THESIS

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MSc. Plant Protection

KESZTHELY,

2023

Hungarian University of Agriculture and Life Science

Georgikon Campus

GENETIC ANALYSIS OF *PHYTOPHTHORA INFESTANS* RESISTANCE IN POTATO

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NEPTUN ID: F0PV7Q

KESZTHELY,

2023

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LIST OF ABBREVIATIONS

AFLP - Amplified Fragment Length Polymorphism

AOX- alternative oxidase

APAF-1 - Apoptotic protease-activating factor 1

ARC - Apaf-1, R protein, CED-4

Avr - Avirulence gene

BLAST - basic alignment search tool

BLAT- The blast-like alignmentl

CC - Coiled coil domain

CDPK- Ca^{2+}-dependent protein kinase

cDNA - Complementary deoxyribonucleic acid

DEG – Differentially expressed genes

DNA - Deoxyribonucleic acid

dsRNA - double-stranded RNA

ER - Extreme Resistance

ERF- Ethylene-responsive transcription factor

ETI - Effector triggered immunity

ET - Ethylene

GMO - Genetically modified organism

HSP - Heat shock proteins

HR - Hypersensitive reaction

IPM - Integrated pest management

JA - Jasmonic acid

LRR - Leucine-rich repeat

MAPK - Mitogen-activated protein kinase

MAS – Marker-assisted selection

mRNA - Messenger ribonucleic acid

NBS - Nucleotide-binding site

NCBI - National Center for Biotechnology Information

NGS – Next- sequencing

NO - Nitric oxide

PAMPs - Pathogen-associated molecular patterns

Pi- *Phytophthora infestans*

PCR - Polymerase chain reaction

Potato-DM - Solanum tuberosum group

PR - Pathogenesis-related genes

PRR - Pattern recognition receptors

PTI - PAMP triggered immunity

QTL - Qualitative trait loci

q-PCR - quantitative- PCR

R-Avr- Resistance-avirulence

RFLP - Restriction fragment length polymorphism

RNA - Ribonucleic acid

ROS- Reactive oxygen species

SA - Salicylic acid

SAGE - Serial analysis of gene expression

SAR - systemic acquired resistance

SNP - Single nucleotide polymorphism

SOLiD - Sequencing by oligonucleotide ligation and detection

STS - Sequence tagged site

TC- transcriptomes

TIR - Toll interleukin-1 receptor domain

TNLs - TIR-NB–LRRs

CHAPTER ONE

1. INTRODUCTION

Despite the huge proportion of potato yield utilized for seed and animal feed, potato is now the world's third most important food crop in terms of human consumption, after wheat and rice (FAOSTAT 2013). Fresh potato consumption accounts for almost two-thirds of the harvest, and approximately 1.3 billion people eat potatoes as a staple diet (more than 50 kg per person per year) CIP (2020). The cultivated potato (*Solanum tuberosum* L.) is an autotetraploid species (2n = 2x = 24) (Raker and Spooner, 2002). Late blight, caused by the pathogen, *Phytophthora infestans*, is a disease that has had a significant historical impact, including causing the Irish famine in 1840. *P. infestans* infects potato and other solanaceous crops, such as tomato (Derevnina *et al.*, 2016, Yuen, 2021) at any developmental stage, causing economic losses of up to $6 billion annually (Derevnina *et al.*, 2016).

P. infestans migrated to Hungary from other European nations, and domestic modifications brought about by sexual and asexual reproduction have also contributed to the introduction of these populations (Sakai 1961). Since late blight is the most serious obstacle to potato production in the world (Yang et al., 2018), resistance to late blight has long been a concern of potato farmers in Hungary and elsewhere. Late blight resistance has been transferred through repeated backcrossing of *Solanum demissum* to cultivars of *S. tuberosum*. R1 is a gene present in many varieties, while R2, R3, and/or R4 are present in other varieties. However, R genes only conferred temporary resistance to late blight, so, new pathotypes can overpower R-gene-mediated resistance (Ballvora et al. 2002; Lokossou et al. 2009). Potato R genes typically create receptors that can detect secretory effector (Avr) proteins made by *P. infestans*. In potato varieties with R genes, these effector proteins strongly promote resistance, but in potato types without R genes, they inhibit resistance Fatty acids, glucans, and elicitins, which are conserved compounds from Phytophthora species, also

operate as elicitors in Solanaceae species. These elicitors from *P. infestans* cause defensive responses, such as phytoalexin buildup and hypersensitive cell death (Vleeshouwers *et al.*, 2008).

Globally, late blight resistance has been achieved through repeated backcrossing of *S. demissum* to *S. tuberosum* cultivars (Paluchowska *et al.*, 2022). However, the introduction of R genes giving race-specific resistance to potato cultivars produced only temporary resistance to late blight. The R-gene-mediated resistance could be overcome by new races that evolve. So, searching for new *Rpi* genes among potato wild relatives and then applying these genes in potato cultivars represents an alternative to the use of fungicides for late blight control. Due to the rapid evolution of new virulent isolates of *P. infestans*, potato breeding for durable late blight resistance is challenging. Using *Rpi* genes recognizing conservative, essential effectors of *P. infestans* and the construction of *Rpi* gene pyramids may help achieve durable, broad-spectrum late blight resistance, which could be accelerated through genetic engineering (Paluchowska *et al.*, 2022).

In the gene bank of the Potato Research Centre at Keszthely, there are different genotypes that convey resistance against the late blight-causing *Phytophthora infestans*.

Research objectives

Considering the importance of *P. infestans* and the resistance sources in potato, the general objectives of the present study are the followings:

1) To explore the genetic background of resistance against late blight (*P. infestans)* of potatoes.
2) To analyze the resistance response to *P. infestans* inoculation of the potato variety, White Lady
3) To contribute to the development of molecular tools, which can be effectively used in the resistance breeding of potato.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Origin and evolution of potato crop

Potato is a crop native to the New World that was unknown to the rest of the world until the 1500s. The most visible domestication occurred in the Andes Mountains of South America. The modern potato was domesticated in present-day Peru and Bolivia and had a vital part in that society, as evidenced by many depictions of potatoes in ceramic artwork from the region (Bamberg and Del Rio, 2005). Hawkes (1990) identified 219 wild tuber-bearing Solanum species that span from the southwestern United States to central Argentina and neighbouring Chile, which cover a wide ecogeographical region. They form a polyploidy sequence ranging from diploid (2n = 2x = 24) to hexaploid (2n = 6x = 72). The diploid species *S. stenotomum* was the product of domestication; six additional cultivated species were descended from it, including *S. tuberosum*, which became South America's most widely cultivated species (Bradshaw, 2008).

S. tuberosum is a tetraploid species (2n = 4x = 48) with tetrasomic inheritance. S. tuberosum subsp. andigena and subsp. tuberosum, often known as Andigena and Chilean Tuberosum potatoes, are genetically distinct populations that have been defined as separate subspecies because they are adapted to shorter days in the Andes and longer days in coastal Chile (Raker and Spooner, 2002). *Solanum demissum* possesses an allohexaploid genome structure (2n = 6x = 72, a genome formula of AADDDdDd; Matsubayashi 1991), yet when *S. demissum* is utilized as the female parent, it is simple to produce hybrids with *S. tuberosum* (cultivated potato). The pentaploid F1 hybrids produce an abundance of normal-looking pollen grains but are ineffective as males and usually produce seeds only when backcrossed with *S. tuberosum* pollen (Dionne 1961). In the 1570s, the potato (tetraploid *S. tuberosum*) was brought to Europe. From there, it was exported to and grown in many other regions of the world (Hawkes and Francisco-Ortega, 1993). Their agroecological distribution

shows *S. tuberosum*'s adaptation to short summer days in the highland tropics and subtropics, then to long summer days in lowland temperate zones, and finally to short winter days in the lowland subtropics and tropics. Potatoes are grown in 149 countries (Hijmans and Spooner, 2001) and are the third most important food crop after wheat and rice (Lang, 2001, FAOSTAT 2013).

2.2. The importance of potato in the world

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world including Hungary. Potato has many advantages as a food source. It is highly nutritious, containing various vitamins, minerals, fibre, and phytochemicals. It has zero percent fat and is low in sodium. It is also very versatile and can be prepared in many different ways (Beals, 2019). Moreover, the potato is a very productive crop that can yield more food per unit of land and water than any other major cereal crop (Devaux et al. 2021). The potato has played a significant role in the history and development of many regions and countries. Potato was vital to European agriculture between 1750 and 1850, providing double the calories per hectare as rye and wheat. It has been a staple food for millions of people, especially in Europe, where it helped to prevent famines and support population growth but the invasion of *P. infestans* in 1844 altered the situation (Beals 2019). It has also been a source of income and livelihood for many farmers and traders. Also, potato has influenced the culture and cuisine of many nations, as well as the scientific and technological advancement of agriculture (Devaux et al. 2021).

The potato is still a vital crop for global food security and poverty alleviation. According to the International Potato Center (CIP), more than one billion people consume potatoes worldwide, and global production exceeds 300 million metric tons (CIP 2020). The potato is especially important for developing countries, where it can provide food, income, and employment for rural communities. The CIP estimates that the demand for potatoes will increase by 40% by 2050 in developing countries, due to population growth, urbanization, and changing diets (CIP 2020). However, the potato also faces many challenges including pests and diseases (Haverkort et al.

2009). In addition to the well-known Irish famine, crop failures in 1845 and 1846 caused an estimated 750,000 fatalities in continental Europe due to hunger (Zadoks, 2008). To address these issues, there is a need for more research and innovation in potato production and utilization. Some of the strategies that can help to improve the sustainability and resilience of the potato sector are: enhancing genetic diversity and breeding new resistant varieties; improving agronomic practices and pest management; reducing post-harvest losses and adding value; promoting market access and competitiveness; and strengthening institutional capacity and policy support (Devaux et al. 2021).

2.3. History of potato research at Keszthely, Hungary

Modern potato research and breeding efforts have been conducted at the Potato Research Centre (PRC) and its legal predecessors in Keszthely since 1950s, building on a 200-year legacy in agricultural research, as Hungary's only institution specialized in potato research and breeding (Ahmadvand, 2013). The PRC conducts research on various aspects of potato science such as genetics, breeding, biotechnology, agronomy, physiology, pathology, entomology, and seed production. The PRC also provides services for potato growers, processors, and consumers such as variety testing, certification, consultation, and education.

Late blight (*Phytophthora infestans*) has been and is still the most damaging potato disease globally, causing significant yield losses and requiring intensive use of fungicides for its control (Haverkort et al. 2009). Therefore, breeding for late blight resistance has been a main objective of potato research at Keszthely and elsewhere. The institute has therefore continued to work and expand its activities to include agronomy, physiology, pathology, entomology, biotechnology, and seed production. The institute also collaborated with national and international partners such as the Hungarian Academy of Sciences, the University of Pannonia, the International Potato Center (CIP), and the European Association for Potato Research (EAPR). Some of these projects aimed to discover novel Rpi genes from wild Solanum species using a combination of genetic mapping,

bioinformatics, and functional genomics approaches (Visser et al. 2009), validate molecular markers for late blight resistance genes, and to use them for marker-assisted selection (MAS) in potato breeding programs (Ghislain et al. 2019).

One of the main sources of late blight resistance genes (Rpi genes) in potato breeding is *Solanum demissum*, a wild relative of potato that originates from Mexico. The PRI has also been a pioneer in using genetic engineering techniques to transfer and pyramid late blight resistance genes from different sources into cultivated potato varieties. The institute used Agrobacterium-mediated transformation to introduce single or multiple Rpi genes from *S. demissum, S. stoloniferum, S. bulbocastanum, S. venturii, S. papita, S. mochiquense, S. schenckii, S. microdontum, S. chacoense, S. tuberosum* subsp. andigena, and *S. tuberosum* subsp. tuberosum into potato cultivars such as Desiree, White Lady, Lady Claire, Lady Rosetta, Lady Anna, Lady Olympia, Lady Amarilla, Lady Felicia (Kondrák et al. 2020). The transgenic potato varieties developed by the PRC showed high levels of resistance to multiple strains of P. infestans in greenhouse and field trials (Kondrák et al. 2020). However, the commercialization of these varieties is hindered by the regulatory and social barriers that limit the acceptance and adoption of genetically modified crops in Europe and elsewhere (Haverkort et al. 2009). Therefore, the institute is also exploring alternative ways to introduce late blight resistance genes into potato cultivars without using genetic engineering techniques, such as cisgenesis, intragenesis, genome editing with base editors or prime editors, or gene transfer via protoplast fusion or grafting (Kondrák et al. 2020).

The institute has developed more than 100 potato varieties for different purposes and markets, such as table, processing, starch, and seed potatoes. Some of the most successful varieties were Keszthelyi rózsa, Keszthelyi bíbor, Keszthelyi korona, Keszthelyi piroska, Keszthelyi holdfény, Keszthelyi csillagfény, Keszthelyi fényes napfény (Esztergályos and Polgár 2020). The Center also has 13 varieties included on the EU list (Arany Chipke, Démon, Balatoni rózsa, Katica, Lorett, Góliát, Rioja, Hópehely, White Lady, Vénusz Gold, Luca XL, Kánkán, and Somogyi Kifli) as a

result of a consistent resistance-breeding effort using wild species material. Except for Somogyi Kifli, these varieties are unique in their class due to their complex resistance, high-yielding potential, and outstanding consumption quality, and some of them are especially recommended for organic production (Ahmadvand, 2013).

2.4. Genetics of potato species

Based on a haploid number of 12, the number of ploidy levels of potato species ranges from diploid (2n = 24) to hexaploid (6n = 72), including triploids, tetraploids, and pentaploids (Watanabe, 2002). There is some indication that polyploidy was crucial in wild potato environmental diversification and range extension (Hijmans et al., 2007). The ploidy levels are related to the phenomena of unreduced gametes. In addition to the conventional haploid gametes (n), certain genotypes produce unreduced gametes (2n) as a result of meiotic abnormalities (Carputo and Barone, 2005). The frequency of 2n pollen generation ranges from 2% to 10%. (Watanabe, 2002). Potatoes grown for food are tetrasomic tetraploids (4n=48), although the vast majority (80%) of wild species are diploid (Carputo and Barone, 2005).

Hijmans et al. (2007) discovered 123 species in diploid cytotypes and only 43 species in polyploids. Almost all of the diploid species and the tetraploid S. tuberosum subsp. tuberosum, outbreed. Based on the presence of S alleles, the incompatibility is gametophytic and multi-allelic in nature (Dodds, 1965). According to research by Hosaka and Hanneman (1998) and De Jong and Rowe (1971), S. chacoense has a dominant self-incompatibility inhibitor that is exploited in breeding. Tetraploids and hexaploids are self-compatible allopolyploids with disomic inheritance (Hawkes, 1990).

2.5. Phytophthora infestans:

Phytophthora infestans is the causal agent of potato late blight. Plant diseases caused by Phytophthora, known as blights, cause wilting, damping-off, chlorosis, root rot, and the rotting of

other organs. Phytophthora species are known to cause at least 27 diseases in over 100 plant species (Watanabe 1998). *Phytophthora infestans* (Montague) de Bary is most known as the cause of the Irish potato famine in the 1840s (Fry 2008). This disease affects not only potato plants but also other Solanaceae plants like tomato.

Late blight is a native potato disease in the potato plant's native Central and South America. The disease was discovered in the United States in 1843 and quickly spread to the east coast of North America (Peterson et al. 1992). It first appeared in Belgium in 1845 and quickly spread to France, Switzerland, the United Kingdom, Ireland, and Scotland, causing extensive devastation (Robertson 1991). Since then, late blight has occurred every year in various parts of Europe, causing catastrophic damage to potato crops in some instances (Sakai 1961).

P. infestans isolates of the A2 mating type were originally discovered in Mexico and were first reported outside of Mexico in Switzerland in 1981. (Hohl and Iselin 1984). It was concluded that the pathogen movement in this case was caused by the export of potatoes from Mexico to Europe in 1977. (Fry et *al.* 1993). A2 isolates were also discovered in Egypt-imported potatoes in England (Shaw *et al.* 1985). In the following years, A2 isolates were repeatedly discovered in Asia (Koh *et al.,* 1994; Mosa *et al.* 1989, 1990; Nishimura et al. 1999; Ogoshi et al. 1988), Africa (Sedegui et al. 2000), Europe (Lebreton and Andrivon 1998; Malcolmson 1985; Schöber and Rullich 1986; Tantius et al. 1986), North and South America, and other places (Deahl et al. 1990; Oyarzun et al. 1997). Genetic analysis proved that the A2 isolate's distribution was not due to natural selection or mutation in these nations, but rather to the global spread of *P. infestans* after the 1980s (Goodwin and Drenth 1997).

The availability of both mating types in numerous regions around the world increased the likelihood that isolates with new genetic features developed through sexual reproduction. P. infestans oospore production has been documented in Europe (Götz 1990; Shattock et al. 1990)

and North America (Chycoski and Punja 1996). Oospores were found in an experimental field in Japan under artificial conditions (Kato et al. 1993), but no progeny were found.

2.6. Genetics of resistance

Plants live in complex environments in which they intimately interact with a broad range of microbial pathogens with different lifestyles and infection strategies. In general, plants defend themselves against pathogens through a combination of active and passive defense. In passive defense, structural characteristics act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant. And in active defense, biochemical reactions take place in the cells and tissues of the plant and produce substances that are either toxic to the pathogen or create conditions that inhibit the growth of the pathogen in the plant (Agrios, 2005).

The evolutionary arms race between plants and their attackers provided plants with a highly sophisticated defense system that, like the animal innate immune system (active defense), recognizes pathogen molecules and responds by activating specific defenses that are directed against the invader (Pieterse et al., 2009). Plants respond to infection using a two-branched innate immune system. The first branch recognizes and responds to molecules common to many classes of microbes, including non-pathogens. In this branch, the resistance is induced by the recognition of pathogen-associated molecular patterns (PAMPs) by plant cell surface pattern recognition receptors (PRR), which initiates PAMP-triggered immunity, that usually prevents the infection of pathogens before invasion. Defense responses activated by PAMPs are collectively termed PAMP-triggered immunity (PTI) or basal resistance (Jones and Dangl, 2006).

In the majority of cases, PTI prevents pathogen growth at an early infection stage due to the induction of pathogen-responsive genes, production of reactive oxygen species, mitogen-activated protein kinase signaling, and deposition of callose to reinforce the cell wall at sites of infection (Schwessinger and Zipfel, 2008). If a pathogen evades this line of defense, it must also overcome

a second line of defense to become pathogenic. The second branch acts primarily inside the cell using disease resistance (R) proteins which recognize pathogen-delivered effectors or their effects on host proteins. R protein-mediated defenses are termed effector-triggered immunity (ETI) or gene-for-gene resistance, in which the protein products of plant resistance (R) genes specifically recognize cognate pathogen avirulence (Avr) gene products and trigger a stronger resistance response. Direct or indirect recognition of effectors by R proteins initiates ETI, which is an amplified and accelerated PTI response resulting in disease resistance (Jones and Dangl, 2006). ETI usually induces a hypersensitive response (HR) with localized cell death and defense gene expression that suppresses the growth and spread of pathogens.

2.6.1. Structure and function of R genes and their distribution in Potato Genome

According to Sacco and Moffett (2009) over 70 R genes have already been cloned (Sacco and Moffett, 2009). However, the focus has been mainly on monogenic dominant resistance to fungal and bacterial pathogens. But there is clear evidence that common mechanisms can be involved in pathogen resistance (Hammond-Kosack and Parker, 2003). The majority of R genes belong to the nucleotide-binding leucine-rich repeat (NB-LRR) family. NB-LRRs contain a C-terminal LRR domain and a central NB domain (Sacco and Moffett, 2009). The NB is part of a larger domain that is called the NB-ARC (Apaf-1, R protein, CED-4) as it is shared between R proteins and the human apoptotic protease-activating factor 1 (APAF-1) and its Caenorhabditis elegans homolog CED- 4 (Takken et al., 2006). NB is proposed to act as a nucleotide-dependent molecular switch regulating the conformation and signaling activity of these proteins. The LRR domain, positioned C- terminally to the NB-ARC, forms an arc-shaped conformation, forming a protein-protein interaction surface that provides recognition specificity (Fig 1.). Based on the identity of the N-terminal domain two main classes of NB-LRR, R proteins can be distinguished. Some contain the toll interleukin-1 receptor (TIR) domain and these R proteins are called TIR-NB-LRRs or TNLs

(Burch-Smith and Dinesh-Kumar, 2007). Non-TIR-NB-LRR proteins contain predicted coiled-coil (CC) motifs, this family is referred to as CC-NB- LRRs (Lupas, 1997).

Direct interaction between the LRR and pathogen effectors has rarely been reported. The guard-theory describes an alternative recognition mechanism by which the NB-LRR proteins known as the guardee are the target of the Avr protein. When the recognition mechanism detects interference with the guardee protein, it activates resistance (Van Der Biezen and Jones, 1998; de Wit, 2002). The guard hypothesis suggests that the host-pathogen interaction is more likely an interaction between the Avr protein and a host recognition complex. This complex must be able to recognize the pathogen and signal a defense response. Complex levels and activation of signaling must be tightly regulated and the recognition complex must be poised to perceive and respond to pathogens.

More than 50 functional NB-LRR genes have been cloned from potato and related members of the Solanaceae (Hein et al., 2009). Recently, based on an amino acid motif-based search of the annotated potato genome 438 NB-LRR type genes were identified among the ~39,000 potato gene models. Of the predicted genes, 77 contain an N-terminal toll/interleukin 1 receptor (TIR)-like domain, and 107 contain an N-terminal coiled-coil (CC) domain (Jupe et al., 2012). After a pathogen is recognized by the LRR, the function of an R protein complex must switch from recognition to signal transduction. Intramolecular interactions, activation of the NBS domain, and changes in signaling components that may associate with the CC or TIR domain and LRR domain have all been implicated during early signaling (Fig 1.) (Caplan and Dinesh-Kumar, 2006; Sessa, 2013).

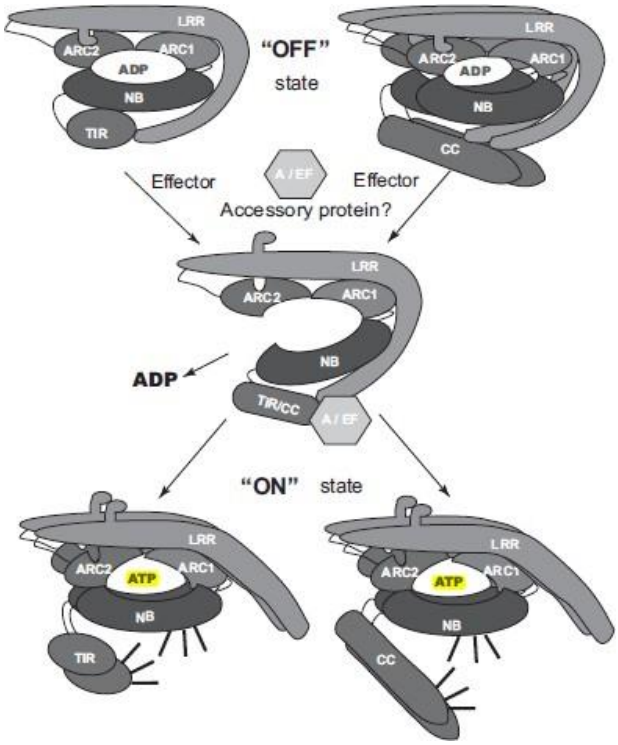

Fig 1. A Model for NB-LRR protein activation. In the resting state, an NB-LRR protein is kept ina closed and auto-inhibited state in which the LRR and N-terminal domain (CC/TIR) fold back onthe NB-ARC core. Effector recognition, often aided by an accessory protein, likely occurs by an interface formed by the C-terminal half of the LRR and the CC/TIR domain. Effector recognition results in a conformational change that is transduced via the N-terminal part of the LRR to the ARC2. This change allows the exchange of ADP for ATP, triggering a second conformationalchange in the NB-ARC resulting in a more open structure in which interfaces on either the NB or the N-terminal domain (CC/TIR) become exposed and activate defense signaling (Sessa, 2013).

2.6.2. Arm Race

Interactions between *R* genes and effectors represent host-pathogen molecular co-evolution when effectors evolve to evade detection and R proteins evolve to establish or retain detection (Hein et al. 2009; Naveed et al. 2020). Potatoes have developed sophisticated surveillance systems, which respond to and prevent pathogenic infections. Among these, the plant cell wall represents the first

protective barrier. Moreover, pattern recognition receptors (PRRs) located on the host cell surface detect the evolutionarily conserved pathogen-associated molecular patterns (PAMPs) or apoplastic effector proteins and initiate the PAMP-triggered immunity (PTI) (Dou and Zhou, 2012; Win et al., 2012). In response, pathogens secrete intracellular effector proteins through haustoria to interfere with the PTI and promote their colonization, a phenomenon referred to as effector-triggered susceptibility (ETS) (Whisson et al., 2007; King et al., 2014; Zheng et al., 2014; Boevink et al., 2016; Wang S. et al., 2018).

During the pathogenesis of *P. infestans*, a key step is the formation of haustorium in potato tissue through which the pathogen secretes effectors. These proteins manipulate and alter the host's immune response to promote infection. Genes encoding pathogen effectors that induce *R* gene response are defined as avirulence (*Avr*) genes (Qutob et al. 2006). Cytoplasmic effectors secreted by *P. infestans* can be divided into two classes, CRN (crinkling, necrosis) and RxLR effectors. The effectors of the RxLR type possess arginine-any amino acid residue-leucine–arginine motifs in the N-terminal region. All known *P. infestans* effectors, which are recognized by the products of corresponding potato *Rpi* genes, belong to the RxLR class (Martynov and Chizhik 2020). The RxLR effectors contain the highly conserved N-terminal RxLR motif involved in the translocation of *P. infestans* effector proteins into plant cells, and the heterogeneous C-terminal region that can be recognized by plant *R* gene products (Dou et al. 2008).

The Arg-X-Leu-Arg (RXLR) is the most studied class of cytoplasmic *P. infectans* effectors, containing a signal peptide followed by the conserved RXLR motif and is associated with the biotrophic phase of *P. infestans* infection (Whisson et al., 2007; Dou et al., 2008; Gilroy et al., 2011; Vleeshouwers et al., 2011; King et al., 2014; Zheng et al., 2014; Boevink et al., 2016). In resistant potato genotypes, effector-triggered immunity (ETI) is induced by direct or indirect recognition of some RXLR effectors by nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins (R

proteins), resulting in localized cell death [hypersensitive response (HR) cell death] (King et al., 2014). The recognized RXLR effectors are referred to as avirulence (Avr) proteins.

Several *Avr* genes belonging to the RXLR class of oomycete effectors have been investigated, since the first cloning of *P. infestans Avr* gene (*AVR3a*) in 2005 (Armstrong et al., 2005). These include *AVR2* (Saunders et al., 2012), *AVR3b* (Li et al., 2011), *AVR4* (Van Poppel et al., 2008), *AVRblb1* (Vleeshouwers et al., 2008), *AVRblb2* (Oh et al., 2009), and *AVRvnt1.1* (Pel, 2010). The RXLR effectors are extremely diverse and can rapidly evolve to evade detection by host R proteins (Birch et al., 2008; Raffaele et al., 2010). *P. infestans* genome encodes 563 RXLR effectors (Haas et al., 2009); thus, cultivating resistant potato cultivars is the most effective way of preventing and controlling potato late blight. However, the host-driven selective pressure causes *RXLR* genes to mutate rapidly, enabling *P. infestans* to escape host defense and establish an infection (Yang B. et al., 2017).

Since pathogen virulence and host resistance are constantly changing, the evolutionary dynamics of the plant–pathogen interactions can be well illustrated by a four-phased "zig-zag" model (Jones and Dangl, 2006). Although publishing the *P. infestans* and *Solanum tuberosum* (*S. tuberosum*) genomes accelerated the characterization of RXLR effectors (Haas et al., 2009; Xu et al., 2011), most of the 563 RXLR effectors are not yet known.

The adaptation of potato to the continuous evolution of the pathogen is through the diversification of *R* genes by recombination, gene conversion, duplication, and/or selection (Jupe et al. 2012). While some of the *S. demissum Rpi* genes were found to be race-specific and rapidly became ineffective, the following genes have been described as providing a broad-spectrum of resistance against *P. infestan*s: *Rpi-blb1, Rpi-blb2* and *Rpi-blb3* from *S. bulbocastanum*; *R8* and *R9* from *S. demissum* and *Rpi-vnt1.1* from *S. venturi* (Vleeshouwers et al. 2011; Vossen et al. 2016). However, these genes have not yet been widely introduced into potato cultivars, in part because of crossing barriers. This

continuous co-evolution of pathogen effectors and plant *R* genes represents a so-called arms race between plants and pathogens (Khavkin 2015).

2.6.3. Signaling mediated resistance

The response of plants to pathogens could also be divided into two major categories such as cellular stress and developmental defects. Comparison of Arabidopsis and *Nicotiana benthamiana* gene expression leads to the conclusion that virus infection causes characteristic changes in gene expression that is similar to stress and defense responses (Whitham et al., 2006). The stress responses are characterized by the induction of heat shock proteins (HSP) and defense responses by the induction of pathogenesis-related (PR) genes and other genes associated with plant disease defense. The induction of HSP and PR genes represent cellular stress responses because of their non-specific nature and the lack of specific elicitors that induce them. The expression of PR genes, mediated by salicylic acid (SA), is increased in many incompatible responses. In general, increasing of SA is required for the high accumulation of PR mRNA transcripts and proteins that occur during resistance response to viruses but not in susceptible interactions (Malamy et al., 1990; Gaffney et al., 1993; Ryals et al., 1996).

The defense-related genes include numerous pathogenesis-related (PR) genes such as PR-1, PR-2 (β-1,3 glucanase), PR-3 (chitinase), PR-4, PR-5 (thaumatin-like protein), genes associated with redox statuses such as superoxide dismutase and GST (glutathione S- transferases), resistance gene homologs. (Robatzek and Somssich, 2001, 2002). Mitogen-activated protein kinase (MAPK) cascades play different roles in plant processes that include cytokinesis, phytohormone signaling, wound responses, osmotic stress, and pathogen resistance (Zhang and Klessig, 2001). Different transcription factor families such as TGA, MYB, and WRKY have been implicated in disease resistance (Caplan and Dinesh-Kumar, 2006). In brief, some important signal transduction pathways involved in disease resistance include:

2.6.3.1. Salicylic acid (SA):

SA plays a main role in the signal transduction pathway that results in the induction of systemic acquired resistance (SAR) and it is required for the localization of pathogens during the HR. SA is required for the expression of a group of proteins that collectively are referred to as pathogenesis-related (PR) proteins. SA can induce inhibition of virus replication, cell-to-cell movement, and systemic movement but the precise effects of SA-induced resistance on the life cycle of a virus can differ between hosts and between viruses (Murphy and Carr, 2002; Love et al., 2007; Wang et al., 2007). SA biosynthesis is induced most strongly during HR lesion development. SA may be necessary to regulate the timing and extent of the HR. During the HR, SA forms a gradient, with SA accumulating to high levels at the center of the HR lesions, moderate levels at the lesion borders, and low levels in healthy tissue (Enyedi et al., 1992).

2.6.3.2. Signaling mediated by Jasmonic acid and Ethylene:

In spite of SA-dependent signaling pathways, the requirement of ethylene (ET) and jasmonic acid (JA) during R gene-mediated resistance is more complex and variable. The crosstalk between ET-, JA-, and SA-dependent signaling pathways can have synergistic or antagonistic effects on each other. ET and JA are secondary signaling molecules that function in microbial defense, wounding, and insect attack.

2.6.3.3. Signaling by reactive oxygen species, calcium, and nitric oxide:

Reactive oxygen species (ROS) have been recognized as signals in defense, most notably during the oxidative burst or bursts that occur very early in the HR during a gene-for-gene response (Heath, 2000). ROS has several roles in HR, but from the signaling point of view, perhaps two are the most important. Firstly, the oxidative burst activates Ca^{2+} ion influx across the plasma membrane via cyclic nucleotide-gated channels, in addition to the mobilization of Ca^{2+} ions from intracellular stores (Torres and Dangl, 2005; Ma and Berkowitz, 2007). A second effect of changes

in Ca^{2+} ion flux in the cytoplasm is the triggering of the activity of calcium-dependent protein kinases, as well as highly complex MAPK cascades. ROS generated in the mitochondrion may also play roles in defensive signaling, particularly with respect to the induction by SA in resistance response to viruses. It has been proposed that alternative oxidase (AOX) is one of the factors that may influence this form, induction by SA, of defensive signaling (Ma and Berkowitz, 2007).

2.6.3.4. Nitric oxide (NO):

NO is an important signal in plant defense. For example, the relative levels of NO and H_2O_2 regulate programmed cell death during an HR (Delledonne et al., 2001), and regulate defense gene expression both at the point of infection and in distal tissues, in part by inducing the biosynthesis of SA (Song and Goodman, 2001). NO also may stimulate changes in nuclear gene expression and defensive signaling indirectly through the inhibition of cytochrome oxidase (Huang et al., 2002).

2.6.4. Types of Resistance to *Phytophthora infestans*

Genetic resistance to *P. infestans* is desirable for potato cultivars to guard against late blight. Late blight resistance has remained a primary concern in potato breeding since the Irish Famine. Early Rose, a descendant of the Chilean clone Rough Purple Chili introduced by C Goodrich in 1851, made an important contribution to potato breeding owing to its earliness, which reduced the severity of late blight (Plaisted and Hoopes 1989). True genetic resistance is divided into two types: hypersensitivity (vertical or race-specific resistance) and field resistance (horizontal, general, or non-race-specific resistance).

2.6.4.1. Hypersensitive reaction (HR)

A necrotic response of the injected cells and surrounding cells characterizes hypersensitive genotypes. Hypersensitivity is influenced by a number of major genes, each of which is activated by different races. In the first years after introduction, these major hypersensitivity genes (R genes)

provide total protection. Since R. N. Salaman discovered late blight resistance in *Solanum edinense* Berth. (a natural hybrid of *Solanum demissum* Lindl. *Solanum tuberosum* L.) in 1906, 11 R genes (R1-R11) have been identified in *S. demissum* (Ross 1986). However, new races that frequently appear may be able to overcome R genes (Fry and Goodwin 1997). As a result, attempts to breed for late blight resistance using R genes have frequently failed.

2.6.4.2. Field resistance (FR)

Field resistance is a collection of diverse components that work together to thwart parasitic attacks on the host. It is governed by several interconnected genes, often known as quantitative trait loci (QTLs). In potato breeding, one common technique for achieving a long-term solution has been to use field resistance rather than R genes. However, this method has not been effective, owing to the unavoidable relationship between resistance and late maturity (Simko 2002).

2.7. The potato late blight resistance genes in potato cultivars and breeding lines

Wild relatives of the potato are unique sources of genetic variation. They are characterized as being highly resistant to various diseases, including late blight, and they have been used in breeding programmes for more than 100 years (Machida-Hirano 2015). To date, more than 70 Rpi genes have been identified and mapped in 32 Solanum species (Paluchowska et al., 2022). Most of the Rpi genes have been derived from tuber-bearing species (25): Mexican (9 species), Bolivian (6), Peruvian (4), Argentine (3), Paraguayan (1), USA (1), and one species found generally in the Andes. Novel Rpi genes were found also in *S. tuberosum* subspecies andigena and in the Hungarian cultivar Sárpo Mira. Six Rpi genes were identified in four non-tuber-bearing species and five from the tomato wild species *S. pimpinellifolium*. Single resistance genes were identified in 15 potato wild species. Frequently, multiple functional Rpi genes have been found within a single species, e.g., *S. demissum* (14 Rpi genes), *S. bulbocastanum* (5), *S. berthaultii* (5), *S. stoloniferum* (4), *S. edinense* (4), *S.*

venturii (4), *S. hjertingii* (3), *S. chacoense* (3), *S. huancabambense* (2), *S. pinnatisectum* (2) *S. schenckii* (2), and *S. tarijense* (2). The Rpi genes were mapped in clusters onto potato chromosomes I, IV, V, VI, VII, VIII, IX, X, and XI (Paluchowska et al., 2022). For instance, on chromosome IV, a total of 13 *Rpi* genes from seven potato wild species were found. Several *Rpi* genes have not yet been mapped, including the following: *Rpi-pta2* from *S. stoloniferum*; $R4^{BI}$ and $R4^{MA}$ from *S. demissum*; *Rpi-ber1.2, Rpi-ber1.3,* and *Rpi-ber1.4* from *S. berthaultii*; *Rpi-tar1.3* from *S. tarijense, Rpi-nrs1* from *S. neorossii* and putative novel *Rpi* genes from *S. jamesii* and *S. tuberosum* subsp. *Andigena* (Paluchowska et al., 2022).

R1, R2, R3a, and R3b have been cloned out of the 11 known race-specific resistance genes (R1-R11) from *S. demissum* (Ballvora et al. 2002; Huang et al. 2005; Li et al. 2011; Lokossou et al. 2009). Interestingly, the QTL for late blight resistance was localized to the same site as R1 in populations derived from multiple Solanum species, using different races of *P. infestans* and different resistance testing methodologies (Simko 2002). Thus, different alleles of the same genetic locus may produce different qualitative and quantitative phenotypes of resistance to P. infestans, and hypersensitive cell death caused by the R1 allele may be an extreme expression of a quantitative defense response brought on by different forms of the same gene product (Gebhardt 1994). A significant late blight resistance locus in potato has been found on the long arm of chromosome 11, where R3, R5-R7, and R9-R11 are located (Jo et al. 2011). Many more genes providing resistance to late blight have lately been identified, including R Pi-ber from Solanum berthaultii Hawkes (Ewing et al. 2000), and R Pi-mcd1 from Solanum microdontum Bitt. (Tan et al. 2008). Others are RB/R Pi-blb1, R Pi-blb2, R Pi-blb3, and R Pi-abpt from Solanum (Park et al. 2005a, b; Song et al. 2003; van der Vossen et al. 2003, 2005).

Recently, using advanced techniques, new *Rpi* genes have been identified. Through genetic linkage analysis and collinearity analysis, a new dominant resistance gene, *Rpi2*, from the Mexican diploid wild species *S. pinnatisectum* was mapped onto potato chromosome VII (Yang et al. 2017).

The *Rpi2* locus is different from the previously reported resistance locus *Rpi1*, which is on the same chromosome. *Rpi2* provides broad-spectrum resistance against various *P. infestans* isolates, including those that overcome resistance conferred by *R9*. Resistance gene enrichment sequencing (RenSeq) was used to finely map onto chromosome X, the *Rpi-rzc1* gene from *S. ruiz-ceballosii*, which confers high and broad-spectrum resistance to 500 diverse Polish *P. infestans* isolates, (Jupe et al. 2013; Brylińska et al. 2015).

The largest collections of potato germplasm are available in International Potato Center (CIP) in Peru, the USDA Potato Genebank in Wisconsin, USA, and IPK Gatersleben Genebank in Germany (Karki et al. 2021b). An analysis of resistance to *P. infestans* carried out over a period of more than 20 years has shown that among 34 potato wild relatives there are accessions characterized by a high level of resistance, but the genes underlying this resistance are still unknown (Pérez et al. 1999; Zoteyeva et al. 2012; Khiutti et al. 2015; Bachmann-Pfabe et al. 2019; Zoteyeva 2020; Karki et al. 2021b). Recently, 189 potato genotypes, from 20 wild species and cultivated *Solanum tuberosum* from Andigenum and Chilotanum groups, were screened for their resistance against *P. infestans* (Duan et al. 2021). Ten genotypes from five wild species originating in Mexico showed a broad-spectrum resistance to all four *P. infestans* used, suggesting that each of these genotypes contains *Rpi* gene(s) other than *R1-R11*. They belong to *S. bulbocastanum* (3 genotypes), *S. cardiophyllum* (4), *S. jamesii* (1), *S. brachycarpum* (1) and *S. trifidum* (1). The other 127 genotypes displayed isolate-specific resistance.

One method to increase resistance level and persistence is pyramiding, which is the consolidation of key R genes into a single genotype (Tan et al. 2010). A different approach was the R gene polyculture strategy, which allows for the use of a variety of cultivars with various R genes in the same genetic background (Dangl and Jones 2001; Mundt 2002). A better understanding of resistance genes and their interactions with different races of *P. infestans* will lead to novel

techniques for managing late blight, allowing potato plants and *P. infestans* to coexist without severe production losses.

Presently, *P. infestans* effectoromics is employed to identify the potential potato germplasm exhibiting late-blight resistance germplasms. The technique involves transforming and transiently expressing RXLRs recombinant plasmids into plant leaves to determine the existence of potential resistance genes in host materials based on the triggered HR reaction (Vleeshouwers et al., 2008; Oh et al., 2009; He et al., 2019; Ren et al., 2019). While this strategy is reliable, it is greatly inefficient. RNA sequencing (RNA-seq) is a newly developed method for a comprehensive understanding of the host-pathogen interactions, involving simultaneous analysis of the gene expression changes in both the pathogen and host genomes (Westermann et al., 2012, 2017; Du et al., 2021).

2.8 Gene expression profiling in plant-pathogen interactions

Gene expression profiling can be used to study how genes respond to different stimuli, such as pathogens, and identify genes involved in various biological processes, such as disease resistance. Gene expression profiling can be performed using different methods, such as DNA microarrays, RNA-Seq, or SAGE. Gene expression profiling is a powerful tool to study plant-pathogen interactions' molecular mechanisms and identify genes involved in disease resistance or susceptibility. Gene expression profiling can reveal the changes in gene expression levels of both the host and the pathogen during infection, and can also compare the responses of different genotypes or treatments to the same pathogen.

One example of gene expression profiling in plant-pathogen interactions is the study of late blight resistance in potato, which is caused by the oomycete pathogen *Phytophthora infestans*. Late blight resistance is a complex trait that involves multiple genes and signaling pathways, and the pathogen can rapidly evolve to overcome the resistance genes. In this context, gene expression profiling can

help to understand how different potato genotypes respond to different isolates of *P. infestans*, and to identify candidate genes and pathways for resistance breeding. For instance, Yang et al. (2020) performed transcriptome analysis of a high late blight-resistant potato genotype SD20 under exogenous ethylene application, a hormone that regulates plant defense responses. They found that ethylene activated immune and defense-related genes and pathways in SD20, such as transcription factors, kinases, defense enzymes, and disease-resistance genes. They also found that ethylene stimulated a similar defense pathway as pathogen infection in SD20, which involved multiple signaling hormones such as salicylic acid, jasmonic acid, abscisic acid, auxin, cytokinin, and gibberellin. These results suggested that ethylene modulates genome-wide transcriptional regulation in SD20 and enhances its resistance to *P. infestans*.

Another example of gene expression profiling in late blight resistance is the study of Yang et al. (2018), who performed a time-course experiment to profile the late blight resistance response genes using RNA-sequencing in SD20 after infection by a super race isolate CN152 of P. infestans, which can overcome nearly all known late blight resistance genes. They identified 3354 differentially expressed genes (DEGs), which mainly encoded transcription factors and protein kinases, and also included four NBS-LRR genes, which are a common class of plant disease resistance genes. They also found that multiple signaling pathways of salicylic acid, jasmonic acid, and ethylene were involved in resistance and defense against P. infestans in SD20.

Gene expression profiling can also reveal the transcriptome changes of *P. infestans* during its asexual development and infection process. For example, Judelson et al. (2008) performed a microarray analysis of P. infestans during sporulation, germination, appressorium formation, and infection of potato leaves. They found that the transcriptome of *P. infestans* was highly dynamic and regulated by developmental cues and environmental signals. They identified genes specifically expressed or repressed at each stage of development or infection, such as metabolism, cell wall synthesis, signal transduction, pathogenicity factors, and effector proteins. These studies demonstrate the potential

of gene expression profiling to uncover the molecular basis of late blight resistance in potato and the pathogenicity mechanisms of *P. infestans*. Gene expression profiling can provide valuable information for developing novel disease management and resistance breeding strategies.

2.8.1. Transcriptome analysis

Large-scale transcriptome data allow examining the expression of tens of thousands of genes over time or over a set of conditions under study such as biotic and abiotic stresses (Ahmadvand, 2013). In recent years, numerous technologies have been developed to analyze and quantify the transcriptome. Initially, a traditional sequencing method was used, but this approach was costly and time-consuming because it involved cDNA library construction, cloning, and labor-intensive Sanger sequencing. The advent of the remarkable technology, Next Generation Sequencing (NGS), allowed direct and cost-effective sequencing of DNA at an impressive speed (Ahmadvand, 2013).

The massively parallel sequencing platform, NGS, was introduced in 2004. Next-generation sequencing (NGS) is a collective term for various sequencing methods that can produce massive amounts of DNA or RNA sequences in a fast and cost-effective manner (Pervez et al., 2022). NGS has many applications in different fields that involve DNA or RNA analysis, such as genomics, transcriptomics, metagenomics, epigenomics, and others. Transcriptomics is the study of the transcriptome, which is the complete set of RNA transcripts expressed by a cell, tissue, or organism under specific conditions. Transcriptomics can reveal gene expression patterns, alternative splicing events, gene fusion events, and other molecular mechanisms that regulate cellular functions and phenotypes. NGS can be used to perform transcriptome analysis by sequencing the RNA molecules extracted from the samples of interest. Depending on the type of RNA molecules to be sequenced, different NGS methods can be applied, such as RNA-seq, small RNA-seq, single-cell RNA-seq, long-read RNA-seq, and others. NGS platforms vary in their template preparation, sequencing chemistry, read length, output per run, and quality of the reads. The read length is the

number of nucleotides that can be sequenced in a single run. The output per run is the total amount of nucleotides that can be sequenced in a single run. The quality of the reads is measured by the accuracy and error rate of the sequencing process. Some of the most widely used NGS platforms are developed by Illumina, Ion Torrent, BGI, PacBio, and Oxford Nanopore. Each platform has its own strengths and weaknesses depending on the research. For example, Illumina platforms have high accuracy and output but short read lengths and high cost. Ion Torrent platforms have fast turnaround time and low cost but lower accuracy and output than Illumina. BGI platforms use different sequencing chemistry based on DNA nanoballs and are similar to Illumina platforms but cheaper. PacBio platforms have long read lengths and can capture complex regions of the transcriptome but have lower accuracy and output than Illumina and BGI. Oxford Nanopore platforms have the longest read lengths and can sequence any type of RNA in real time but have lower accuracy and higher error rates than other platforms (Pervez et al., 2022). Table 1 shows the comparison of the different available NGS technologies.

Table 1. Comparison of available NGS technologies.

Company	Template Preparation	Sequencing Chemistry	Read Length (bp)	Total Output per Run (Gbp)	Applications	Website
Illumina	Random fragmentation or PCR amplification	Reversible terminator-based sequencing by synthesis (SBS)	50-300 (single-end) or 100-600 (paired-end)	1.8-6 (MiSeq), 120-600 (NextSeq), 600-3000 (NovaSeq)	Whole-genome sequencing, transcriptome sequencing, metagenomics, epigenomics, etc.	http://www.illumina.com/
Ion Torrent	Random fragmentation or PCR amplification	Semiconductor sequencing by pH detection of hydrogen ions released during DNA polymerization	200-400 (single-end) or 100-200 (paired-end)	0.3-2 (Ion S5), 10-80 (Ion Proton)	Targeted sequencing, whole-exome sequencing, transcriptome sequencing, etc.	http://www.thermofisher.com/us/en/ home/life-science/sequencing/next-generation-sequencing.html/
Pacific Biosciences (PacBio)	Circular consensus sequencing (CCS) or continuous long-read (CLR) sequencing of single molecules in zero-mode waveguides (ZMWs)	Real-time sequencing by detection of fluorescent pulses emitted by DNA polymerase during DNA synthesis	10,000-20,000 (CCS), >20,000 (CLR)	60-160 (Sequel IIe)	Whole-genome sequencing, transcriptome sequencing, metagenomics, epigenomics, etc.	http://www.pacb.com/
Oxford Nanopore Technologies (ONT)	Linearization or PCR amplification of single molecules and addition of motor proteins	Real-time sequencing by detection of changes in electric current as DNA	>10,000 (MinION/GridION/ PromethION), >100,000 (Flongle)	4-30 (MinION), 120-1500 (GridION), 6000-9000 (PromethION), 1.8-3.6 (Flongle)	Whole-genome sequencing, transcriptome sequencing, metagenomics, epigenomics, etc.	http://nanoporetech.com/

	and adapters for nanopore translocation	strands pass through nanopores				
BGI Genomics	Random fragmentation or PCR amplification of DNA nanoballs (DNBs) on patterned arrays	Sequencing by synthesis with combinatorial probe-anchor ligation chemistry and fluorescent imaging on DNBs	50-150 (single-end) or 100-300 (paired-end)	1800-7200 (MGISEQ-T7), 900-3600 (MGISEQ-2000), 180-720 (MGISEQ-200), 60-240 (DNBSEQ-G400)	Whole-genome sequencing, transcriptome sequencing, metagenomics, epigenomics, etc.	https://www.bgi.com/global

Reference: (Pervez et al., 2022)

2.8.1.1. *De novo* transcriptome analysis

De novo transcriptome assembly is often the preferred method to study non-model organisms since it is cheaper and easier than building a genome, and reference-based methods are not possible without an existing genome. The transcriptomes of these organisms can thus reveal novel proteins and their isoforms that are implicated in such unique biological phenomena (Ahmadvand, 2013).

To create a transcriptome database in plant species without a sequenced genome (*de novo* sequencing), many researchers have used 454 NGS since it produces the longest reads among current NGS technologies. Because during the assembly of contigs, single reads are assessed for their overlapping sequence. The assembly becomes increasingly more difficult when the read length gets shorter and shorter (Pop and Salzberg, 2008). Indeed, two different strategies are possible for *de novo* sequencing. If the non-model species is closely related to a species with a sequenced genome, the sequence reads can be mapped onto the reference. In this case, the type of mapping software can influence the results. In the second strategy, the normalized cDNA libraries from all conditions to be analyzed later and non-normalized libraries from these conditions should be assembled into a reference transcriptome. Using a long-read sequencing technology for at least the normalized cDNA library will facilitate the assembly (Pop and Salzberg, 2008).

2.8.1.2. Transcriptome analysis of the sequenced genomes

Prior to the development of transcriptome assembly computer programs, transcriptome data were analyzed primarily by mapping onto a reference genome, which is a robust way of characterizing transcript sequences. Transcriptome analysis of species with a fully sequenced genome identifies novel transcripts and identifies splicing isoforms. A much higher sequencing depth at a comparable cost can be achieved using short-read technology such as the Illumina Genome Analyser or the Applied Biosystems SOLiD system, producing over 100 million sequencing reads. These reads are

directly mapped to the genome sequence (Cloonan et al., 2008; Lister et al., 2008; Mortazavi et al., 2008). Splice isoforms can be identified by reads reaching over predicted exon boundaries (Mortazavi et al., 2008).

Novel genes and incorrectly annotated 5' or 3' untranslated regions are discovered if reads map to genomic regions for which no elements were annotated. The abundance of a transcript can be measured simply by counting how many maps onto a given gene reads. In contrast to microarray experiments, which report a ratio of fluorescence in arbitrary units, NGS measurements are absolute (Ahmadvand, 2013). To compare the abundance of transcripts within a sequence library these read counts are often normalized to the transcript length, e.g., reads per kilobase (RPK) of the transcript. To compare the abundance of transcripts in two different libraries generated in two different conditions of an organism, the read counts are normalized to one million reads. According to this method, the abundance of a certain transcript in a certain cDNA population/sequence library, obtained by NGS, is generally given as reads per kilobase per million mapped sequence read (RPKM), meaning reads counted per 1000 bp of this transcript and per one million total reads from the sequence library (Mortazavi et al., 2008). This way, not only relative but absolute abundance values are determined for a given condition.

Mapping the huge amounts of short read sequences produced by NGS to a given reference sequence is challenging. A traditional and well-established sequence alignment tool like basic alignment search tool (BLAST) (Altschul et al., 1997) can be used for mapping these short reads, but BLAST is not optimized to cope with high numbers of reads; therefore, such mappings are very time-consuming. The blast-like alignment tool (BLAT) was developed to perform alignment tasks much faster (Kent, 2002). BLAT is suitable to map reads from the 454 platform, but short read sequencing technologies can produce over ten times more data within a single run, thus new bioinformatics tools capable of dealing with such huge amounts of data have been developed (Flicek and Birney, 2009). Currently, there is much progress in the development of such software,

leading to the publication of several new programs within the last few years. Since experience with these programs and also comparative investigations is limited at the moment, it is difficult to predict if and which of these tools will become accepted as the standard. Perhaps it will turn out that, depending on the number of reads, read length and genome complexity of the organism investigated, a different program is favored (Palmieri & Schlotterer 2009). The high throughput, short-read NGS systems have been successfully used in several studies for quantitative and qualitative transcriptome analysis in animal, plant, and microbial model systems (Cloonan et al. 2008; Mortazavi et al. 2008). An example of a particularly comprehensive study comes from Lister et al. (2008). By combining different techniques, they assessed the strand-specific transcriptome, small RNA-s, and cytosine methylation in Arabidopsis on the genome scale, using short-read sequencing with the Illumina Genome Analyser. The comparison of wild-type plants, DNA methyl transferase, and DNA demethylase mutants allowed analysis of the interactions between DNA methylation, small RNA function, and effects on transcriptional regulation within the experiment. Publication of the potato genome sequence has provided the possibility to apply genome sequence as a reference sequence for the transcriptome analysis of this crop. In this study, the transcriptome profile of the resistant potato cultivar, White Lady in response to *P. infestans* was generated by NextSeq 500 (Illumina, USA) type sequencer, using High Output 150 sequencing kits (Illumina, USA).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Plant materials used in PI inoculation test

To examine the reaction of White Lady (Hungarian potato variety) to *Phytophthora infestans*, disease free minitubers were used in this experiment at the Potato Research Centre (Hungarian University of Agriculture and Life Sciences, Keszthely, Hungary). The plants were grown under vector-free greenhouse and phytotron conditions. Previously, the White Lady cultivar showed resistance to *P. infestans,* displaying a hypersensitive response (HR).

3.2. Methods of the *P. infestans* inoculation tests

For the *P. infestans* resistance tests, the MP-1548 isolate was used. This isolate was obtained upon request from Dr. Jadwiga Sliwka, IHAR, Mlochow, Poland. It was collected in 2012 from the potato variety Irys in the Mazowieckie Region. From the obtained information, the isolate belongs to the A1 mating type, and its type of virulence is 1, 3, 4, 6, 7, 8, 10, and 11. However, we found that the isolate produced oospores, which is not a characteristic of the A1 mating type, and its virulence was found to be 1, 2, 3, 4, 5, 6, 7, 8, 10, and 11 types. All of these indicated that the obtained isolate is a mixed A1-A2 type. Nevertheless, we used this isolate for infection tests because of the shortage of time in the project.

Infection with the *P. infestans* isolate was done directly on the abaxial leaf surface of living plants (whole plant assay) and detached leaf assay (DLA). For each infected leaf, a structure was built at first to keep the leaf in a horizontal position up with its abaxial side. For the infection solution, *P. infestans* was grown on 1.0-1.5 cm thick tuber slices of the variety Hópehely, which have been previously sterilized in 10% sodium hypochlorite solution for 10 minutes and rinsed several times

before inoculation. Tuber slices were incubated at 17°C for six days to let the mycelium grow. Then, from 20 tuber slices, the phytophthora mycelium was moved with sterile tools into 10 ml of sterile distilled water and steered vigorously to let the sporangia be separated from the mycelium. After filtering the solution through four sheets of gauze, sporangium density was measured with a Bürker chamber, adjusted to 15,000 sporangium/mL, and incubated for 2 hrs at 5-6°C. Before infection, the solution was left to warm to 20°C. The inoculation was done by applying 50 μL in one drop from the solution on the leaf surface. The infection solution was removed the next morning by pipetting it off. Five plants were infected, and two leaves of each plant were used for infection.

Samples for transcriptome analyses were taken at four different time points (18-, 24-, 48-, and 72-hours post-infection, hpi) from uninfected leaflets of infected leaves. The samples were collected on dry ice and were stored at -80°C until RNA extraction.

It is worth mentioning that because of the discrepancy between the description of the MP-1548 isolate and our observations, with the contribution of József Bakonyi (Plant Protection Institute, Centre for Agricultural Research, HAS), a pure culture of the MP-1548 isolate that is not producing oospores and also a monosporic culture of the isolate has been produced in these days. This monosporic culture does not produce oospores and belongs to the A1 mating type. Whole genome sequencing of these three isolate types will be performed soon to explore their differences.

3.3. Wet lab methods of transcriptome analyses.

50 mg of leaf samples of the control and of the treated four time-points samples were smashed in 2 mL tubes in the presence of 100 μL TRIzol using a DNA mill (Retsch, Germany). Then, 400 μL TRIzol was added, and total RNA was extracted with the Direct-zol RNA Microprep kit (Zymo Research, USA) following the protocol of the producer. Poly-A enrichment was done with the Poly(A) RNA Selection kit (Lexogen, Austria). RNA integrity was checked with 2100 Bioanalyzer

(Agilent, USA) using the RNA 6000 Pico kit. For further processing RNA with RIN value higher than 5.5 was used. Sequencing libraries were prepared with the NEXTFLEX Rapid Directional RNA-Seq kit 2.0 (PerkinElmer, USA) according to the producer's guidelines. Sequencings were done on a NextSeq 500 (Illumina, USA) type sequencer, using High Output 150 sequencing kits (Illumina, USA).

3.4. Transcriptome construction and Data analysis methods

De novo reconstruction of the transcriptomes was done using the SOAPdenovo program. For gene expression analyses, the *de novo* transcriptome of White Lady was used as a reference. The "index" and "quant" commands of the Salmon program package were used to quantify RNA-Seq data. Quantified transcripts were analyzed and visualized with the DESeq2 package in 'R' environment. Further analyses of the transcript sequences are done with the seqkit program, supplemented with the "grep", "awk", "join", and "comm" commands.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1. Results of the late blight resistance analyses

The White Lady's transcriptomes [one control and four treated (18, 24, 48 and 72 hours post infection, hpi)] were reconstructed. In control, 92,079 transcripts were obtained in total. 2198, 845, 1100, and 1210 transcripts showed significant upregulation at 18, 24, 48, and 72 hpi, respectively. Since the transcriptomes are not yet completely annotated, we chose the most upregulated first 200 transcripts and checked them one by one in the NCBI and then in the UniProt database to identify the resistance gene-like sequences among them. Gene Ontology and KEGG pathway enrichment analysis were also used to gain mechanistic insight into the resistance gene lists.

The immune-responsive genes showed time-point-specific induction/repression. Out of the most upregulated 200 transcripts that were checked, 15, 19, 4, and 3 differentially expressed genes (DEGs) were linked to immune response at 18, 24, 48, and 72 hpi, respectively, while 9 DEGs were linked to immune response were continuously upregulated at all the four time points (Tables 2, 3, 4, 5, & 6). These DEGs include transcription factors, protein kinases, and 13 NBS-LRR genes.

Two of the NBS-LRR coding genes were annotated as *S. tuberosum* Disease resistance protein R3a, and Disease resistance protein RGA2, *S. bulbocastanum* Blight resistance protein RPI, RGA2-blb (which could play a role in broad-spectrum resistance to Pi infection in White Lady variety). Other NBS-LRR coding genes were annotated as CC-NB-LRR protein, NB-ARC domain-containing protein, Cc-nbs-lrr resistance protein, Hero resistance protein 1 homologue, and ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase, 3.2.2.6. (Tables 2, 3, 4, 5, & 6)

Studies have reported that several protein kinases (PKs) also regulate the resistance to late blight in potatoes (King et al., 2014; Ren et al., 2019). PKs were found to be differentially regulated.

These PKs were from families including serine/threonine protein kinase (STK), Leucine-rich repeat transmembrane protein kinase, histidine kinase (HK), calcium-dependent protein kinase (CDPK), and receptor-like kinase (RLK), among others (Tables 2, 3, 4, 5, & 6). Other defense response genes that were upregulated include heat shock protein 90 family (Tables 2 and 6), and WRKY (Table 2) transcription factor. Stress responses are characterized by the induction of heat shock proteins (HSP) and defense responses by the induction of pathogenesis-related (PR) genes and other genes associated with plant disease defense (Whitham et al., 2006). The members of the WRKY transcription factor family, which is a type of zinc-finger family proteins, are involved in the regulation of various physiological processes that are unique to plants including pathogen defense and senescence (Eulgem, 2005). They are important regulators of salicylic acid-dependent defense responses. It is demonstrated that they bind to promoter elements of defense-related genes and regulate, activate, or repress their expression (Eulgem et al., 2000).

Whole transcriptome shotgun sequencing, known as RNA-seq is becoming a widely used high-throughput powerful tool for transcriptome profiling, comparative gene expression analysis, and gene identification (Chen *et. al*, 2016, Muthusamy *et al*., 2016, Li *et al*., 2017). Several studies have used RNA-seq to identify genes involved in the potato response to late blight at the genome level.

The result of our study, therefore, corresponds with the findings of Yang *et al.,* 2018, who reported that late blight-responsive genes showed time-point-specific induction/repression by identifying DEGs, which mainly encoded transcription factors and protein kinases, and also included four NBS-LRR genes. A similar result was reported by Li *et al.,* 2022, stating that resistant potatoes activated a set of biotic stimulus responses and phenylpropanoid biosynthesis genes, including kirola-like protein, NBS-LRR, disease resistance, heat shock proteins, and kinase genes. Yang *et al.,* 2018, also reported that forty-three DEGs were involved in the immune response, of which 19 were enriched in hypersensitive response reaction, which could play an important role in broad-spectrum resistance to Pi infection.

Table 2: Defense Response DEGs at 18 hours post infection (hpi)

*****N/A: Not Available, PPI: Plant-Pathogen Interactions

S/N	Transcript ID	UniProt ID	Protein Name	Organisms	Protein Families	Gene Ontology	KEGG Pathway
1	14007	M1ACA7	Cc-nbs-lrr resistance protein	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	N/A
2	117423	M1A6U3	Leucine-rich repeat family protein	*Solanum tuberosum* (Potato)	N/A	defense response to other organism	N/A
3	30710	M1BN91	NB-ARC domain-containing protein	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	PPI (sot04626)
4	247	A0A1S3XEG1	putative leucine-rich repeat-containing protein DDB_G0290503	*Nicotiana tabacum* (Common tobacco)	N/A	N/A	N/A
5	31786	M1B8U6	Leucine-rich repeat family protein / protein kinase family protein	*Solanum tuberosum* (Potato)	N/A	plasma membrane; ATP binding; protein serine/threonine kinase activity; protein phosphorylation	N/A
6	32051	M1B054	leucine--tRNA ligase, 6.1.1.4, Leucyl-tRNA synthetase	*Solanum tuberosum* (Potato)	class-I aminoacyl-tRNA synthetase family.	leucyl-tRNA aminoacylation; post-embryonic development; reproductive structure development	N/A
7	10058	M1CWC8	Pleiotropic drug resistance protein 1	*Solanum tuberosum* (Potato)	ABC transporter superfamily. ABCG family. PDR (TC 3.A.1.205) subfamily.	membrane; ABC-type transporter activity; ATP binding	N/A
8	101835	A0A2G3BUT0	non-specific serine/threonine	*Capsicum chinense*	protein kinase superfamily.	ATP binding; protein serine/threonine kinase activity; protein phosphorylation	N/A

			protein kinase, 2.7.11.1	(Bonnet pepper)	CMGC Ser/Thr protein kinase family. GSK-3 subfamily.		
9	10546	M1AP53	Serine/threonine protein kinase	*Solanum tuberosum* (Potato)	N/A	cytoplasm; ATP binding; protein kinase activity; protein serine/threonine kinase activity; protein phosphorylation; signal transduction	N/A
10	11456	A0A2G2WQ68	Putative serine/threonine protein kinase IRE3	*Capsicum baccatum* (Peruvian pepper)	N/A	ATP binding; protein serine/threonine kinase activity; protein phosphorylation	N/A
11	115071	M1C004	Phosphoglycerate kinase, 2.7.2.3	*Solanum tuberosum* (Potato)	phosphoglycerate kinase family.	cytosol; ADP binding; ATP binding; phosphoglycerate kinase activity; gluconeogenesis; glycolytic process	N/A
12	42433	M1D6Q4	Aspartate kinase	*Solanum tuberosum* (Potato)	homoserine dehydrogenase family.; aspartokinase family.	aspartate family amino acid biosynthetic process; homoserine biosynthetic process; lysine biosynthetic process via diaminopimelate; methionine biosynthetic process; phosphorylation; threonine biosynthetic process	N/A
13	68824	M1C047	WRKY DNA-binding protein	*Solanum tuberosum* (Potato)	N/A	nucleus; DNA-binding transcription factor activity; sequence-specific DNA binding	PPI (sot04626)
14	94622	M1D6X7	Calcium-dependent protein kinase 8	*Solanum tuberosum* (Potato)	N/A	intracellular signal transduction; peptidyl-serine phosphorylation; protein autophosphorylation	PPI (sot04626)
15	63579	M1ALZ6	Endoplasmin homolog	*Solanum tuberosum* (Potato)	heat shock protein 90 family.	protein folding	PPI (sot04626)

Table 3: Defense Response DEGs at 24 hours post infection (hpi)

***N/A: Not Available, PPI: Plant-Pathogen Interactions

S/N	Transcript ID	UniProt ID	Protein Name	Organisms	Protein Families	Gene Ontology	KEGG Pathway
1	41009	A0A1U8E4I9	Putative LRR receptor-like serine/threonine-protein kinase	*Capsicum annuum* (Capsicum pepper)	protein kinase superfamily. Ser/Thr protein kinase family.	membrane; ATP binding; protein serine/threonine kinase activity; defense response to other organism; protein phosphorylation	N/A
2	51914	M1BMQ8	Cc-nbs-lrr resistance protein	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	PPI (sot04626)
3	33394	A0A3Q7EG58	NB-ARC domain-containing protein	*Solanum lycopersicum* (Tomato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	N/A
4	47642	A0A3Q7GDL1	NB-ARC domain-containing protein	*Solanum lycopersicum* (Tomato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	N/A
5	48613	A0A3Q7GDL1	NB-ARC domain-containing protein	*Solanum lycopersicum* (Tomato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	N/A
6	11784	M1CVN8	Hero resistance protein 1 homologue	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	N/A
7	47619	Q7XBQ9	Disease resistance protein RGA2, Blight resistance protein RPI, RGA2-blb	*Solanum bulbocastanum* (Wild potato)	disease resistance NB-LRR family.	ADP binding; ATP binding; defense response to other organism	N/A
8	108359	M1BF53	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase, 3.2.2.6	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	nucleus; ADP binding; defense response to fungus; signal transduction	N/A
9	30616	A0A3Q7EQZ7	Protein kinase domain-containing protein	*Solanum lycopersicum* (Tomato)	protein kinase superfamily. Ser/Thr protein kinase family.	membrane; ATP binding; protein serine/threonine kinase activity; defense response to other organism; protein phosphorylation	N/A
10	37353	M1CMS1	Somatic	*Solanum tuberosum*	protein kinase	membrane; ATP binding; protein kinase	N/A

			embryogenesis receptor-like kinase 1	(Potato)	superfamily. Ser/Thr protein kinase family.	activity; defense response to other organism; protein phosphorylation	
11	111390	M1C004	Phosphoglycerate kinase, 2.7.2.3	*Solanum tuberosum* (Potato)	phosphoglycerate kinase family.	cytosol; ADP binding; ATP binding; phosphoglycerate kinase activity; gluconeogenesis; glycolytic process	N/A
12	117076	M1BKB3	DNA binding protein	*Solanum tuberosum* (Potato)	N/A	nucleus; DNA-binding transcription factor activity; protein dimerization activity; transcription cis-regulatory region binding; regulation of DNA-templated transcription	N/A
13	129676	M1CG26	CCG-binding protein 1	*Solanum tuberosum* (Potato)	N/A	cytosol; nucleus; mediator complex binding; pollen tube guidance	N/A
14	29420	M1DMR8	Kinase	*Solanum tuberosum* (Potato)	N/A	plasma membrane; ATP binding; protein kinase activity; transmembrane receptor protein tyrosine kinase activity; protein autophosphorylation	N/A
15	35935	M1BJJ1	Pantothenate kinase 2, 2.7.1.33	*Solanum tuberosum* (Potato)	type II pantothenate kinase family	cytosol; nucleus; ATP binding; pantothenate kinase activity; coenzyme A biosynthetic process; phosphorylation	N/A
16	40060	M1A965	Nucleic acid binding protein	*Solanum tuberosum* (Potato)	N/A	metal ion binding; RNA binding	N/A
17	56368	Q9AWA5	Alpha-glucan water dikinase, chloroplastic, 2.7.13.3, 2.7.9.4, Starch-related R1 protein	*Solanum tuberosum* (Potato)	PEP-utilizing enzyme family.	chloroplast; ATP binding; maltodextrin water dikinase; metal ion binding; protein histidine kinase activity; starch, H2O dikinase activity; carbohydrate metabolic process	N/A
18	64879	M1D6G5	Sensory transduction histidine kinase	*Solanum tuberosum* (Potato)	ARR-like family.	nucleus; cytokinin-activated signaling pathway; phosphorelay signal transduction system; rhythmic process	N/A
19	66131	M1BGA7	Receptor-like serine/threonine-protein kinase, 2.7.11.1	*Solanum tuberosum* (Potato)	protein kinase superfamily. Ser/Thr protein kinase family.	membrane; ATP binding; protein serine kinase activity; protein serine/threonine kinase activity; protein phosphorylation	N/A

Table 4: Defense Response DEGs at 48 hours post infection (hpi)

*****N/A: Not Available

S/N	Transcript ID	UniProt ID	Protein Name	Organisms	Protein Families	Gene Ontology	KEGG Pathway
1	**10211**	M1BF53	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase, 3.2.2.6	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	nucleus; ADP binding; defense response to fungus; signal transduction	N/A
2	27379	M0ZXF4	Leucine-rich repeat transmembrane protein kinase	*Solanum tuberosum* (Potato)	N/A	membrane; ATP binding; protein kinase activity; receptor serine/threonine kinase binding; defense response to other organism; protein phosphorylation	N/A
3	13842	M1ASW2	Nucleotide binding protein	*Solanum tuberosum* (Potato)	N/A	N/A	N/A
4	86293	M1CK90	3,4-dihydroxy-2-butanone kinase	*Solanum tuberosum* (Potato)	N/A	cytosol; ATP binding; glycerone kinase activity; glycerol catabolic process; phosphorylation	N/A

Table 5: Defense Response DEGs at 72 hours post infection (hpi)

*****N/A: Not Available

S/N	Transcript ID	UniProt ID	Protein Name	Organisms	Protein Families	Gene Ontology	KEGG Pathway
1	10211	M1BF53	ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase, 3.2.2.6	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	nucleus; ADP binding; defense response to fungus; signal transduction	N/A
2	23182	M1E040	Pollen-specific kinase partner protein	*Solanum tuberosum* (Potato)	N/A	guanyl-nucleotide exchange factor activity	N/A
3	59321	A0A2G2WEZ0	Serine/threonine-protein kinase 24	*Capsicum baccatum* (Peruvian pepper)	N/A	ATP binding; protein kinase activity; protein phosphorylation	N/A

Table 6: Defense Response continuously upregulated DEGs at all four time points

*****N/A: Not Available; PPI: Plant-Pathogen Interactions

S/N	Transcript ID	UniProt ID	Protein Name	Organisms	Protein Families	Gene Ontology	KEGG Pathway
1	36939	M1ABJ9	CC-NB-LRR protein	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	PPI (sot04626)
2	21923	A0A2G2W4X3	NB-ARC domain-containing protein	*Capsicum baccatum* (Peruvian pepper)	disease resistance NB-LRR family.	cytoplasm; membrane; ADP binding; plant-type hypersensitive response	N/A
3	4557	M1BLZ6	Disease resistance protein R3a	*Solanum tuberosum* (Potato)	disease resistance NB-LRR family.	ADP binding; defense response to other organism	N/A
4	71273	M1CS93	Verticillium wilt disease resistance protein	*Solanum tuberosum* (Potato)	RLP family.	plasma membrane; defense response to fungus	N/A
5	23954	M1ANN3	Ubiquitin-protein ligase	*Solanum tuberosum* (Potato)	N/A	defense response to other organism	N/A
6	50823	A0A3Q7HWV8	Phosphatidylinositol 4-phosphate 5-kinase, 2.7.1.68	*Solanum lycopersicum* (Tomato)	N/A	plasma membrane; 1-phosphatidylinositol-4-phosphate 5-kinase activity; ATP binding; phosphatidylinositol phosphate biosynthetic process; phosphorylation	N/A
7	56591	M1AI77	NAK-like ser/thr protein kinase	*Solanum tuberosum* (Potato)	N/A	membrane; ATP binding; protein serine/threonine kinase activity; protein phosphorylation	N/A
8	87856	A0A3Q7EGA4	Protein kinase domain-containing protein	*Solanum lycopersicum* (Tomato)	N/A	ATP binding; protein kinase activity; protein phosphorylation	N/A
9	115801	M1BQI2	Heat shock protein 90	*Solanum tuberosum* (Potato)	heat shock protein 90 family.	unfolded protein binding; de-etiolation; protein folding; protein import into chloroplast stroma; response to chlorate; response to heat; response to salt stress; response to water deprivation	PPI (sot04626)

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The Pi-responsive genes and their expression patterns will aid in our understanding of critical resistance genes to late blight and give a biological basis for plant-pathogen interactions. This study employed a time course RNA-seq to evaluate the resistance response of the White Lady potato variety to *P. infestans* inoculation. The result of this present study showed that the White Lady potato cultivar exhibited time-point-specific induction/repression of the late blight response genes and biotic stimulus responses. These results provide valuable information for understanding the late blight resistance mechanism of potato.

5.2. Recommendations

The result of this study showed that the White Lady potato variety exhibited late blight resistance response genes in which induction/repression was time-point specific. However, there is a need to validate the results of RNA-seq data by analyzing the expression levels of the DEGs through qRT-PCR. Comparing the transcription profiling data from the RNA-seq analysis and qRT-PCR will help us determine the reliability of our result. For instance, Yang *et al.,* 2018, and Li *et al.,* 2022, employed this method to show that their transcriptome profiling data were highly reliable.

Another tool that could be used for genetic mapping, searching, and testing the functionality of resistance genes in cultivars and breeding lines is dRenSeq (Armstrong et al. 2019). dRenSeq has been used to identify and validate all currently known NLRs effective against potato virus X, the potato cyst nematode *Globodera pallida,* and *P. infestans*.

The introgression of *Rpi* genes into susceptible potato cultivars is limited by long breeding cycles and the high level of heterozygosity across the potato genome (Jo et al. 2014). To facilitate the transfer of resistance to late blight to potato cultivars, hybrid breeding, using existing elite material and marker-assisted introgression allows for obtaining resistant plants in a relatively short time. This enables obtaining plants with single or pyramided *Rpi* genes without disrupting the genetic composition of the parental breeding lines that have good agronomic performance. Su *et al.* (2020).

Molecular markers that are genetically connected to R genes can be used to select resistance genotypes at early stages of selection without requiring a pathogen inoculation test. As molecular markers, DNA fragments amplified by polymerase chain reaction (PCR) from regions closely connected to an R gene or from the R gene-coding region itself are utilized. For instance, specific primers are available for the gene R1, which is found on potato chromosome 5 (Leonards-Schippers et al. 1992, Ballvora et al. 2002). Furthermore, Ohbayashi *et al.* (2010) designed a PCR marker, R2-800, that can be used to identify R2 holders by combining sequence information for AFLP markers strongly connected to R2, which has been localized to potato chromosome 4 (Li et al. 1998). Mori et al. (2011) devised a multiplex PCR approach for selecting resistance genes to late blight (R1 and R2), cyst nematode pathotype Ro1 (H1), Potato virus X (Rx1), and Potato virus Y. (Ry chc). Using this approach, five genotypes with all five resistance gene markers were swiftly and cheaply selected from 96 inter-varietal hybrid genotypes.

An alternative approach involves genetic engineering, which significantly shortens the long time to introgress resistance genes through the breeding cycle for tetraploid potato plants (Van Esse et al. 2020). One such method is cisgenesis, i.e., the introduction of genetic material from the same species or from a crossable species (Hou et al. 2014). Gene editing techniques are an alternative approach to introducing Rpi genes into potato cultivars by conventional methods or by genetic engineering. Gene editing can be used to repair non-functional alleles of Rpi genes (Paluchowska et al., 2022).

6.0 SUMMARY

Late blight caused by the oomycete fungus, *Phytophthora infestans* (*Pi*), is the most devastating disease affecting potato (*Solanum tuberosum*) production in the world. This disease is hard to control because the Pi races possess high evolutionary potential, and can overcome known resistance genes. Recently, farmers have controlled late blight primarily with chemicals, but the chemical sprays are expensive and result in environmental pollution. The general purpose of this research project is to explore the genetic background of resistance against *P. infestans*. In the gene bank of the Potato Research Centre at Keszthely, there are different genotypes that convey resistance against the late blight-causing *P. infestans*. The final result of this project should be the development of molecular tools, which can be effectively used in resistance breeding of potatoes. For this goal, we use high-throughput molecular technologies with the following approaches: 1. Highly saturated maps of the potato genome were constructed based on 31,190 SNP markers which are identified by microarray analysis. 2. The haplotype-resolved whole genome sequence of the tetraploid cultivar, White Lady, was reconstructed from short (150 bp) Illumina reads and long (8,000 bp) PacBio HiFi reads. 3. For each genotype, transcriptome datasets of samples taken before and after the infections were generated.

In the present study, as a part of the project the resistance response to *P. infestans* inoculation of the variety, White Lady, was analyzed. This variety contains the R1, R2, R3a, R4, R5, R6, R7, R8, R10, and R11 genes that convey strain-specific resistance against *P. infestans*. The result of this study showed that the White Lady potato cultivar exhibited time-point-specific induction/repression of the late blight response genes. These results provide valuable information for understanding potato's late blight resistance mechanism. However, there is a need to validate the results of RNA-seq data by analyzing the expression levels of the DEGs through qRT-PCR. Comparing the transcription profiling data from the RNA-seq analysis and qRT-PCR will help us determine/validate the reliability of our result.

ACKNOWLEDGEMENTS

I return all glory to Almighty God for successfully seeing me through my MSc program. By His grace, I am what/who I am (1 Cor.15:10) for I do not see myself as capable enough to do anything in my own strength, for my true competence flows from God's empowering presence (2 Cor. 3:5).

I would like to express my sincere gratitude to the Food and Agriculture Organization (FAO) of the United Nations, the Hungarian Ministry of Agriculture, and the British Society for Plant Pathology (BSPP) for their generous funding towards the completion of my master's degree. Additionally, I appreciate everyone who have supported me through this journey, starting with my primary supervisor, Prof. János Taller, for his invaluable mentorship, encouragement, and feedback. He has been a great mentor and a source of inspiration to me. I am also grateful to my co-supervisor, Dr. Krisztián Frank, for his invaluable input and constructive comments that helped me improve the quality of my work. I also thank Erzsi, my lab technician at the Institute of Genetics and Biotechnology of the University, including Melinda, Tamas, and Dr. Istvan Wolf of the Potato Research Centre of the University, for their guidance and assistance during the research. Thanks to Prof. András Takács, and other faculty of the Plant Protection Institute at Keszthely.

Furthermore, I would like to acknowledge the contributions of my fellow graduate students, especially, Siraj, Esther, Evans, Daniel, Rita, and Ghani, who have provided me with a supportive and collaborative community during this program. Their friendship, encouragement, and intellectual curiosity have been instrumental in shaping my academic interests and pursuits.

Lastly, I would like to thank my family and friends for their unwavering love, support, and encouragement throughout my academic journey. Their belief in me and my abilities has been a constant source of inspiration, and I am indebted to them for their unwavering support. Special thanks to my mom, for her prayers and love, my friend turned brother, Rasak, for his selfless support, and Oluwabunmi, my fiancée, for her sacrificial love and support through this journey.

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