2022): *In silico* characterization and expression analysis of *GIGANTEA* genes in potato. Biochemical Genetics. (IF:2.4) 60(6), 2137-2154. https://doi.org/10.1007/s10528-022-10214-7

Odgerel, K., **Jose, J**., Karsai-Rektenwald, F., Ficzek, G., Gergely, Simon, G., Végvári G., Bánfalvi Z. (2022): Effects of the repression of *GIGANTEA* gene *StGI.04* on the potato leaf transcriptome and the anthocyanin content of tuber skin. BMC Plant Biology (IF:5.3), 22(1):24922-249. https://doi.org/10.1186/s12870-022-03636-3

Book Chapter

Jose, J. and Éva, C., 2023. Plant Biotechnology: Its Importance, Contribution to Agriculture and Environment, and Its Future Prospects. Biotechnology in Environmental Remediation, pp.9-30. ePDF ISBN: 978-3-527-83904-9

Articles and abstracts published in conference books

Jose, J., Bozsó, Z., Fábián, A., Éva, C., and Sági, L. (2024): The *Arabidopsis* sulfate transporter *AtSultr1;2* is a negative regulator of resistance to *Ralstonia solanacearum*. XXX. Növénynemesítési Tudományos Napok. Máy. p.104-108. ISBN:978-963-8351-50-0

Jose J., Éva C., Kyrpa T., Bozsó Z., Bakonyi, J., Sági, L. (2024): Role of miR396 in regulating plant defense mechanisms against pathogens: insights from CRISPR/CAS9 editing in potato. 6th National Conference of Young Biotechnologists. 4-5. April 2024, Martonvásár, Hungary. In Book of abstracts (2024), p.28.

Jose, J., Éva, C., Bozsó, Z., Bakonyi,, J., Balázs E. and Sági, L. (2023): Employing molecular techniques to confer resistance against Phytophthora Infestans in potato. 12th International

Congress of Plant Pathology 20-25th August 2023, France. In ICPP 2023 Book of abstracts (2023), pp 185-186.

Jose, J., Sági, L., Crean, L., Danila, F., and Rathjen, J. (2023): Employing protein-interaction techniques to identify pathogen effectors to better devise targeted genome editing in plants. 4th PlantEd conference, 18th-20th September 2023, Portugal. In COST Action CA18111 Genome Editing in Plants: book of abstracts (2023), p. 67.

Éva, C., **Jose, J**., Bozsó, Z., Bakonyi, J., Kyrpa, T., Hamow, K.Á., Balázs, E., Sági, L. (2023): Studying potato resistance and susceptibility factors against pathogens with the use of genome editing. In COST Action CA18111 "Genome Editing in Plants: book of abstracts (2023), p. 43.

Mészáros, K., **Jose, J**., Szakács, A., Kunos, V., Cséplő, M., Bányai, J., Pál, M., Bakonyi, J., Éva, C. (2023): The role of ora59 transcription factor in the hormonemediated defence mechanism during *Pyrenophora teres* f. teres infection in barley. In: Mihály-Langó, Bernadett; Bona, Lajos; Tóth, Beáta; Börner, Andreas Cereal Breeding - Challenges and Opportunities for Global Improvement Book of Abstracts of the Eucarpia Cereals Section Conference Szeged, Hungary : Cereal Research Nonprofit Ltd. (2023) 134 p. pp. 69-69. , 1 p. Scientific

Karsai-Rektenwald, F., OdgereL, K., **Jose, J**. (2021): Regulation of *GIGANTEA* genes in potato. Proceeding of the “Scientific Conference of PhD. Students - 2021”. Nitra, Slovakia. Nov. pp.14

Bánfalvi, Z., Odgerel, K., Csákvári, E., **Jose, J**., Kondrák, M. (2019): Studying the relationship between tuber bulking and canopy growth in potato. Proceedings of the XXV. Plant Breeding Scientific Day. Budapest, Hungary. Mar. pp. 149-153

Karsai-Rektenwald, F., Odgerel, K., **Jose, J**., Bánfalvi, Z. (2022): Regulation of GIGANTEA genes in potato. Proceeding of the “Scientific Conference of PhD. Students - 2021”. Nitra, Slovakia. Nov. pp.14

Karsai-Rektenwald, F., Odgerel, K., **Jose, J**., Bánfalvi, Z. (2022): A GIGANTEA gének szerepe és szabályozása burgonyában. XXVIII. Növénynemesítési Tudományos Napok. Május. Összefoglaló kötet. p.59

Bánfalvi, Z., Karsai-Rektenwald, F., Odgerel, K., **Jose, J**. (2022) Regulation and function of gigantea genes in solanum tuberosum cultivar ’Désirée’. 4th International Conference on Plant Biology (23rd SPPS Meeting). October 6-8, Belgrad, Serbia, Abstract p. 40

Éva, C., Majláth, I., **Jose, J**., Tajti, J., Reinoso, L.G., Dernovics, M., Sági, L. (2021): Első eredményeink arabidopsis és árpa gyökéren történő szervetlen szénfelvételéről.In:

Györgyey, János (szerk.) XIII. Magyar Növénybiológiai Kongresszus : Összefoglaló kötet (2021) p. 25. Paper: E2-04 , 1 p.

Éva, C., **Jose, J**., Bozsó, Z., Moncsek, B., Hamow, K.Á., Reinoso, L.G., Balázs, E. and Sági, L. (2021): Edition of potato for reduced PPO activity confers resistance to *Ralstonia solanacearum*. 2nd PlantEd Conference Plant genome editing 20-22 September 2021, Lecce, Italy. ISBN 9788880804741. p.38

Jose, J., Éva, C., Bánfalvi, Z., Bozsó, Z., Hamow, K.Á., Balázs E., and Sági, L. (2021): Molecular and metabolomic analysis of resistant potato varieties as a way forward to generate resistance to *Ralstonia solanacearum*, at 2nd PlantEd Conference Plant genome editing 20-22 September 2021, Lecce, Italy. ISBN 978-88-8080-475-8. p.39

Jose, J., Éva, C., Bánfalvi, Z., Bozsó, Z., Balázs E., and Sági, L. (2022): Harnessing S-gene candidates for conferring resistance against Ralstonia solanacearum in potato. 3rd PlantEd Conference Plant genome editing 5-7 September 2022, Dusseldorf, Germany. COST Action CA18111 "Genome Editing in Plants" Book of Abstracts

Jose, J., Éva, C., Bozsó, Z., Moncsek, B., Reinoso, L.G., Balázs, E. and Sági, L. (2021): Towards conferring resistance to *Ralstonia solanacearum* by the edition of PPO genes in potato. FIBOK 2021, August, Szeged, Hungary ISBN: 9786150123509. p. 69

Jose, J., Bánfalvi, Z. (2020): Expressional and functional analysis of gigantea in potato (Solanum tuberosum l.). FIBOK 2020, Debrecen Hungary. ISBN:9789634902720. p.29

Bánfalvi, Z., Odgerel, K., Csákvári, E., **Jose, J**., Kondrák, M. (2019): Studying the relationship between tuber bulking and canopy growth in potato. In: Proceedings of the XXV. Plant Breeding Scientific Day, Budapest, Hungary, March 6-7, (2019) ed: Karsai I, pp. 149-153.

Oral and Poster presentations

Jose, J., Bozsó, Z., Fábián, A., Éva, C., and Sági, L. (2024): The *Arabidopsis* sulfate transporter *AtSultr1;2* is a negative regulator of resistance to *Ralstonia solanacearum*. XXX. Növénynemesítési Tudományos Napok. Máy. p.104-108. ISBN:978-963-8351-50-0

Jose, J., Éva, C., Bozsó, Z., Bakonyi,, J., Balázs E. and Sági, L. (2023): Employing molecular techniques to confer resistance against Phytophthora Infestans in potato. 12th International Congress of Plant Pathology 20-25th August 2023, France. In ICPP 2023 Book of abstracts (2023), pp 185-186.

Jose, J., Sági, L., Crean, L., Danila, F., and Rathjen, J. (2023): Employing protein-interaction techniques to identify pathogen effectors to better devise targeted genome editing in plants. 4th PlantEd conference, 18th-20th September 2023, Portugal. In COST Action CA18111 Genome Editing in Plants: book of abstracts (2023), p. 67.

Jose, J., Éva, C., Bánfalvi, Z., Bozsó, Z., Balázs E., and Sági, L. (2022): Harnessing S-gene candidates for conferring resistance against Ralstonia solanacearum in potato. 3rd PlantEd Conference Plant genome editing 5-7 September 2022, Dusseldorf, Germany. COST Action CA18111 "Genome Editing in Plants" Book of Abstracts

Jose, J. (2021): To confer resistance against *Ralstonia solanacearum* in potato focusing on the susceptibility genes at FIAtal NÖVénybiológusok ELŐadássorozatát PLANT BIOLOGISTS lecture series on March 2021, Debrecen, Hungary.

Jose, J., Éva, C., Bozsó, Z., Moncsek, B., Reinoso, L.G., Balázs, E. and Sági, L. (2021): Towards conferring resistance to *Ralstonia solanacearum* by the edition of PPO genes in potato. FIBOK 2021, August, Szeged, Hungary ISBN: 9786150123509. p. 69

Jose, J., Éva, C., Bánfalvi, Z., Bozsó, Z., Hamow, K.Á., Balázs E., and Sági, L. (2021): Molecular and metabolomic analysis of resistant potato varieties as a way forward to generate resistance to *Ralstonia solanacearum,* at 2nd PlantEd Conference Plant genome editing 20-22 September 2021, Lecce, Italy. ISBN 978-88-8080-475-8. p.39

Jose, J. (2021): Transcriptome and targeted metabolite analysis of resistant potato varieties as a way forward to generate resistance to *Ralstonia solanacearum.* Plant Genomes at a changing environment October, 2021, Cambridgeshire, UK.

Jose, J., Bánfalvi Z. (2020): Expressional and functional analysis of gigantea in potato (*Solanum tuberosum* l.). FIBOK 2020, Debrecen Hungary. ISBN:9789634902720. p.29

Jose, J. (2019): 'Enhancing the intrinsic ability of plants to combat abiotic stresses' at the International Seminar in Environmental Issues and Challenges in the 21st century at Bareilly, India;

Jose, J., Éva, C., Bozsó, Z., Bakonyi, J., Balázs, E. and Sági, L. (2023): Employing molecular techniques to confer resistance against *Phytophthora Infestans* in potato. 12th International Congress of Plant Pathology 20-25th August 2023, France. In ICPP 2023 Book of abstracts (2023), pp 185-186.

Jose, J., Sági. L., Crean, L., Danila, F., and Rathjen, J. (2023): Employing protein-interaction techniques to identify pathogen effectors to better devise targeted genome editing in plants. 4th PlantEd conference, 18th-20th September 2023, Portugal. In COST Action CA18111 Genome Editing in Plants: book of abstracts (2023), p. 67.

10. ACKNOWLEDGEMENTS

I am deeply thankful to God, the Almighty, for blessing me with good health, intellectual acumen, and determination, which have propelled me forward in my research endeavours.

The privilege of conducting my research at HUN-REN ATK alongside the constant support and encouragement from MATE and the support of Tempus Public Foundation has provided me with an unparalleled research environment conducive to growth. I am profoundly indebted to this opportunity, as it has significantly contributed to my professional development.

I extend my sincere appreciation to numerous individuals whose guidance and support have been instrumental in the successful completion of this project. Foremost among them are my esteemed supervisors, Dr. Csaba Éva, Dr. László Sági, and Dr. Zsófia Bánfalvi. Their mentorship, along with the invaluable assistance of the HUN-REN Centre for Agricultural Research and my fellow lab members, particularly Dr. Éva Csaba, have been pivotal at every stage of my research journey.

I am also immensely grateful to Dr. Zoltán Bozsó of the Plant Protection Institute for his invaluable contributions to the project. Dr. Zoltán Bozsó's expertise in integrating *GFP* into the *Rs* strain, executing infection processes, and meticulously documenting results has been indispensable to our research outcomes. Dr. Jozsef Bakonyi also has provided his invaluable help with infections with *Pi*, which form a cardinal aspect of my presented work and I am extremely thankful for it. Furthermore, I extend my heartfelt thanks to Dr. Kamirán Áron Hamow for generously sharing his expertise in UPLC-MS/MS. Dr. Attila Fábián deserves special recognition for his generous support in microscopy work. His pivotal role in sample collection, fixation, staining, and guidance on microtome sectioning has enriched my learning experience and contributed significantly to the visual components of my thesis.

I am profoundly grateful to my family and friends whose unwavering support has been a constant source of strength throughout this journey. Their encouragement and understanding have been invaluable, and I extend my heartfelt appreciation to them.

In conclusion, I am profoundly thankful to all those who have contributed to my academic and personal growth, including those whom I may have inadvertently overlooked. Without their unwavering support and guidance, this achievement would have remained beyond reach.