UNIVERSITAT DE BARCELONA

Polyamine metabolism during the defense response to *Pseudomonas syringae* in *Arabidopsis thaliana*

Nazanin Arafaty

UNIVERSITAT DE BARCELONA

Polyamine metabolism during the defense response to *Pseudomonas syringae* in *Arabidopsis thaliana*

Nazanin Arafaty

Spring 2021

UNIVERSITAT DE BARCELONA
FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

Polyamine metabolism during the defense response to *Pseudomonas syringae* in *Arabidopsis thaliana*

Nazanin Arafaty

Spring 2021

UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA I CIÈNCIES DE L'ALIMENTACIÓ

DOCTORAT EN BIOTECNOLOGIA

Polyamine metabolism during the defense response to *Pseudomonas syringae* in *Arabidopsis thaliana*

Memòria presentada per Nazanin Arafaty per optar al títol de doctor per la

Universitat de Barcelona

Rubén Alcázar Hernández

Director i Tutor

Nazanin Arafaty

Doctorand

To Maman and Baba

To my little sister, Shiva

Acknowledgements

I would first like to thank my supervisors, Dr. Rubén Alcázar Hernández and Prof. Dr. Antonio Fernández Tiburcio for giving me this opportunity to study in Polyamines Lab at University of Barcelona. My special thanks to Dr. Rubén Alcázar Hernández, for supervising me during last five years. I'm honored to be your student, your thoughtful comments pushed me to think differently and brought my work to a higher level, which would help me in my professional life. Thank you, a lot.

I would like to thank my colleagues, Kostadin E. Atanasov, Chang Xin Liu, Ester Murillo, Anna Fleitas, Fernando Yelamo, Milena Acosta, Víctor Rodríguez and Marta Carrión for your wonderful collaborations. Specially, I'm so thankful to Kosta and Chang Xin for your always help and support. I learned a lot from you. You always were there for me patiently. Thank you, a lot, for being with me in this period of my life.

Thanks to the University of Barcelona to financially support my study in Barcelona. I would like also to thank Mari Carmen Molina and Angel Gutiérrez for their help and support during my study.

I also thankful to my friends at the Department of Biology, Healthcare and Environment, Section of Plant Physiology, Rafa, Luciana, Diego, Edgar, Ainoa and Miquel, I had a really great time with you.

I would like to express my thanks to my Iranian friends for their support even from a distance. Thank you Samaneh, Mahnaz, Shirin and Shahin for being next to me and encouraging me in difficult moments.

I would love to thank Marta from the bottom of my hearth, whose friendship makes me feel happy, strong, loved and supported. Thank you, Marta, you have been with me all the moments of this journey. I couldn't do it without your support. Love you, and your friendship is the most valuable thing that I have here.

I would love to thank the support and great love of my family, my mother, my father, my brother and my sister. I could not be able to do it without your love. You always believed on me and supported me from the long distance. You might not be here, but

you are here in my hearth. Having you as my family is the most precious thing that I have in my life, love you.

Last but not least, my special thanks go to my family here, Romero family. Montse, Andreu, Pati, Sílvia and Duc. I am so thankful for your love and support during all these years. Thank you Montse and Andreu, you loved me as your daughter. It is my pleasure to be part of Romero family, and your support made it easier for me to finish this journey. You are and you will be always in my hearth, here or in another place. Love you.

نازنین عرفاتی، بهار هزار و چهارصد

Nazanin Arafaty
Spring 2021

In the memory of Prof. Dr. Antonio Fernández Tiburcio

I will remember Antonio in all the positive ways possible. I would love him to be in my thesis defense, but he always will be here with us, in our hearts. Rest in peace Antonio.

List of Abbreviation

ABC	ATP-binding cassette
ACL5	Thermospermine synthase
ADC	Arginine decarboxylase
AIH	Agmatine iminohydrolase
AOs	Amine oxidases
Arg	Arginine
avr	Avirulence gene
BAK1	Brassinosteroid Insensitive Associated Receptor Kinase 1
Cad	Cadaverine
CDPKs	Calcium-dependent protein kinases
cNIL	complemented NIL line
CNL	CC-NBS-LRR
Col	Colombia
CPA	*N*-carbamoylputrescine amidohydrolas
CuAOs	copper-containing amine oxidases
DAP	1.3-diaminopropane
dcSAM	Decarboxylated S-adenosyl-Met
dsp	Disease-specific genes
ED	β -Estradiol
EDS1	Enhanced disease sussceptiblity 1
EF-Tu	Elongation factor Tu
EFR	EF-Tu receptor
ET	Ethylene
ETI	Effector-triggered immunity
ETS	Effector-triggered susceptiblity
Flg22	Flagelline 22
FLS2	Flagelline sensing 2
GWAS	Genome-wide association study
HCCA	Hydroxycinnamic acid amide
HPLC	High performance liquid chromatography
HR	Hyperssensitive response

HRC	HR and conserved gene
HRP	HR and pathogenicity gene
HTD	1.7-diaminoheptane
ICS1	Isochorismate synthase1
IM	Inner membrane
JA	Jasmonic acid
Kas-2	Kashmir
Ler	Landsberg
MAMPs	Microbial-Molecular Associated Pattern
MAPKs	Mitogen-activated protein kinases
MPK4	Mitogen-activated protein kinases 4
MS	Murashige and Skoog
NADP	Nicotinamide adenine dinucleotide phosphate
NDR1	None-race -specific disease resistance 1
NIL	Near isogenic line
NO	Nitric oxide
NPR1	Non-expressor of pathogenesis-related 1
ODC	Ornithine decarboxylase
OM	Outer membrane
Orn	Ornithine
PAD4	Phytoalexin-deficient 4
PAMPs	Pathogen-Molecular Associated Pattern
PAOs	Polyamines oxidases
PAs	Polyamines
pat	Pathogenicity genes
Paucine	Caffeoyl- putrescine
PCD	Programmed cell death
PM	Plasma membrane
PR1	Pathogenesis-related gene 1
PRRs	Pattern recognition receptor
PSII	Photosystem II

Pst	*Pseudomonas syringae*
PTI	Pathogen-triggered immunity
Put	Putrescine
RBOH	Respiratory burst oxidase homolog
RIN4	RPM1-interacting protein 4
RIPK	RPM1 induce protein kinase
RLKs	Receptor-like cytoplasmic kinases
ROS	Reactive oxygen specie
RPM1	Resistance to *Pseudomonas syringae maculicola* protein 1
RPP1	Recognition of *Pernospora parasitica* 1
RPS2	Resistance to *Pseudomonas syringae* 2
RPS4	Resistance to Pseudomonas syringae 4
SA	Salicylic acid
SAG 101	Senescence associated gene
SAM	S-adenosylmethionine
SAMDC	SAM decarboxylase
SAR	Systemic acquired resistance
SID2	SA-induction deficient 2
SNC1	Suppressor of npr1-1, Constitutive 1
Spd	Spermidine
SPDS	Spermidine synthase
Spm	Spermine
SPMS	Spermine synthase
t-Spm	Thermospermine
T1SS	Type 1 secretion system
T2SS	Type 2 secretion system
T3SS	Type 3 secretion system
T4SS	Type 4 secretion system
T5SS	Type 5 secretion system
T6SS	Type 6 secretion system
TNL	TIR-NBS-LRR
WAK	Wall-associated kinase

Contents

List of Figures

List of Tables

Introduction

World agriculture is facing myriad challenges in recent decades. Today's population is expected to grow up to 9 billion by 2050 (United Nations, 2009). Rising demands on agricultural land are estimated to increase rapidly with continued population growth (Bommarco et al., 2013). Although, crop productivity is not raising in parallel with the food demand (Parihar et al., 2015). It is well known that about one third of the earth's land is under agriculture, far more than any other human activities (Rohila et al., 2017). Based on FAO report, global agricultural land area is 4.9 billion hectares (Gha) or 38% of the 13 Gha global land surface. One third of total agricultural land is cropland (1.6 Gha), which includes both temporary (e.g., annuals) and permanent (e.g., perennials) crops. Over the decades 2007-2016, the largest world agricultural land area was in Asia (34 %), Americas (25 %) and Africa (23 %), with Europe and Oceania representing each about 8-10 % of the total (FAOSTAT, 2019; Available at: www.fao.org/faostat/en/, 2019). As stated in the FAO report, irrigation of agricultural crops entailed 70 % of all water withdrawn from aquifers, streams and lakes (FAOSTAT, 2019). Moreover, about 13.5% of global greenhouse gas (GHG) emissions is directly produced by agricultural contribution (IPCC, 2007). Enlarging agricultural lands due to forests and grasslands conversion has such huge negative impacts on environment like biodiversity reduction, additional GHG emissions, soil quality degradation and water pollution (Bommarco et al., 2013; Hooper et al., 2005; Moss, 2008; Potts et al., 2010; Rohila et al., 2017).

Thus, to meet the global food demand for increasing population, there is no doubt for enhancing crop production. In particular, producing stress tolerant/ resistant plants needs to improve markedly. The study of plant stresses, their response to different kinds of stress and stress management processes in plants, will provide knowledge to make plants ready for climate change and environmental challenges.

1. Stress Physiology

In both natural and agricultural conditions, plants are constantly exposed to a broad range of stresses. Some factors such as temperature can become stressful after some minutes and some others, like soil mineral deficiencies, may take months to become stressful (Taiz & Zeiger, 2006).

Moreover, stress plays an important role in determining how soil and climate can limit the distribution of plant species and their production. Thus, understanding the physiological

processes of stress that affect plant adaptation and production is of immense importance to both agriculture and the environment.

Hans Selye described the original concept of stress in 1936 as an unfavorable and environmental limitation in plants (Selye. H, 1936). **Stress** usually defined as any external factor that negatively affects plant growth, development, production and adaption to the environment and it is measured in correlation to plant growth (biomass accumulation), survival, crop yield, nutrient assimilation (CO_2 and mineral uptake), which are related to overall growth (Vickers, 2004).

Lichtenthaler developed the stress concept in plants. On one hand, he focused on the regeneration phase of plants after removing the stressors and on the other hand, the evolving difference between eustress and distress (Lichtenthaler, 1988; Lichtenthaler, 1996). Eustresses enhance function and are a positive factor for plant development, whereas distresses refer to persistent stresses that are not resolved through coping or adaptation, and negatively affect plants and cause damages. Sensitivity and tolerance may be defined as stress elements that have a negative (distress) or positive (eustress) effect. For instance, deficiency of water in vegetative tissues of vascular plants causes distress (except for resurrection plants) and is lethal below the constant wilting point, whilst water deficit above the constant wilting point or for a short time may persuade hardening (**Table 1**) (Kranner et al., 2010).

Table 1. Abiotic stress factors and their effects on plants (Based on Kranner et al., 2010).

Effect on whole plant		
Stress factor	**Distress**	**Eustress**
Water deficiency	Lethal below the permanent wilting point (Hsiano, 1973)	Above the permanent wilting point may induce hardening, for example in Zea mays leaves (Chazen & Neumann, 1994).
Temperatures	Extreme temperature may be lethal, for example heat stress in Triticum aestivum resulted in leaf senescence (Harding et al., 1990)	May induce hardening, for example acclimation of Spinacea oleracea to cold stress (Somersalo & Krause, 1989).
Fire	Lethal to most vegetative tissues of nonpyrophytes (Tyler, 1996).	Competitive advantage for pyrophytes due to removal of competitors, for example in the Chaparral (Tyler, 1996).
Nutrients	Imbalances may cause malfunction/malformation, for example iron deficiency leading to chlorosis in rice (Jolley et al., 1996).	Deficit may stimulate root growth, for example lateral root proliferation in Arabidopsis in nitrate-rich patches (Zhang & Forde, 1998).
Contamination, for example by nonessential heavy metals	Toxic to nontolerant plants, for example can result in sterility in rice contaminated by arsenic (Wells & Gilmour, 1977).	Competitive advantage for heavy metal-tolerant plants and hyperaccumulators with specific adaptations, for example in the arsenic hyperaccumulator Pteris vittata (Zhao et al., 2002).

1.1. The Different Phases Induced by Stress

Stand on the original concept of the stress of Selye and its progress by Lichtenthaler, plant stress responses split into four phases. Before stress exposure, the plants are in optimum

physiology conditions of growth, light, water and mineral supplement. Stressors will cause the first three phases of stress responses and after removal of the stressors, if the damage has not been too severe, it will lead to the regeneration phase (phase 4) (Wang et al., 2006). These phases are mentioned below and have also been summarized in **Figure 1**.

Phase 1. Response phase, which occurs at the beginning of stress and it is indicated by an alarm reaction such as deviation of normal functionality, raising catabolism process rather than anabolism and decline of vitality.

Phase 2. Restitution or resistance phase, in which the stress continues, and it consists of adaptation, repair and reactivation processes.

Phase 3. End phase. Stage of exhaustion and also known as long-term stress, in which the stress severity is too high and with the overload of adaptation capacity can lead to chronic disease or death.

Phase 4. Regeneration phase. When the stressor is removed, and the damage has not been too high. The physiological function can be partial or fully regenerated (Lichtenthaler, 1988 (Wang et al., 2006).

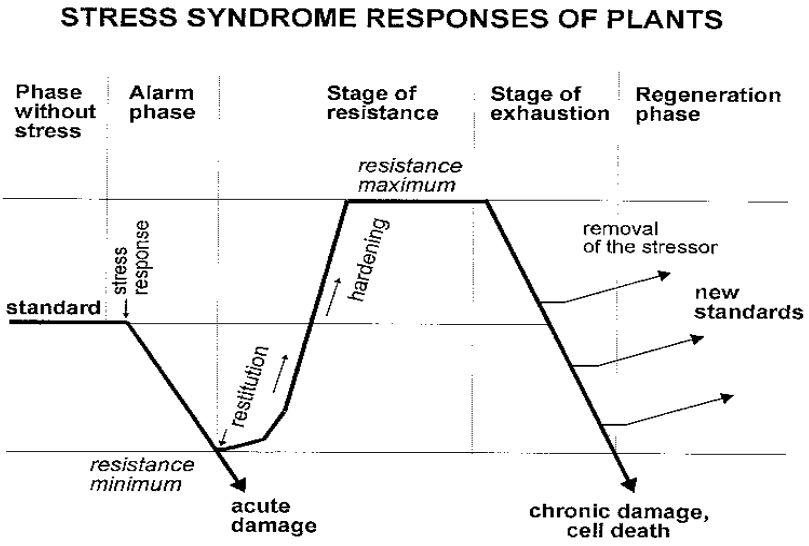


Figure 1. General concept of the phase sequences and responses induced in plants by stress exposure (Based on Lichtenthaler 1998). Plants growing under stress begin at a physiological standard condition to respond and cope with stress. Various responses and defense mechanisms will be activated. After removal of the stressor(s), new standards of physiology can, however, be reached in the plant depending on the time of the stressor removal as well as on the duration and intensity of the stress.

Crop yield is adversely affected by various biotic and abiotic stresses. The major abiotic stresses that cause reduction in plants growth and crop production are well studied (Cavanagh et al., 2008; Munns & Tester, 2008; Chinnusamy & Zhu, 2009; Mittler & Blumwald, 2010). Salinity, drought, cold, heat, freezing, chilling, high light intensity, nutrient and anaerobic stresses are posing a severe threat to agriculture and ecosystems (Chaves & Oliveira, 2004; Agarwal & Grover, 2006; Nakashima & Yamag- uchi-Shinozaki, 2006; Hirel et al., 2007; Bailey-Serres & Voe- senek, 2008).

1.2. Biotic stresses

In addition to the abiotic stress factors, a huge amount of economic losses are due to plant disease caused by plant pathogens. Plant pathogens are parasitic organisms such as bacteria, fungi, oomycetes, viruses and nematodes that can cause severe damage or destroy plants (Baker et al., 1997; Gimenez et al., 2018; Karim, 2007; Moustafa-Farag et al., 2020). Pathogens do not just take up nutrients from host plants but cause more damage by secreting enzymes, growth regulators, toxins and other substances that disturb cell metabolism. Host tissue damages result in biochemical and functional changes, metabolic and physiological disorders which lead to partial destruction or even complete death of the plant (Agrios, 2005). Plant pathogens are divided based on their mode of nutrition. Biotrophs or obligate parasites, which drive nutrients from living cells, should keep the host alive. Other plant pathogens, termed as necrotrophs, promote the destruction of host cells to feed from their contents (Stone, 2001). A third group, hemibiotrophs, live mostly on living hosts and can shift to necrotrophy at later stages of disease. The duration of the biotrophic and necrotrophic phases differ amongst hemi-biotrophic pathogens (Laluk & Mengiste, 2010).

1.3. Bacteria

1.3.1. Introduction

Among the 7100 classified bacterial species, 150 species cause mild to severe symptoms in a wide range of plants throughout the entire world (Buonaurio, 2008; Kannan, V., Bastas, K., and Devi, 2015; Strange & Scott, 2005). These organisms, known phytopathogenic bacteria result in devastating damage in crops and have a negative impact in agriculture due to their economic losses (Mansfield et al., 2012), particularly in tropical and subtropical countries,

where humidity and warm temperature are ideal conditions for bacterial growth (Kannan, V., Bastas, K., and Devi, 2015). They cause losses of over $1 billion dollars worldwide annually (Mansfield et al., 2012).

Bacterial infection produces symptoms such as leaf and fruit spots, twig blights, tissue rots, canker and/or hormone imbalances which result in stunning, root branching, plant overgrowth and leaf epinasty (Burkholder, 1948; Strange & Scott, 2005). Except for rare cases, phytopathogenic bacteria induce disease by penetrating into the host cells through natural openings like stomata, stigma, lenticels, hydathodes or by wounds. Bacteria colonize the apoplast and once inside, they execute two attack strategies for exploiting host nutrients: Biotrophy, in which bacteria extract nutrients of living cells and keep them alive; and necrotrophy, in which bacteria kill plant cells and extract nutrients from dead ones (Buonaurio, 2008).

In general, damage by pathogenic bacteria in plants associated with Xanthomonadaceae, Pseudomonadaceae, and Enterobacteriaceae families. The most destructive plant pathogens belong to genera like *Erwinia*, *Pectobacterium*, *Pantoea*, *Agrobacterium*, *Pseudomonas*, *Ralstonia*, *Burkholderia*, *Acidovorax*, *Xanthomonas*, *Clavibacter*, *Streptomyces*, *Xylella*, *Spiroplasma*, and *Phytoplasma* (Kannan, V., Bastas, K., and Devi, 2015).

1.3.2. Bacterial pathogenicity and virulence

The ability of a pathogen to cause disease is termed as “pathogenicity”, while “virulence” is the measure of pathogenicity of a given pathogen. Accordingly, for instance a bacterium could be pathogenic yet have varying degrees of virulence. Plant pathogens possess diverse classes of genes that cause disease (pathogenicity genes) or enhance virulence in the host cells (virulence genes). “Pathogenicity genes” (*pat*) and “disease-specific genes” (*dsp*) encode pathogenicity factors that are crucial for the establishment of disease, attachment of the pathogen to the plant surface, germination, infection structure formation, penetration and colonization of the host tissue (Agrios, 2005).

Pathogenicity and/or virulence of Gram-negative plant pathogenic bacteria are closely related to secretion apparatuses in their cells. They secrete proteins and nucleoproteins entailed in their virulence into apoplast or host cell (Buonaurio, 2008).

1.3.3. Bacterial Secretion Systems

Prokaryote organisms apply secretion, as a crucial task, to interact with the surrounding environment. Bacteria have several secretion systems to produce surface structures for aggregation, adhesion, bacterial mobility and, more importantly, to translocate enzymes, proteases, effectors and other molecules to the host cell (Chang et al., 2014). In gram- negative bacteria, six secretion pathways (I–VII) have been described. Amongst them, type III, IV and VI, specifically move effector proteins into the plant cell (Alvarez-Martinez & Christie, 2009; Arnold et al., 2010; Chang et al., 2014; Hayes et al., 2010; Records, 2011; Zalguizuri et al., 2018). Type I and type III directly translocate an unfold substrate into the extracellular space by one-step transport mechanism which does not require any periplasmic intermediators. Type II and Type V primarily transport the substrate into the periplasm for folding before a second transition step occurs across the outer membrane (Kanonenberg et al., 2013).

Type I secretion system

Mostly all plant pathogenic bacteria have type I-SS and carry out toxins secretion such as hemolysins, cyclolysin, and rhizobiocin. Type I-SS consists of three components: an ATP-binding cassette (ABC) transporter, a membrane fusion protein, and an outer membrane protein (Chang et al., 2014). ATP-binding cassette (ABC) proteins are involved in import and export of different compounds through hydrolysis of ATP (Agrios, 2005; Desvaux et al., 2004).
Proteins secreted by Type I -SS which important for pathogenicity include leukotoxins, hemolysins and bacteriocins (Bleves et al., 2010; Dirix et al., 2004). The other groups of proteins secreted via Type I-SS, are extracellular lipases, proteases and iron scavenger protein HasA (Akatsuka et al., 1995; Duong et al., 1992; Letoffe et al., 1994).

Type II secretion system

This type of secretion system is prevalent in gram-negative bacteria, and it is composed of 12-15 different proteins which often secrete cell wall-degrading enzymes into the apoplast (Chang et al., 2014; Szczesny et al., 2010). Type II-SS is required for the export of various enzymes, proteins, toxins, and virulence factors. Export of proteins are processed in a two-step process.
In the initial step, unfolded proteins cross the inner membrane to the periplasm via the Sec pathway, then folded proteins cross the outer membrane through the periplasm via an apparatus containing 12-14 proteins (Sandkvist, 2001). Type II secretion system is essential for

pathogenicity of the genera *Xanthomona, Ralstonia, Dickeya*, *Pectobacterium* and *Erwinia* (Kang et al., 1994; Ray et al., 2000; Szczesny et al., 2010; Toth et al., 2003).

Type IV secretion system

Type IV secretion system (T4SS) is the most versatile family of secretion systems with a vast variety of functions. The T4SSs are found in gram negative and gram positive bacteria as well as in Archaea (Bhatty et al., 2013), and mediate transport of macromolecules like DNA and proteins across the cell envelope (Rêgo et al., 2010). This translocation is provided by three cytoplasmic ATPases, that may cause conformational changes in the translocation complex (Walldén et al., 2012). Some T4SSs are used to transfer plasmid DNA from one cell to the other during conjugation, that is the main mechanism to expand antibiotic resistance genes among pathogenic bacteria (Wallden et al., 2010). Another T4SS transferring DNA is T-DNA strand of *Agrobacterium tumefaciens,* that is transmitted from bacterium to plant cell cytoplasm by proteins encoded by *vir*B. These proteins form an organized structure that expands from the bacterial inner membrane through the outer membrane and terminates a pilus-like structure that protrudes from the bacterial cell (Desvaux et al., 2004; Kannan, V., Bastas, K., and Devi, 2015). Moreover, the other T4SSs that are mostly found in pathogenic bacteria displace virulence proteins into the host cell and play important roles in host-pathogen interactions (Wallden et al., 2010).

Type V secretion system

This secretion system is an autotransporter, which contains the genes responsible for surface adhesins. Many autotransporters are easily recognized through their N and C- terminus sequences (Preston et al., 2005). The highest number of autotransporter-like proteins belong to *P. syringae* with nine candidate proteins, while *R. solanacearum* and *E. carotovor* possess two. Mammalian pathogens have similar autotransporter, critical for adhesion to epithelial cells (Agrios, 2005).

Type VI secretion system

The type VI secretion system (T6SS) is the most recently discovered mechanism in gram-negative bacteria for translocation of toxic proteins into the different target cells (Jani & Cotter,

2010; Schwarz et al., 2010; Silverman et al., 2012). The core apparatus of T6SS consists of a set of 13 proteins, that are conserved among pathogenic and non-pathogenic bacteria, and a set of conserved accessory proteins (Bingle et al., 2008; Cascales, 2008). These accessory proteins might be associated with the regulation or contribution to complementary apparatus function (Silverman et al., 2012).

The components of T6SS are encoded by tightly clustered genes (Boyer et al., 2009). In the survey of 500 bacterial genomes, at least in 100 genomes including three rhizobacteria, four symbionts and 13 plant pathogens, the presence of the T6SS loci was identified. In plant pathogenic bacteria like *Pectobacterium atrosepticum, Agrobacterium tumefaciens* and *Pseudomonas syringae* functional role of T6SS has been proved (Bingle et al., 2008; Records, 2011).

1.3.4. Pathogenicity and Type III secretion system

In terms of pathogenicity, T3SS (also called injectisome) is the most important bacterial secretion system in the genera *Pseudomonas, Xanthomonas, Ralstonia, Erwinia, and Pantoe* that colonize in plant intracellular spaces (apoplast) (Agrios, 2005; Alfano & Collmer, 2004). The earliest discovery of Type III secretion system goes back to 1997 in gram-negative bacterial pathogens in plants (He, 1998; Lindgren, 1997). The T3SS is encoded by *hrp* (HR and pathogenicity) and *hrc* (HR and conserved) genes (Bogdanove et al., 1996), which are required to cause disease in susceptible plants and to induce hypersensitive response in resistant plants (Lindgren et al., 1986). Plant pathogenic bacteria are extracellular pathogens (Sigee, 1993). The type III secretion system is used to modulate host-cell processes through secreted virulence factors, which include: (i) phytotoxins, plant hormones and hormone analogs that are secreted into the apoplast; (ii) protein-virulence factors (effectors); and (iii) cell wall degrading enzymes that are secreted through a *sec*-dependent type II secretion system (Alfano & Collmer, 1997; Sandkvist, 2001). Effector proteins are delivered into the plant cell from the cytoplasm of gram-negative bacteria through the T3SS, which requires to cross multiple physical barriers: the plasma membrane of the plant cell and the two bacterial membranes that are spaced by a peptidoglycan layer (**Figure 2**) (Büttner & He, 2009) and modulate host cell physiology in favor of the pathogen to develop disease level. It is noteworthy that if the effector leads to the development of disease symptoms in the host, it is called virulence protein, while if it triggers defense response and HR, it is referred to as an avirulence protein (Buonaurio, 2008).

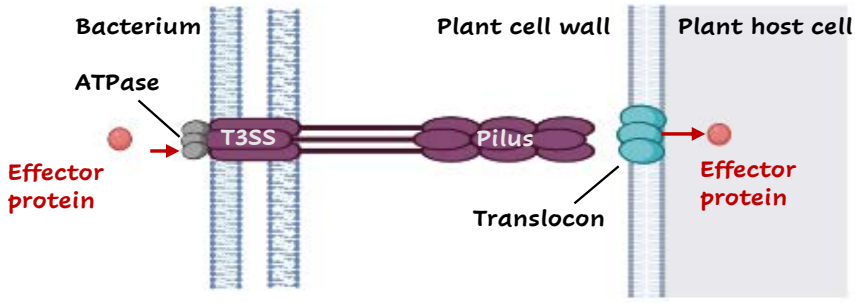


Figure 2. Schematic representation of the T3SS from plant pathogenic bacteria. The secretion apparatus spans both bacterial membranes and is associated with a cytoplasmic ATPase. The T3SS from plant pathogenic bacteria is connected to an extracellular pilus that pre- sumably spans the plant cell wall. (IM, Inner membrane; OM, outer membrane; PM, plasma membrane). Adopted from Daniela Buttner and Sheng Yang, 2009.

1.3.5. *Pseudomonas syringae*

In the scenario of plant-pathogen interactions, *Pseudomonas* is one of the well-studied genus of Gram-negative, rod-shaped bacteria, which includes both beneficial and pathogenic species (Passera et al., 2019; Pieterse et al., 2014). In the 1980s, *P. syringae*, as important pathogen species, was identified to infect the model plant *Arabidopsis thaliana* in the laboratory and cause disease symptoms. The Arabidopsis - *P. syringae* pathosystem provides a model for understanding bacterial pathogenicity and molecular mechanisms underlying plant-pathogen interactions (Dangl & Jones, 2001; Xin et al., 2018). In the late 1980s, a number of strains belonging to pathovars *tomato*, *pisi*, *maculicola* and *atropurpurea* of *P. syringae* were described to infect the *Arabidopsis* (Crute et al., 1994). The two virulent strains, *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *maculicola* ES4326 which are used extensively, originated from these early studies. In addition, avirulent strains like *P. syringae* pv. *maculicola M2* and *P. syringae* pv. *tomato JL1965* were also identified as a source of *avr* genes (Dong et al., 1991; Whalen et al., 1991).

Up to now, more than 50 pathovars of *P. syringae* have been identified, that infect almost all economically important crop species (Xin et al., 2018). Among them, *P. syringae* pv. *tomato* and the closely related pathogen *P. s*. pv. *maculicola* are widely used to elucidate several key elements of the plant-pathogen interaction (Jones & Dangl, 2006; Preston, 2000). Almost all strains of *P. s*. pv. *maculicola* have been shown to be pathogenic on tomato and crucifers, although many of *P. syringae* pv. tomato strains are pathogenic in tomato.

1.3.6. *Pst* DC3000 as a model pathogen

P. syringae pv. *tomato* DC3000 (*Pst* DC3000) belongs to the Pseudomonadaceae family; *Pseudomonas* genus and *Pseudomonas syringae* species. In 1991, *P. syringae* pv. *tomato (Pst*) strain DC3000 was reported to infect not only tomato, its natural host, but also *Arabidopsis* in the laboratory (Whalen et al., 1991). This led to intensive efforts to describe the molecular mechanisms of infection by this strain. The complete sequenced genome of *Pst* DC3000 was released in 2003 and, based on 5,763 open reading frames of this strain, various functional categories were estimated (Buell et al., 2003). *Pst* DC3000 encodes a wide range of virulence proteins (Preston, 2000), such as specialized protein secretion systems, toxins, flagella, bacterial surface attachment factors and type III effectors (T3Es), which are the most important virulence proteins (Alfano & Collmer, 2004). Approximately around 5% (298 genes) of the genome encodes virulence-related genes, unveiling a high genetic potential of *Ps*t DC3000 as a successful plant pathogen (Buell et al., 2003).

1.4. Viruses

It is well known that viral diseases affect agriculture all over the world in terms of quantity and quality of productions (Hernan Garcia-Ruiz, 2019; Nicaise, 2014). Although it is difficult to have an exact estimation of financial impact of viruses in agriculture, yield losses caused by these pathogens cost more than $30 billion annually (Sastry and Zitter, 2014).

Viruses consist of DNA or RNA genomic segments that encode few genes, and a protein shell which called capsid (coat protein). The main function of capsid is to encapsidate viral genomes to protect, transport and release them into the host cell (Roos et al., 2007). A successful infection by a plant virus results in the advent of symptoms that can vary greatly. These range from mosaic patterns, yellowing of the leaves, to developmental abnormalities, chlorosis and even systemic necrosis which somewhen lead to plant death (Culver & Padmanabhan, 2007; Ghoshal & Sanfaçon, 2015; Roossinck, 2010). Because of natural physical barriers (cell wall and cuticle) viruses should enter host cell by wounds or using an organism vector which feeds or infects plants like insects, nematodes and fungi.

As strict intracellular pathogens, chemical control cannot be efficient. Prophylactic measures mostly include demolition of infected plants and increased pesticide applications to control vectors population which have a huge impact on the environment (Nicaise, 2014). In this

domain, the use of genetically resistant plants is one of the beneficial and sustainable strategies to combat with virus infections (Gallois et al., 2018).

1.5. Fungi

Fungi are a large and heterogeneous eukaryotic group of living organisms, which have chitinous cell wall (González-Fernández et al., 2010). Most of the diseases occurring in agricultural and horticultural productions worldwide, are caused by plant fungal pathogens. Among the 100,000 known fungal species, over 10,000 fungi can cause diseases in plants such as mildew, canker, coils, leaf spot, rust, wilt, gall, anthracnose and blight (Agrios, 2005; Jain et al., 2019).

Fungal plant diseases in both pre and post-harvest processes lead to economic loses of 200 billion US dollars (Birren et al., 2002; González-Fernández et al., 2010; Horbach et al., 2011). Moreover, contamination of food and forage by mycotoxins, which are highly toxic secondary metabolites, demonstrate the importance of the problem in agriculture and fungal biology (Horbach et al., 2011; Shuping & Eloff, 2017).

Generally, plant fungal pathogens can also be classified in biotrophs, hemi-biotrophs and necrotrophs (Shuping & Eloff, 2017).

Not all the plant fungal pathogens are able to cause disease in the same host. Some have wide range host capacity, while others have limited host range (Agrios, 2009). Phytopathogens use different strategies to attack and enter to their hosts. Some pathogens release chemicals and exert mechanical pressure to enter, whereas some others enter through wounds and stomata (Knogge, 1998). Fungal pathogen interaction with plants starts with spore attachment to the host surface and continues with spore germination, host cognition, formation of infection structure and penetration (Knogge, 1998). Spores will break dormancy and start germination once exposed to appropriate conditions like suitable host, low molecular nutrients and humidity (Osherov & May, 2001; Sephton, 2018). Fungal pathogen spores can be spread by wind, insects, birds, humans, water and some parts of infected plants (Rossman, 2009). On top of that, spores can survive for many years using self-inhibitors to stop germination until favorable conditions occur (Chitarra et al., 2004). Knowledge of pathogenic cycle and plant pathogen interaction is critical to expand adequate strategies for crop protection, including the development of plant resistant genotypes by genetic engineering, classical plant breeding, fungicide or biological control (González-Fernández et al., 2010; Yang et al., 2009).

1.6. Oomycetes

Oomycetes are filamentous microorganisms from the Stramenopile kingdom (Dick, 2001). Based on fossil records, the first evidence of oomycete existence goes back to 400 to 360 Ma (Krings et al., 2011). Oomycetes are divided in two subclasses, "water mold" or Saprolegniomycetidae which consists of Saprolegniales, Eurychasmales and Leptomitales, and Peronosporomycetidae that contains Peronosporales, Pythiales and Rhipidiale (Fawke et al., 2015). Several oomycetes are well known as destructive pathogens, with massive economically impacts on agriculture, horticulture, aquaculture and ecosystem (Kamoun et al., 2015; Wang et al., 2019). Among them, the Phytophthora genus, with 100 species that infects a vast variety of plants has been well-studied (Kroon et al., 2012). For instance, *Phytophthora infestans* the cause of potato late blight, and *P. ramorum* which causes the most destructive disease on oak trees and other plant species, had a historical impact in agriculture (Kamoun *et al*., 2015).
Oomycetes have different lifestyles to infect their hosts, namely, downy mildew species classified as obligate biotrophic pathogens. However, Phytophthora genus have a hemi-biotrophic life style (Herlihy et al., 2019). The success key of pathogenic oomycetes is based on in their high evolutionary potential which results in their capacity to adapt and overcome host resistance. Their flexible mating system, rapid proliferation, large population sizes and their ability to encode diverse virulence effectors enable them to adapt and modulate their hosts (Bozkurt et al., 2012; McDonald & Linde, 2002; Schornack et al., 2009; Thines, 2014).

1.7. Nematodes

Over 4100 species plant parasitic nematodes have been recognized (Decraemer and Hunt, 2006 book reference). Plant nematodes caused damages of about $US80 billion per year (Nicol et al., 2011). Most nematode damages occur directly through their interference with the normal cell cycle or by withdrawing the contents of plant cells (Dropkin, 1955; Palomares-Rius et al., 2017). However, some groups can act as virus vectors like nep- and tobraviruses (Decraemer and Robbins, 2007). Besides, nematodes can interact with other plant pathogens to increase plant damage or break plant resistance (Back et al., 2002). Plant parasitic nematodes also have a wide variety of interactions with their hosts. All have a stylet (a hollow mouth spear, like a hypodermic needle) which in length and shape is highly variable. Nematodes use stylets to penetrate plant cells and to feed from all plant parts, including roots, stems, leaves, seeds and flowers. Root nematode damage symptoms are unspecific and associated with nutrition

deficiency, wilting, stunning and sometimes plant death. Galls in roots or stems and deformation in some hosts, are symptoms which certainly link to specific species of plant nematodes (Gimenez et al., 2018; Palomares-Rius et al., 2017).

Some nematodes, migratory ectoparasites, never enter the host, but simply migrate along the soil, using roots as flimsy food source. Others, called migratory endoparasites, enter the host and migrate through tissues causing considerable damage. Semi-endoparasitic nematodes may have migratory stage, but also penetrate the host plant partially to feed at one stage of life cycle. Although, the root knot and cyst nematodes which are obligate sedentary endoparasites, are most important economically nematodes (Jones et al., 2013; Sijimons et al., 1991). Of particular importance was the finding that showed both root knot and cyst nematodes can infect the model plant *Arabidopsis thaliana*.

1.8. Plant pathogen interactions

According to fossil records, the first land plant was established approximately 480 million years ago. Even so, based on molecular- clock estimation, the first land plant occurred more than 700 million years ago (Heckman et al., 2001). Fascinating to know that the interaction between symbiotic fungal associations and early land plants simplified their establishment, which suggests the coevolution of plants with microbes since their first emersion on the land (Gehrig et al., 1996), which has led to the establishment of the current plant immune system (Brown & Tellier, 2011).

Plants, no matter whether in nature or in agriculture, are continuously exposed to a vast variety of microbes. The first step of infection is to access the plant interior by penetration, natural opening like stomata or through wounding. Once inside, microbes are encountered with another obstacle: the plant cell wall, a cellulose-based and firm support surrounding every cell. Microbes encounter the host plasma membrane through penetration of the cell wall (Chisholm et al., 2006; Miedes et al., 2014).

In addition to these mechanisms, plants possess two branches of immune system. One of such branches relies on cell surface recognition of Microbial- or Pathogen-Molecular Associated Patterns (MAMPs or PAMPs) such as flagellin, by transmembrane pattern recognition receptor (PRRs) (Zipfel & Felix, 2005). This recognition leads to what is known as MAMP or PAMP-Triggered Immunity (MTI or PTI). The second layer acts largely inside the cell, recognizing pathogen effectors by using the polymorphic NB-LRR protein products encoded by *Resistance*

(*R*) genes which results in Effector-Triggered Immunity (ETI) (**Figure 3**). (Dangl & Jones, 2001). NB-LRR mediated disease resistance is effective against obligate biotroph or hemi-biotrophic pathogens, but not necrotrophs (Glazebrook, 2005).

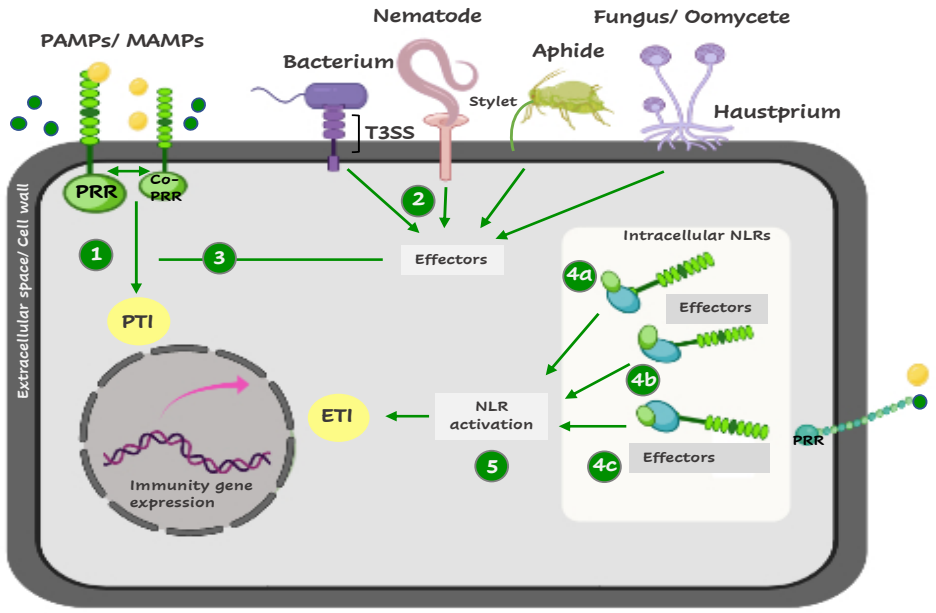


Figure 3. Schematic of the plant immune system. Pathogens of all lifestyle classes express PAMPs and MAMPs as they colonize plants. Recognition via extracellular PRRs triggers PTI (step 1). Pathogens deliver virulence effectors to both the plant cell apoplast to block PAMP/MAMP perception (not shown) and to the plant cell interior (step 2). These effectors are addressed to specific subcellular locations where they can suppress PTI and facilitate virulence (step 3). Intracellular NLR receptors can sense effectors in different ways (step 4). Adapted from Dangl et al., 2013.

Based on the co-evolution of plants with pathogens, the plant immunity system can be simplified as a four phased "zigzag" model (**Figure 4**) (Jones & Dangl, 2006). Recognition of PAMPs or MAMPs by PRRs results in PTI response (phase 1). Successful pathogens deliver effectors that contribute to pathogen virulence, which can disrupt PTI and delay plant immune responses (Toruño et al., 2016). This results in Effector-Triggered Susceptibility (ETS) (phase 2). Once the pathogen acquired the ability to suppress primary defense activation, plants evolved to have more specialized mechanism of recognition, termed ETI (phase 3), (Chisholm et al., 2006). ETI activation results in disease resistance often associated with cell death at the site of infection, which inhibits the pathogen growth. Not surprisingly, pathogens seem to interfere with ETI through pathogen effectors diversification or by attaining additional effectors (phase 4), (Torres et al., 2006). This way, pathogens evade plant recognition until a new *Resistance* gene or allelic variant evolves in the population and it increases in frequency due to positive selection. Nonetheless, *Resistance* genes are amongst the most variable genes in *A. thaliana* populations.

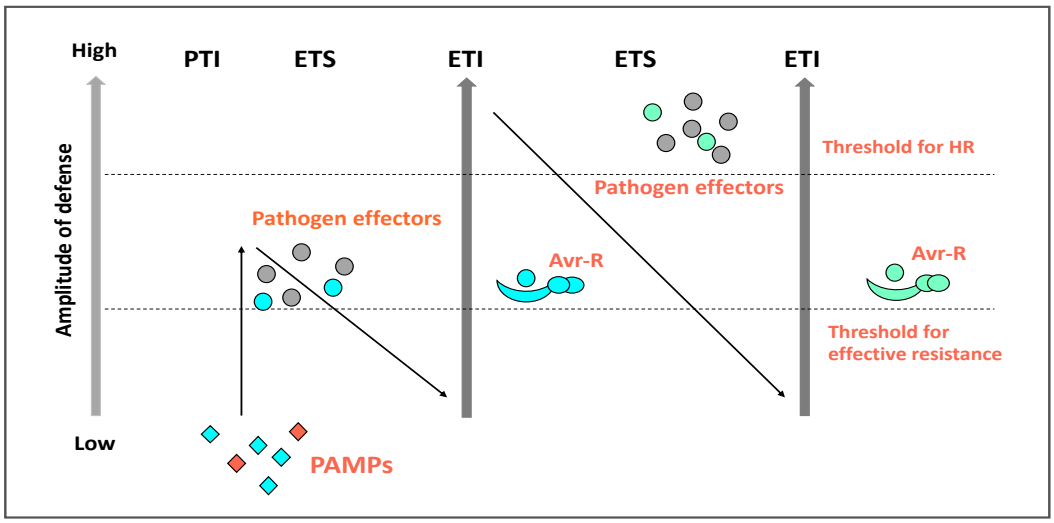


Figure 4. A zigzag model illustrates quantitative output of plant immune system. Adapted from Jones and Dangl, 2006.

1.8.1. PAMP recognition and PAMP-Triggered Immunity

The first active plants response to a pathogen is PAMPs-triggered immunity (PTI), which includes an early and broad array of specific immune responses (An et al., 2017). Surface-localized pattern recognition receptors (PRRs) perceive conserved bacterial molecules described as pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) like Flagellin 22 (flg22), Elongation Factor Tu 18 (elf18), lipopolysaccharides or peptidoglycan (PGN) (Dangl et al., 2013; Schulze-Lefert & Panstruga, 2011). Flagellin sensing 2 (FLS2) and the elongation factor Tu receptor (EFR), are the most well-characterized PRRs (Toruño et al., 2016).

Activation of PRRs leads to phosphorylation and activation of receptor-like cytoplasmic kinase (RLKs) (Lin et al., 2013; Macho & Zipfel, 2014). Downstream immune responses of PTI are associated with the generation of reactive oxygen species (ROS), activation of mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs), expression of immune-related genes and deposition of callose to strengthen the cell wall at the site of infection (**Figure 5**) (Boller & Felix, 2009; Nürnberger et al., 2004; Tena et al., 2011).

To attain an effective infection, pathogens develop diverse mechanisms to disrupt host cellular and physiological processes or to dampen the host immune system. Many gram-negative bacteria have acquired the potential to inject their virulence effectors through type 3 secretion system (T3SS) into the host cell to block immune responses (Feng & Zhou, 2012; Lin et al.,

2013). Several *Pseudomonas syringae* effectors target FLS2, EFR and their co-receptor BAK1 (*Brassinosteroid Insensitive Associated Receptor Kinase 1*) to repress plant immune responses (Macho & Zipfel, 2015). For instance, the effector AvrPto increases bacterial virulence, inhibits kinase activity of FLS2 and EFR. Some others like HopF2, AvrPto and AvrPtoB block downstream immune responses by targeting BAK1 (Shan et al., 2008; Xiang et al., 2008; J. Zhou et al., 2014). Plants have evolutionary obtained polymorphic intracellular receptors, that limit or even eliminate bacterial infection (Lin et al., 2013). Pathogenic bacterial effectors are directly or indirectly recognized by R proteins, which elicit a second line of plant inducible defense defined as effector-triggered immunity (ETI) (Dodds & Rathjen, 2010). For instance, gram-negative phytopathogenic bacteria like *P. syringae* can deliver 20-30 effectors through T3SS during infection, which are recognized by specific disease resistance (*R*) genes (Baltrus et al., 2011; Block & Alfano, 2011; Chang et al., 2005; Cunnac et al., 2009).

1.8.2. Effector-Triggered Immunity (ETI)

Arabidopsis- *P. syringae* pathosystem

The confirmation of the plant-pathogen interaction with "gene-for-gene" hypothesis, which is the specific interaction between pathogen *avr* (avirulence) gene and its corresponding *R* gene, was the landmark of pathosystem development (Nimchuk et al., 2003; Van Der Biezen & Jones, 1998). When both genes are present in the host and pathogen, disease resistance occurs. Conversely, in the absence of either one of them, disease results (Dangl & Jones, 2001). Tremendous efforts have been made from several laboratories in the genetic isolation of *avr* genes and their corresponding *R* genes in *Arabidopsis*, which led to the identification of *avr-R* combinations, including *avrB-RPM1* (Bisgrove et al., 1994), *avrRpm1-RPM1* (Dangl et al., 1992), *avrRps4-RPS4* (Hinsch & Staskawicz, 1996), *avrRpt2-RPS2* (Kunkel et al., 1993; Yu et al., 1993), *avrPphB-RPS5* (Simonich & Innes, 1995) and *avrPphB-PBS1* (Warren et al., 1999). All of the forenamed *Arabidopsis R* genes belong to the nucleotide binding site-leucine reach repeat (NBS-LRR) classes of *R* genes but not *PBS1*. NBS-LRRs are the largest group of the intracellular immune receptors, which structurally consist of a central Nucleotide-Binding domain (also known as NB-ARC), a carboxy-terminal LRRs and a variable N-terminal domain (Dangl & Jones, 2001; Takken & Goverse, 2012; Warmerdam et al., 2020). Within the NB-LRR class, RPS2, RPM1, and RPS5 are the most characterized members. These R proteins

specify resistance to *P. syringae* bearing the bacterial effectors AvrRpt2, AvrRpm1/AvrB, and AvrPphB, respectively (Chisholm et al., 2006).

NBS genes based on the presence of Toll/IL-1 Receptor-like (TIR) domain in the protein amino terminus are subdivided in TIR-NBS-LRR (TNL) and non-TIR-NBS- LRR (nTNL) (Hofberger et al., 2014; Meyers et al., 1999; Zhou et al., 2004). Most of nTNL genes encode a coiled-coil (CC) domain at the N terminus, thus also termed as CC-NBS-LRR (CNL) genes. Recent studies have revealed that apart from CNL, a small group of genes carry a special N-terminal domain, RPW8, (RPW8-NBS-LRR, RNL) and represent a discrete class of NBS genes (Meyers et al., 2003; Shao et al., 2016; Zhang et al., 2016). In pathogen resistance, CNLs and TNLs have different genetic requirements. Although many CNLs receptors require plasma membrane-associated protein Non-Race-Specific Disease Resistance 1 (NDR1), all TNLs receptors signal via the nucleocytoplasmic lipase-like protein, Enhanced Disease Sussceptibility 1 (EDS1) for resistance (Cesari et al., 2014; Dangl & Jones, 2001; Day et al., 2006; Falk et al., 1999; Jacob et al., 2013; Wiermer et al., 2005) (**Figure 5**).

In *Arabidopsis*, the RPP1 TNL type R-protein confers resistance to *Hyaloperonospora arabidopsidis* (downy mildew) (Boisson et al., 2003). The TNL type R-proteins RPS1 and RPS4 provide resistance to *Ralstonia solanacearum* (soil microbe) in *Arabidopsis* (Deslandes et al., 2003; Narusaka et al., 2009). Moreover, the RPS5 CNL type R-protein activate innate immune response to *Pseudomonas syringae* through avrPphB effector recognition (Warren et al., 1998).

The distribution of NBS-LRR genes is species specific, while RNL and CNL genes are present in both monocots and dicots, TNL genes are restricted in dicots (Andolfo et al., 2013; Meyers et al., 2003; Shao et al., 2014). For instance, *Arabidopsis*, as a dicotyledonous plant, contains ~ 150 NLR genes which encode TNL and CNL proteins, while the genome of *Oryza sativa*, a monocotyledonous plant contains ~ 480 NLR genes that code only CNL proteins (Meyers et al., 2003; Yang et al., 2006). Monocot species that receive interfamily NLRs genes from dicots confer broad-spectrum disease and pest resistance (Li et al., 2019).

The number of recognized NLR genes does not seem sufficient to mediate direct recognition of all kind of virulence factors (Mackey et al., 2003). To combat with that, it was postulated that R proteins might “guard” a limited set of key cellular targets of pathogen virulence factors, which are manipulated by effectors (Dangl & Jones, 2001; Rafiqi et al., 2009; Van Der Biezen & Jones, 1998).

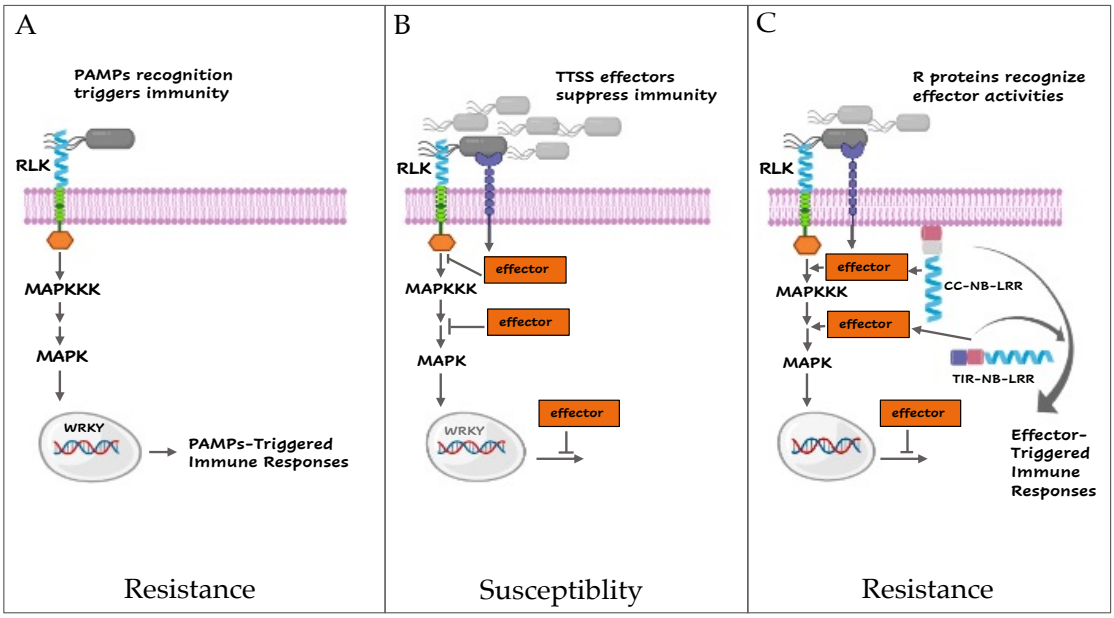


Figure 5. Plant pathogen interactions. A. PAMPs recognition by RLKs, which triggers basal immunity through MAPK signaling and transcriptional reprogramming mediated by plant WRKY transcription factors. B. Effector proteins delivered into the host cell via type III secretion system target multiple host proteins to suppress basal defenses. **C.** Plant resistance proteins (represented by CC-NB-LRR and TIR-NB-LRR; see text) recognize effector activities and restore resistance through effector-triggered immune responses. Limited accumulation of bacteria occurs prior to effective initiation of effector-triggered immune responses. Adapted from Chisholm et al., 2006.

The best characterized guard-guardee example is the plasma membrane RIN4, which phosphorylation leads to the activation of RPM1 and RPS2 through effector recognition (Axtell & Staskawicz, 2003; Block & Alfano, 2011; Jones & Dangl, 2006; Mackey et al., 2003; Marathe & Dinesh-Kumar, 2003). *R* gene activation mostly results in an oxidative burst, rapid production of reactive oxygen species (ROS), which is required for hypersensitive response (HR) and ultimately ETI (Tsuda & Katagiri, 2010). ROS production may have antimicrobial effect, and it also acts as a signal for activation other defense responses (Glazebrook, 2005; Nimchuk et al., 2003; Torres et al., 2006).

1.9. Plant disease resistance *R* genes

Since the first identification and cloning *R*-gene in 1990's, considerable efforts have been made to identify novel *R* genes in plants (Garzón et al., 2013; Liu et al., 2007; Rouxel & Balesdent, 2013; Sanseverino et al., 2010; Yang et al., 2013). One of the first works of resistance transfer involving *R*-genes, was performed in tomato *(Solanum lycopersicum) Pto* gene, which induces resistance to *P. syringae pv. tomato* strain (Martin et al., 1993). In another pioneer work, *Rpg1* from barely *(Hordeum vulgare cv. Morex)* successfully transferred to a susceptible barley cv.

Golden Promise conferred resistance to stem rust fungus *(Puccinia gramini)* (Horvath et al., 2003).

R-genes trigger resistance by different mechanisms. Some *R*-genes encode wall-associated kinases (WAKs) and some others encode detoxification enzymes, while ETI is often associated with the hypersensitive response (HR), that is rapid cell death at the point of pathogen infection (Hurni et al., 2015; Johal & Briggs, 1992). HR is mainly due to the genetic interaction between a *R*-gene and its related *avr* gene. Although cell death can be effective against necrotrophic pathogens, HR is blocking diseases provoked by hemi-biotrophic pathogens like *P. syringae,* and can be involved in susceptibility to necrotrophic diseases (Nelson et al., 2018). *R*-genes have been identified by combination of several methods like mutation screening (Jones et al., 1994; Liu et al., 2005), fine- mapping and positional cloning (Dixon et al., 1996) and systemic identification and testing of NLR genes (Collins et al., 1999). Most *R*-genes are extremely specific and provide resistance to solely one or a few strains of the specific pathogen. Some *R*-genes, NLRs specifically, are closely linked in clusters (Hulbert et al., 2001), which enable diversification of the recognition specificities of *R genes* (Hulbert et al., 2001; Shao et al., 2014) and ultimately confer resistance to multiple pathogen species (Meyers et al., 1998) and/ or different races of the same pathogen (Hulbert, 1997).

Effector recognition results in intracellular ETI (Effector- Triggered Immunity) and molecular processes connecting R-activation to downstream defenses pathways (Cui et al., 2015), which is often accompanied by several early defense responses such as mitogen-activated protein kinase (MAPKs) cascades, reactive oxygen activity (ROS) production and calcium influx. Many MAPKs such as SIPK and WIPK stimulate defense gene expression and polyamines (PAs) biosynthesis that leads to H_2O_2 production. H_2O_2 acts as a signaling molecule and mediate hypersensitive response (HR) (Jiménez-Bremont et al., 2014). Novel approaches in genomic technology to identify *R*-genes and relevant pathogen effectors, may provide a deeper understanding of host-pathogen interactions.

1.9.1 The AvrRPM1 / RPM1 gene-for-gene model

AvrRpm1

Pseudomonas syringae pv. maculicola strain M2 (Psm M2), carrying avirulence gene *avrRpm1* elicits strong ETI and HR by altering RIN4 activity (Kim et al., 2009; Mackey et al., 2002). Among twenty *P. syringae pv. maculicola* strains analyzed, *avrRpm1* is present in only

5 strains. However, other strains are still pathogenic (Dangl et al., 1992). In Arabidopsis, HR induced by AvrRpm1 depends on the presence of RPM1, although in plants lacking RPM1, AvrRpm1 causes symptoms (Nimchuk et al., 2000), which require defense signaling genes *NPR1, PAD4, SID2* and *RAR1* (Kim et al., 2009). Bacterial type III effectors can provoke defense signaling through more than one NLRs protein. For example, AvrRpm1 as a main activator of RPM1, also induces RPS2- dependent response that results in effective defense (Eitas et al., 2008; Kim et al., 2009). In addition, some data demonstrated that type III effectors like AvrRpm1 enhance bacterial virulence by repressing host defenses activated by MAMPs (Kim et al., 2005; Shang et al., 2006).

RPM1 gene enabling dual specificity disease resistance

In *Arabidopsis,* Resistance to Pseudomonas syringae pv. maculicola 1 (RPM1) is an NLR receptor that confers resistance to *Pseudomonas syringae AvrRpm1* and *AvrB* effectors (Bisgrove et al., 1994; Grant et al., 1995). RPM1 is a well characterized plasma membrane associated protein (El Kasmi et al., 2017; Noman et al., 2019), which does not directly recognize its corresponding effectors, AvrRpm1 and AvrB, but is activated indirectly by perceiving the phosphorylation of the guard protein RPM1-interacting protein 4 (RIN4). A plasma membrane localized protein, RIN4, negatively regulates both RPM1 and RPS2 function (Kim et al., 2009). AvrRpm1 and AvrB induce RIN4 phosphorylation, which is mediated through RPM1 induced protein kinase (RIPK) and related kinases (Chung et al., 2011; Liu et al., 2012). Moreover, RIN4 is associated with plasma membrane-anchored integrin- like protein (NDR1) that is required for ETI induced by CNLs like RPM1 and RPS2 (Knepper et al., 2011). RIN4 is needed for the accumulation of RPM1, as well as the activation of RPM1-dependent HR. However, its phosphorylation occurs independently of RPM1 (Mackey et al., 2002). Although, plasma membrane localization of RPM1 is essential for its activation, RPM1 function does not require nuclear translocation (El Kasmi et al., 2017; Z. Gao et al., 2011). Activation of RPM1 provokes downstream signal transductions, including calcium influx, accumulation of ROS by NADPH oxidases, kinase activation and HR to inhibit growth of Pseudomonas strains expressing AvrRpm1 or AvrB (Chiang & Coaker, 2015; Cui et al., 2015; El Kasmi et al., 2017; Lolle et al., 2020; Song et al., 2020). In addition, some data indicates the membrane fusion of the central vacuole and the plasma membrane after activation of RPM1 and RPS2, which leads to the release antimicrobial proteins to the apoplast with cell death inducing activity (Hatsugai et al., 2009).

Extracellular ROS production is mediated by NADPH oxidases called *Respiratory Burst Oxidase Homologs* (*RBOHs*). The *Arabidopsis atrbohD/F* double mutant displays reduction in ROS production and HR in response to AvrRpm1 but with no effect on bacterial growth (Torres et al., 2002). But intracellular ROS is not only involved in cell death during HR, also acting as a signaling molecule to stimulate defense gene expression (Straus et al., 2010). Contribution of multiple organelles such as peroxisomes, chloroplasts and mitochondria result in intracellular ROS production, and chloroplasts have a key role in ROS production during HR (Doyle et al., 2010; Shapiguzov et al., 2012).

1.9.2 The AvrRps4 / RPS4 gene-for-gene model

AvrRps4

The effector AvrRps4 originally found in *Pseudomonas syringae* pv. pisi, consists of 221 amino acids in length (Hinsch & Staskawicz, 1996; Sohn et al., 2009). Based on N-terminal fragment (AvrRps4^{N}, amino acids 1–133), AvrRps4 shares 75% identity with HopK1, which is a native effector of *Pseudomonas syringae* pv. tomato strain DC3000 (DC3000) that significantly participates in the virulence of *Pst* DC3000. The virulence activity is highly reduced in *hopk1* mutants. HopK1 and AvrRps4 contain a chloroplast transit peptide on their N-terminus, and their ability to suppress PAMPs- Triggered Immunity (PTI) response (ROS production and callose deposition) requires chloroplast localization (Guo et al., 2009; Halane et al., 2018; Li et al., 2014). Even so, nuclear accumulation of RPS4 is necessary to trigger immune response (Wirthmueller et al., 2007). It has been shown that C-terminal 88 amino acids of AvrRps4 are adequate to trigger an HR in turnip but not *Arabidopsis* (Sohn et al., 2009).

RPS4

In *Arabidopsis*, *Resistant to P. Syringae 4 (RPS4)* was first reported as disease- resistance gene in *Arabidopsis* that recognizes AvrRps4 in the susceptible accession RLD (Hinsch & Staskawicz, 1996). *RPS4* functions in pair with *Resistant to Ralstonia Solanacerum1 (RRS1)* to recognize the bacterial effectors AvrRps4 and PopP2 via WRKY transcriptional factor domain in the RRS1-C terminus (Ma et al., 2018). RPS4 and RRS1 belong to the TNL subclass of NB-LRR proteins (Saucet et al., 2015). The TIR domain of the RPS4 is more likely to have a crucial role in cell death signaling pathway (Swiderski et al., 2009). WRKY transcriptional factors are key regulators of plant defense that are targeted by AvrRps4 and PopP2 to suppress

host defense and enhance bacterial pathogenicity. The interaction of AvrRps4 with WRKY domain and its acetylation via PopP2 results in activation of *RPS4-RPS1* complex and defense responses (Le Roux et al., 2015; Sarris et al., 2015). Activation of *RPS4-RPS1* complex also depends on its interaction with EDS1, that is required for ETI mediated by TNLs receptors (Bhandari et al., 2019; García et al., 2010; Wirthmueller et al., 2007). EDS1 interaction with RPS4 and AvrRps4 has been reported in several studies. It was hypothesized that EDS1 is guarded by RPS4. AvrRps4, as a virulence effector (Bhattacharjee et al., 2011; Heidrich et al., 2011), can disrupt this association, pointing that EDS1 could be a target of pathogen effector in the absence of RRS1(Halane et al., 2018; Huh et al., 2017). In addition to EDS1, PAD4 is also required for defense activation by *RPS4-RPS1* complex (Feys et al., 2001) by blocking EDS1- AvrRps4 interaction (Huh et al., 2017).

1.10. SA-Dependent Signaling

Plant defense system is regulated through a complex network of multiple signaling molecules. Three signal molecules, salicylic acid (SA), jasmonic acid (JA) and ethylene, have long been recognized. (Yang et al., 2013). Although, the SA-dependent signaling is effective against biotrophic pathogens like *Hyaloperonospora arabidopsidis*, JA/ethylene-dependent defenses are acting more versus necrotrophic pathogens such as *B. cinerea* (Glazebrook, 2005; Thomma et al., 2001). ETI activation triggers secondary defense responses, including SA-dependent signaling, which is crucial to initiate local and systemic acquired resistance (SAR) (Maruri-López et al., 2019). SA accumulation induces the expression of the defense related genes such as *PR-1* through *NPR1* activation (Birkenbihl et al., 2017; Fu & Dong, 2013). NPR1 is described as a key immune regulator, which acts downstream of SA (Wu et al., 2012). Increasing level of SA induces NPR1-complex dissociation to monomers and translocation into the nucleus, where they interact with TGA-type transcription factors to rise transcriptional activation of SA-regulated genes (Fu & Dong, 2013; Maruri-López et al., 2019). For activating SA accumulation, expression of *EDS1* and *PAD4* are essential (Glazebrook, 2005). *SID2* encodes isochorismate synthase, an enzyme that is needed for SA biosynthesis. In *sid2* and *eds5* mutants, the level of SA is decreased due to biosynthesis blocking (Nawrath & Métraux, 1999). Besides, pathogen inducible expression of *EDS5* requires *EDS1* and *PAD4,* which means that *EDS5* acts downstream of the *EDS1* and *PAD4* in SA signaling pathway (**Figure 6**) (Glazebrook et al., 2003; Nawrath et al., 2002).

1.11. SA-defense signaling pathway

1.11.1. *EDS1*

The plant immune regulator *Enhanced Disease Susceptiblity1 (EDS1)* is an essential component of plant basal resistance against biotrophic and hemi-biotrophic pathogens. EDS1 is also required for resistance mediated by TNL- type *R* genes to induce a robust *EDS1*-dependent immune response called effector- triggered immunity (ETI) (Bhandari et al., 2019; Bhattacharjee et al., 2011; García et al., 2010; Heidrich et al., 2011; Wirthmueller et al., 2007). *Arabidopsis* EDS1 forms separate heterodimer structures with its defense co-regulators *Phytoalexin Deficient 4 (PAD4)* and Senescence Associated Gene 101(*SAG 101*) (Feys et al., 2001, 2005; Rietz et al., 2011; Wagner et al., 2013). Despite of the cytoplasmic localization of EDS1 dimers, EDS1-PAD4 heteromeric complexes accumulate in nuclei and cytoplasm and EDS1- SAG101 are limited to nuclei (Feys et al., 2005). Several pieces of data suggest that EDS1- SAG101 heterodimers stimulate HR cell death in TNL receptors, whilst EDS1- PAD4 heterodimers are necessary for basal immunity and full Systemic Acquired Resistance (SAR), mediated by Salicylic Acid (SA) (Feys et al., 2005; Gantner et al., 2019; Lapin et al., 2019; Neubauer et al., 2020; Rietz et al., 2011). EDS1 and PAD4 also contribute in resistance conditioned by other pathogen sensing protein or intracellular receptors (Chandra-Shekara et al., 2004; Venugopal et al., 2009; Xiao et al., 2001).

Salicylic Acid contributes to PTI and ETI responses, and is regulated by SA biosynthetic enzyme gene *Isochorismate synthase1 (ICS1)* and SA metabolic genes (Seyfferth & Tsuda, 2014). In basal and TNL immunity, EDS1 and PAD4 stimulate *ICS1* expression and SA accumulation, which positions EDS1/ PAD4 upstream of SA signaling (Feys et al., 2001; Rietz et al., 2011; Wagner et al., 2013; Wiermer et al., 2005). Early function of EDS1/PAD4 signaling is independent of ICS1-generated SA (Cui et al., 2017). Accumulated SA reinforces the expression of EDS1, PAD4 and other genes in a feedback loop, that appears to be important in defense amplification (Feys et al., 2001; Vlot et al., 2009).

1.11.2. *PAD4*

Arabidopsis genetic studies identified *Phytoalexin Deficient 4 (PAD4)* as an important modulator of basal immunity, ETI response mediating via TNLs receptors, and also activation of SAR response, which promotes the accumulation of SA and the phytoalexin camalexin (Feys

et al., 2001; Jirage et al., 1999; Louis et al., 2012; Rietz et al., 2011; Zhou et al., 1998). PAD4 physically interacts with EDS1, that was found to be necessary for its function in basal immunity but not for ETI response. For instance, the presence of PAD4 and EDS1, but not their physical interactions, is adequate for TNL-triggered cell death and local restriction of *Hyaloperonospora arabidopsidis*. Direct interaction of PAD4-EDS1 is correlated with up-regulation of *PAD4* and mobilization of SA defense pathway (Rietz et al., 2011).

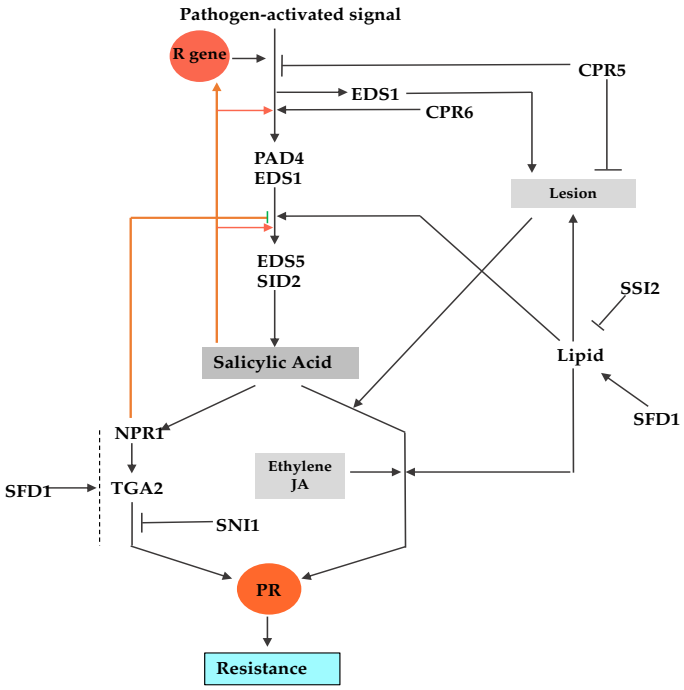


Figure 6. Salicylic Acid Signaling model in *Arabidopsis thaliana*. Adapted from Shah., 2003.

Data point to the placement of *PAD4* downstream of *EDS1* in the *R*-gene mediated defense pathway leading to maximal SA accumulation to affect *PR-1* expression and camalexin synthesis (Feys et al., 2001; Zhou et al., 1998). *pad4* mutation affects the response to SA accumulation and consequently, causes pleiotropic effects on gene expression during the defense response.

PAD4 is also required for activation of camalexin synthesis in response to different elicitors. Signal transduction pathway of camalexin synthesis in challenge by *P. s.* pv. *maculicola* ES4326 and DC3000 requires *PAD4* but in response to AvrRpt2 and *C. carbonum* (non-host fungal pathogen) is *PAD4*- independent (Glazebrook et al., 1997; Zhou et al., 1998).

1.11.3. *NPR1*

The *Nonexpressor of Pathogen- Related gene 1 (NPR1)*, is a master key in signal transduction pathway leading to activation downstream SAR components, such as PR genes (Pokotylo et al., 2019). Moreover, a cross-talk between SA and Jasmonic acid/ Ethylene (JA/ET) in plant defense signaling network is mediated by *NPR1* (Backer et al., 2019; Pieterse et al., 1998). In non-stress conditions, *NPR1* is present as a large cytoplasmic oligomer. Upon SA increase, *NPR1* monomerizes and is translocated to the nucleus, where it can indirectly activate *PR* gene expression through TGA transcription factors (Birkenbihl et al., 2017; Fu & Dong, 2013; Mou et al., 2003). The nuclear localization of *NPR1* is crucial for *PR* gene expression (Després et al., 2000; Mou et al., 2003).

Arabidopsis npr1 mutant, which is impaired in SA signal transduction, shows enhanced disease susceptibility and reduction in *PR1* and *PR5* expression (Cao et al., 1994; Glazebrook et al., 1996). However, overexpression of At*NPR1* or its orthologs display increased disease resistance to a wide variety of pathogens (Dutt et al., 2015; Kumar et al., 2013; Molla et al., 2016; Parkhi et al., 2010; Yuan et al., 2007).

Efficient defense responses depend on the correct activation of SA and JA response pathways, which have antagonistic cross-talk (Glazebrook, 2005; Spoel et al., 2003). *Arabidopsis* infection with both biotrophic and necrotrophic pathogens leads to enhanced susceptibility to the latter through JA defense suppression via SA pathway, that is mediated by cytoplasmic *NPR1* (Ndamukong et al., 2007; Spoel et al., 2007; Yuan et al., 2007). Noteworthy, some pathogens can manipulate this cross-talk to develop disease symptoms (El Oirdi et al., 2011). Further analyses demonstrate the role of *NPR1* in preventing the accumulation of SA during herbivory attack (Rayapuram & Baldwin, 2007). Altogether, *NPR1* is an essential component in plant defense response with a pivotal role in cross-communication between SA and JA/ET pathways (Backer et al., 2019)

1.11.4. *SID2*

The *Arabidopsis Salicylic Acid- Induction Deficient 2 (SID2)* gene encodes isochorismate synthase (*ICS1)*, that converts chorismate to isochorismate, and plays a central role in SA biosynthesis (Imran & Yun, 2020; Lefevere et al., 2020). *sid2* mutants carry mutations in the isochorismate synthase, which indicate the requirement of *ICS1* for SA synthesis following pathogen infection (Wildermuth et al., 2002). Several studies confirmed the importance of

ICS1 in *Arabidopsis* SA accumulation (Garcion et al., 2008; Wildermuth et al., 2002; Yokoo et al., 2018). In addition to strong reduction in SA level, *sid2* mutants display enhanced susceptibility to different pathogens. These symptoms can be rescued through exogenous SA application (Imran & Yun, 2020; Nawrath & Métraux, 1999; Wildermuth et al., 2002; Yang et al., 2015).

Total accumulated SA in *sid2* mutants is about 5-10% of wild-type levels in response to biotrophic pathogens. Besides, *PR1* expression, which is the SAR molecular marker, it is induced at very low level in *sid2* mutants. Almost 1-10 % of wild-type levels, which indicates that SAR defense responses require SA synthesis through *SID2* (Dewdney et al., 2000; Nawrath & Métraux, 1999; Wildermuth et al., 2002). This strong evidence places isochorismate pathway as the main route of defense- associated SA (Brodersen et al., 2005; Zhang & Li, 2019). Multiple efforts have been done to uncover SA roles in plant growth and development by using *sid2* mutants. It has been shown that SA deficiency diminishes Photosystem II (PSII) damage during senescence, which results in extended life span and seed production (Abreu & Munné-Bosch, 2009; García-Heredia et al., 2008).

1.12. Polyamines

1.12.1. General Introduction

The history of the polyamines goes back to more than 300 years ago when Antonie van Leeuwenhoek observed the phosphate crystals of tetramine spermine (Spm) in human semen (Van Leeuwenhoek, 1678), although it was named "spermine" by Ladenburg and Abel in 1888. The discovery of diamine putrescine (Put) and cadaverine (Cad) was about 100 years ago. With the work of Rosenheim in 1924, correct chemical structure of polyamines was determined. At about this time, the triamine spermidine (Spd) was also uncovered (Galston & Sawhney, 1990) (**Table 2**). The early phase of polyamines studies finished via the synthesis of Put, Spd and Spm by Rosenheim (Rosenheim, 1924). Meanwhile, many experiments were designed after the establishment of polyamine's structure to unravel their biological functions. This resulted in interesting findings of their involvement during growth and development processes (Bachrach, 2010), such as the stimulating bacteriophage growth (Ames et al., 1958), accumulation of Spd, Spm and RNA during liver regeneration (Raina et al., 1970) and polyamines enrichment in cancer cells (Bachrach et al., 1967).

Table 2. The history of Polyamines (Based on Bachrach, 2010).

History of Polyamines	
First discovery of Polyamines	Observetion of the phosphate crystals of tetramine spermine (Spm) in human semen (Van Leeuwenhoek, 1678).
Polyamines in plants	The first report of Put occurrence in *Datura stramonium* (Ciamician & Ravema, 1911).
Structure of polyamines	Determination of correct chemical structure of polyamines (Rosenheim, 1924).
Discovery of Diamine Oxidase	(Zeller, 1942)
Polyamines as an stimulator of bacterial growth	Growth of fastidious bacteria such as Hemophilus parainfluenza , Neisseria perflava and Pasteurella tularensis (Herbst & Snell, 1948; Mager et al., 1954; Martin et al., 1952).
Secondary structures of polyamines and their association with DNA	(Liquori et al., 1967)
The first International congress of Polyamines	New York Academy of Science, 1970
Polyamines and cancer	(Russell, 1971)
Synthesis of DFMO; Inhibitor of Polyamine biosynthesis	(Metcalf et al., 1978).
First charachterization of Polyamines biosynthesis and catabolism enzymes	(Gupa & Coffino, 1985; Kahana & Nathans, 1985; Shirahata & Pegg, 1986).
ODC-antizyme complex in plants	(Koromilas & Kyriakidis, 1988)

Parallel to these studies, the function of polyamines in plant growth and development was also surveyed. The early study of polyamines in higher plants (Smith, 1991) led to further investigations that concluded in the biological functions of polyamines (Bagni & Fracassini, 1974; Kaur-sawhney et al., 1978; Richards & Coleman, 1952; Smith, 1971). Nowadays, it is believed that polyamines are ubiquitously present in all living cells and tissues and are essential for organism life (Alcázar, Altabella, et al., 2010). Therefore, genetic or chemical changes that cause depletion in polyamines level, specially Put and Spd, is lethal for yeast, protists and plants (Hamasaki-katagiri et al., 1998; Imai et al., 2004; Roberts et al., 2001; Urano et al., 2005), whilst Spm deficiency results in different grades of organism dysfunctions like hypersensitivity to drought (Pegg & Michael, 2011; Yamaguchi et al., 2007). Moreover, many results revealed the importance of polyamines in plant physiology, such as organogenesis, embryogenesis, foral initiation and development, leaf senescence and biotic and abiotic stress responses (Alcázar et al., 2006; Bagni & Tassoni, 2001; Bouchereau et al., 1999; Galston & Sawhney, 1990; Groppa & Benavides, 2008; Kumar et al., 1997; Kusano et al., 2008; Walden et al., 1997). They do so by modulating the vast variety of cellular processes like differentiation,

cell division, DNA and protein synthesis, cell proliferation, gene expression and signal transduction. Due to their cationic nature in cellular H, this diversity of functions drive from their ability to interact with anionic molecules such as DNA, RNA, phospholipid and proteins (Chen et al., 2019; Handa et al., 2018; Masson et al., 2017; Tiburcio et al., 2014). The major polyamines in plants are Put, Cad, Spd, Spm and thermospermine (t-Spm) (Mattoo et al., 2010; Nahar et al., 2016; Nowicka, 2017; Takahashi et al., 2018; Tiburcio et al., 2014; Wang et al., 2019). Thermospermine is a structural isomer of Spm, which is encoded by a gene named *ACAULIS5 (ACL5*), and ubiquitously present in the plant kingdom. Recent studies show that t-Spm is required for normal growth and development and in the repression of xylem differentiation (Kakehi et al., 2008; Takano et al., 2012; Yoshimoto et al., 2016). In higher plants, polyamines are mostly present in their free forms. In addition to their free forms, polyamines can be conjugated to hydroxycinnamic acids that are referred to hydroxycinnamic acid amides (HCCAs) (Alcázar, Altabella, et al., 2010). The distribution pattern of polyamines show specificity in different plant organs. For example, Put was found to be the most abundant polyamines in leaves (Takahashi et al., 2018). Also, their localization are varying within the cells, for instance in carrot cells, Put was found to localize more in the cytoplasm, whereas Spm was found to be more accumulated in the cell wall (Cai et al., 2006). In general, more plant growth and increased metabolism is associated with greater polyamines contents. Molecular biology techniques provide useful tools to identify target genes in polyamines biosynthesis and signaling pathways which gives new insights into polyamines molecular mechanisms and functions.

Table 3. Abiotic stresses and Polyamines

Polyamines in response to Abiotic stresses		
Abiotic stress	**Induction of Polyamine biosynthesis gene family**	**Additional note**
Salinity	ADC2, SPMS (Urano et al., 2003)	Higher accumulation of Put and Spd, No significant change in Spm level
Drought	ADC2, SPMS, SPDS1 (Alcazar et al., 2006)	Higher accumulation of free and conjugated soluble Put, Higher accumulation of free Spd and Spm
Cold	ADC1, ADC2, SAMDC1 (Hummel et al., 2004; Vergnolle et al., 2005; Cuevas et al., 2008, 2009; Urano et al., 2003)	Higher accumulation of Put, Slightly decrease in Spm level, No significant change in Spd content
Wounding	ADC2 (Pe´rez Amador et al., 2002)	Higher accumulation of Put

1.12.2. Polyamine biosynthesis

Metabolic studies illustrate that polyamine homeostasis is governed by the balance between biosynthesis and catabolism (Kim et al., 2014; Podlešáková et al., 2019; Tiburcio & Alcazar, 2018). Polyamines biosynthetic pathways are conserved from bacterial ancestors (Tabor & Tabor, 1984), and initiates from two amino acids, arginine (Arg) and ornithine (Orn). In animals, Arg is first converted to Orn by mitochondrial arginase, and then decarboxylated by ODC to form Put. In plants and many bacteria, including the intestinal flora but not in mammals (Coleman et al., 2004; Pegg, 2016), there is an alternative pathway to produce Put, which involves the decarboxylation of arginine (Arg) through Arg decarboxylase (ADC), followed by two steps catalyzed by agmatine iminohydrolase (AIH) and *N*-carbamoylputrescine amidohydrolase (CPA). Moreover, it has been demonstrated that ADC/ODC pathways show different evolutionary origins. ODC could arise from bacterial genes of the cyanobacterial endosymbiont, while the origin of ADC, AIH and CPA in plants may derive from a cyanobacterial ancestor of chloroplast (Alcázar, Altabella, et al., 2010; Illingworth et al., 2003). Put is converted to Spd by an aminopropyltransferase reaction catalyzed by spermidine synthase (SPDS). In this reaction an aminopropyl group is transferred to Put from decarboxylated S-adenosyl-Met (dcSAM). Decarboxylation of S-adenosylmethionine (SAM), a universal methyl donor, is catalyzed by SAM decarboxylase (SAMDC) (Ge et al., 2006; Majumdar et al., 2017). Then, Spd is converted to Spm or T-Spm, by a reaction catalyzed by spermine synthase (SPMS) or thermospermine synthase (ACL5), which are encoded by *SPMS* and *ACAULIS5*, respectively (**Figure 7**).

Polyamine biosynthesis in *Arabidopsis thaliana,* a model flowering plant, is initiated by Arg, due to loss of the *ODC* gene (Hanfrey et al., 2001). Accordingly, Put is produced only through the ADC pathway. In *Arabidopsis,* ADC is encoded by *ADC* genes (*ADC1* and *ADC2*) (Alcázar et al., 2006), and only one gene for each AIH and CPA (Janowitz et al., 2003; Piotrowski et al., 2003). Besides, the *Arabidopsis* genome possesses two genes encoding spermidine synthase (*SPDS1* and *SPDS2)* (Hanzawa et al., 2002), one single gene coding spermine synthase (*SPMS*) (Panicot et al., 2002), another one coding for t-Spm synthase (*ACL5*) (Kakehi et al., 2008; Knott et al., 2007), and at least four coding for SAM decarboxylases *(SAMDC1-4)* (Majumdar et al., 2017; Urano et al., 2003).

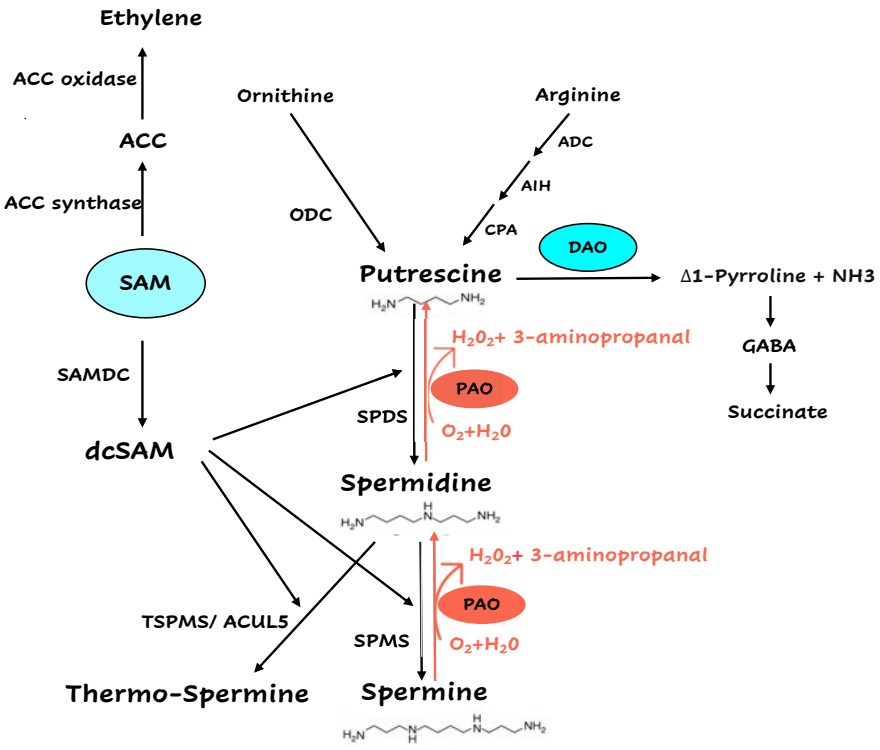


Figure 7. Polyamine biosynthesis and catabolism networks in plants. ACC, 1-amino-cyclopropane-1-carboxylic-acid; SAMDC, S-adenosylmethionine decarboxylase; ODC, ornithine decarboxylase; ADC, arginine decarboxylase, AIH, agmatine iminohydrolase; CPA, N- carbamoylputrescine amidohydrolase; SPDS, spermidine synthase; SPMS, spermine synthase; PAO, polyamine oxidase; DAO, diamine oxidase.

1.12.3. S-adenosylmethionine decarboxylase

S- adenosylmethionine decarboxylase is a key enzyme in the production of higher polyamines, which is encoded by multigene family in angiospermae (Franceschetti et al., 2001). The enzymic function of SAMDC is to raise decarboxylate group of SAM to produce dcSAM, that is the main aminopropyl group donor. The enzyme carries out a critical limiting step in the production of Spd and Spm, thus acting as a major regulator of polyamine biosynthesis. dcSAM is exclusively used in polyamine production and no other function for dcSAM is known (Majumdar et al., 2017). Furthermore, SAM is the major methyl group donor in transmethylation reactions of RNA, DNA, proteins, and lipids, besides it is a precursor of the biosynthesis of ethylene, biotin and nicotianamine in plants (Roeder et al., 2009). Hence, polyamines and ethylene could act antagonistically competing for SAM as a common substrate (Bitrián et al., 2012). *Arabidopsis thaliana* has four *SAMDC* genes, *SAMDC1* (At*3g02470*), *SAMDC2* (At*5g15950*), *SAMDC3* (At*3g25570*), and *BUD2* or *SAMDC4* (At*5g18930*), which show high sequence similarity among them. SAMDCs translation are regulated via

5'untranslated regions (5'UTRs). The 5'UTR contains two upstream open reading frames (uORFs), that govern SAMDC mRNA translation (Franceschetti et al., 2001; Ivanov et al., 2010). Transcripts of SAMDCs have been described in a vast variety of plant species, mostly with higher expression in reproductive organs in contrast to vegetative organs (Ge et al., 2006; Hao et al., 2005; Marco & Carrasco, 2002; Sinha & Venkat, 2013; Urano et al., 2003). In carnation (*Dianthus caryophyllus)* by constructing a promoter::5'UTR(- SAMDC)::GUS, moderate activity of GUS in the stem and the cotyledonary veins of tobacco seedlings was detected, whilst in the pollen, stigma, petals and stamens high activity of GUS was observed (Kim et al., 2004). The transcripts of *AtSAMDC1*, *AtSAMDC2*, *AtSAMDC3* and *AtSAMDC4* in vegetative and reproductive organs were detected (Franceschetti et al., 2001; Ge et al., 2006; Jumtee et al., 2008; Urano et al., 2003). The results showed the expression of *SAMDC1* in all organs of mature plants, high expression of *SAMDC2* in leaves, flowers and roots, weak expression of *SAMDC3* in all except in siliques and, ubiquitous and low expression of *SAMDC4*, which may concluded in diverse distribution pattern of AtSAMDC RNAs (Majumdar et al., 2017). Up regulation of *SAMDC* has been reported in response to diverse biotic and abiotic stresses. The overexpression of *SAMDC* mostly results in enhanced tolerance to different stresses (Wi et al., 2006). Although, in some cases, increased *SAMDC* expression is not correlated with higher Spd and/or Spm contents, probably due to tight regulation of the enzyme activity (Tiburcio et al., 2014).

1.12.4. Polyamine conjugation

In nature, polyamines occur not only as free amines, but also conjugated to different macromolecules like proteins (bound form) or to small molecules such as phenolic acids (conjugated forms) (Martin-tanguy, 1997; Tiburcio et al., 1997). The most common conjugated polyamines in plants are hydroxycinnamic acid amides (HCCAs), which synthesis is catalyzed by a class of enzyme that termed transferases (Martin-tanguy, 2001). HCCAs are classified as secondary metabolites which are extensively distributed in plant species (Elejalde-palmett et al., 2015; Luo et al., 2009). The first discovery of Caffeoyl- putrescine (paucine) goes back to 1983 (Tiburcio et al., 1990) in some leguminous seeds, but further studies revealed the presence of other HCCAs (Coumaroylputrescine, feruloylputrescine, coumaroylagamatine, dicoumaroylspermidine, diferuloylspermidine, diferuloylspermine and feruloyltyramine) in different plant species (Martin-tanguy, 1997). When the plant initiates flowering, conjugated

polyamines accumulate more in the floral organs by moving from leaves to young floral buds (Havelange et al., 1996). In tobacco *(Nicotiana tabacum*) polyamine conjugates (caffeoylputrescine and caffeoylspennidine) accumulate in the last initiated leaves and shoot apices during floral induction (Martin-tanguy, 1997). Recently, it has been revealed the existence of hydroxycinnamic acid conjugates of Spd in *Arabidopsis thaliana* flower buds (Fellenberg et al., 2009). The occurrence of HCCAs is not limited only to floral organs, but also in seeds and roots (Luo et al., 2009). The accumulation of feruloyltyramine, diferuloyputresine, and diferuloyspermidine were detected in notable quantities in rice (*Oryza sativa)* seeds (Bonneau et al., 1994). Moreover, tyramine-derived HCCAs were discovered at high levels in the bark of *Lycium chinense* roots (Lee et al., 2004), and also tobacco roots (Hagel & Facchini, 2005). In addition, HCCAs have been largely reported in plant defense response and particularly against biotic stresses (Elejalde-palmett et al., 2015). Bacterial and fungal infection in some Solanaceae plants induced the feruloyl and coumaroyl tyramine synthesis (Keller et al., 1996; Negrel & Martin, 1984; Newman et al., 2001; Zacarés et al., 2007). In like manner, *Arabidopsis* and barley in response to pathogen attack produced more coumaroyl and feruloyl agmatine (Muroi et al., 2009; Ropenack et al., 1998).

1.12.5. Polyamine catabolism

Amine oxidases (AOs) participate in essential physiological processes involving plant growth and development, response to abiotic stresses like salinity, drought, heavy metals, chilling and frizzing, as well as in plant defense response to pathogen attack (Angelini et al., 2010; Cona et al., 2006). Plants battle these stresses by regulating polyamine levels though their biosynthesis and catabolism. Two classes of enzymes, copper-containing amine oxidases (CuAOs) and flavin-containing polyamines oxidases (PAOs) are involved in plant polyamine oxidation (Alcázar et al., 2006).

Copper containing amino oxidases

Plant CuAOs usually exist in high level in dicotyledons (Cona et al., 2006), and prefer diamines as substrates. Diamine oxidase that relies on Cu^{2+} and pyridoxal phosphate as its cofactors, and catalyze the oxidation of putrescine at primary amino groups, which generate 4-aminobutanal,

H_2O_2, and ammonia (Alcázar et al., 2010; Moschou et al., 2012), although some results indicate the activity of some CuAO on Spd oxidation in *Arabidopsis* (Planas-portell et al., 2013). *Arabidopsis* possess 12 *CuAO* genes (Alcázar et al., 2006), however, only five of them (*AtAO1*, *AtCuAO1*, *AtCuAO2*, *AtCuAO3*, and *AtCuAO8*) have been characterized biochemically (Ghuge et al., 2015; Groß et al., 2017; Møller & Mcpherson, 1998; Planas-portell et al., 2013).

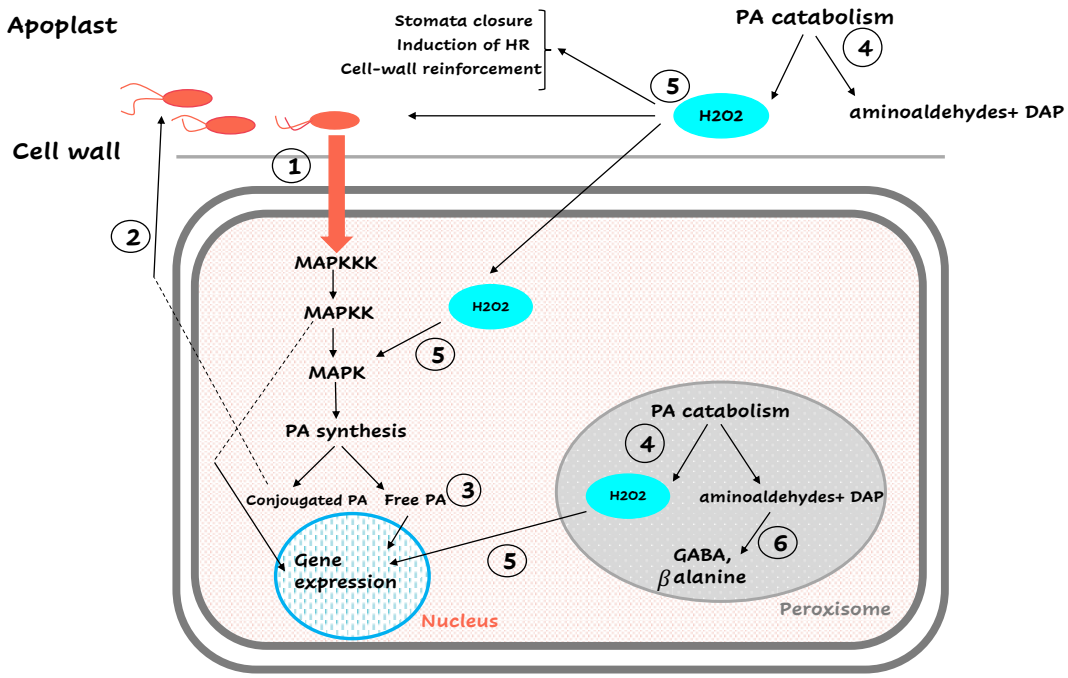


Figure 8. Polyamine metabolism in plant-microbe interaction. 1, Recognition of plant pathogens via specific receptors activate signaling transduction pathway with active participation of MAP kinases, such as WIPK and SIPK, which stimulate defense gene expression and PA biosynthesis. This process leads to the accumulation of free and conjugated PAs; 2, Some conjugated PAs have an antimicrobial effect; 3, Free PAs may contribute directly to the activation of gene expression or indirectly through their catabolism; 4, PAs oxidation by DAO and PAO occur in the apoplast and peroxisome that generate H_2O_2, DAP and aminoaldeydes; 5, H_2O_2 acts as signaling molecule and stimulates the expression of defense-related gene. Also, H_2O_2 has effect of pathogen growth, induces stomata closure and HR. Other products of polyamine catabolism may have defensive roles. The precursors of GABA biosynthesis are aminoaldehydes. GABA participates in plant-bacterial interaction and plant defense. DAP is a substrate for the synthesis of uncommon PAs. β-alanine has an important role in biotic stress. Adapted from Jiménez-Bremont et al., 2014.

Plant CuAOs are classified in two groups based on their localization (Zarei et al., 2015). Members of the first group carry an N-terminal signal peptide and are usually extracellular proteins. *Arabidopsis thaliana* (AtAO1 and AtCuAO1), *Malus domestica* (MdAO2), *Pisum sativum* (PsCuAO) and *Citrus sinensis* (CsCuAO4,CsCuAO5 and CsCuAO6) belong to this

group (Møller & Mcpherson, 1998; Planas-portell et al., 2013; Tipping & McPherson, 1995; Wang & Liu, 2015; Zarei et al., 2015). The second group possess C-terminal peroxisomal targeting signal (PTS1), which includes *Arabidopsis thaliana* (AtCuAO2 and AtCuAO3), *Malus domestica* (MdAO1), *Nicotiana tabacum* (NtMPO1 and NtCuAO1) and *Citrus sinensis* (CsCuAO2 and CsCuAO3) (Naconsie et al., 2014; Planas-portell et al., 2013; Wang & Liu, 2015; Zarei et al., 2015).

Flavin containing polyamines oxidases

In contrast to CuAOs, PAOs are enzymes that have strong affinity to Spd and Spm as well as their acetylated forms (Alcázar, Altabella, et al., 2010). In relation to catabolic functions and subcellular localization, PAOs are classified into two classes. The first class of PAOs catalyze terminal catabolism of Spd and Spm, producing 1,3-diaminopropane (DAP), H_2O_2 and 4-aminobutanal (Spd catabolism) or N-(3-aminopropyl)-4-aminobutanal (Spm catabolism) (Bordenave et al., 2019; Cona et al., 2006; Moschou et al., 2012; Tavladoraki et al., 2016). Maize *PAO* gene (*ZmPAO*) is the best characterized *PAO* gene of the first class (Cona et al., 2006; Tavladoraki et al., 1998). The second class of PAOs catalyze polyamine back-conversion reactions that convert Spd to Put and Spm to Spd and generate H_2O_2 and 3-aminopropanal. This class of plant PAOs are likely to the mammalian Spm oxidase (SMO) which catalyzes the back-conversion of Spm to Spd (Moschou et al., 2008). Although PAOs occur at high levels in monocotyledons (Sebela et al., 2001), some studies have revealed the presence of *PAO* genes in both monocots and dicots, including maize (Cervelli et al., 2006), rice (Ono et al., 2012), barley (Cervelli et al., 2006), *Arabidopsis* (Fincato et al., 2011), tobacco (Yoda et al., 2006), grapevine (Paschalidis et al., 2009), apple (Kitashiba et al., 2006), sweet orange (Wang & Liu, 2015), tomato (Ono et al., 2012) and cotton (Chen et al., 2015). In the *Arabidopsis* genome, five *PAO* genes (*AtPAO1-AtPAO5*) have been characterized, which predominantly catalyze the back-conversion of polyamines (Ahou et al., 2014; Alcázar et al., 2006; Fincato et al., 2011; Tavladoraki et al., 2006). Besides, PAOs display substrate specificities, in which, the AtPAO1 catalyzes the oxidation of Spm but not Spd (Tavladoraki et al., 2006), whereas AtPAO3 prefers Spd rather than Spm (Moschou et al., 2008). AtPAO2 and AtPAO4 show similar preference for Spd and Spm (Fincato et al., 2011). AtPAO5 catalyzes t-Spm back conversion to Spd (Kim et al., 2014). According to the cellular localization, polyamines back-conversion mostly takes

place in peroxisomes, whilst the polyamines terminal catabolism occurs in the apoplast space (Wang et al., 2019).

Polyamine terminal catabolism and back-conversion leads to higher level of H_2O_2, an important signaling molecule, which modulates a span of physiological or biological processes (Wang & Liu, 2015). For instance, H_2O_2 derived from Spd catabolism, provokes Ca^{2+} influx and regulates pollen tube growth (Wu et al., 2010). Moreover, H_2O_2 produced via PAOs oxidation, has been revealed to be involved in the defense response against biotic and abiotic stresses (Rodrıguez et al., 2009; Wang & Liu, 2015). In addition, the involvement of PAO-derived H_2O_2 in hypersensitivity reaction (HR) and programmed cell death (PCD) has been demonstrated (Fu et al., 2011; Moschou et al., 2008). Moreover, polyamine metabolism is tightly related to the other metabolic pathways, such as proline metabolism, ethylene biosynthesis, and the synthesis of nitric oxide (NO) (Freitasa et al., 2018; Lasanajak et al., 2014; Mellidoua et al., 2017; Takahashi, 2016). Recent evidence suggests the relation between polyamines metabolism and nitric oxide (NO), a crucial signaling compound, which has some overlapping physiological roles with polyamines in plants (Agurla et al., 2018; Pál et al., 2015; Yamasaki & Cohen, 2006).

1.13. Polyamines and plant responses to biotic stress

The active participation of polyamines has been documented in plant-pathogen interactions (Jiménez-Bremont et al., 2014). Incompatibility, which is mostly an important form of resistance to biotrophic pathogens (Glazebrook, 2005), leads to ETI response which is accompanied by polyamine accumulation in plants. Increment of PA levels (free Put and Spd), with enhanced enzymic activity of ODC, ADC and SAMDC were observed in response to powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (Cowley & Walters, 2002). Moreover, ETI activation in response to *Fusarium graminearum*, increased ODC and ADC gene expression and Put accumulation in wheat plants (Gardiner et al., 2010; Rampersad, 2020). Other research demonstrated that polyamines, mainly Put, accumulate in response to pathogenic strain of *Fusarium culmorum* in flax seedlings and this was correlated with higher ADC expression. Moreover, the cell wall-bound polyamines content enhanced remarkably compared to the free and conjugate level of polyamines, which indicates the possible role of polyamine in cell wall strengthening and inhibition of *Fusarium* growth, however, these results

were not evidenced in non-pathogenic strain of *Fusarium* (Wojtasik et al., 2015). Other works revealed an increase in ODC activity and polyamine levels during HR and PCD in response to *Tobacco mosaic virus* (TMV) (Negrel et al., 1984) and bacterium *Xanthomonas campestris* (Kim et al., 2013). It has been reported that enhanced apoplastic polyamines, Spm particularly, and the activity of PAOs and DAOs play critical roles in the defense response to biotrophic bacterium *Pseudomonas viridilava* (Marina et al., 2008) and *Cucumber mosaic virus* (CMV) (Mitsuya et al., 2009). Although, the occurrence of enzymic activity of PAOs and DAOs as an very early response has been suggested by Walters (Walters, 2003).

In parallel, some studies explored the contribution of polyamines in compatible interactions. Greenland and Lewis reported the accumulation of Spd in response to *Puccinia hordei* in barley leaves (Greenland & Lewis, 1984). The increased polyamine content was also reported in wheat plants inoculated with *Puccinia graminis* f. sp. *tritici* (Foster & Walters, 1992; Yin et al., 2019).

Table 4. Polyamines and Defense response

Polyamines in defense resposne				
Incompatible Interaction	**Plant Species**	**Induction of Polyamine biosynthesis gene**	**Additional note**	**Reference**
Fungus, *Fusarium graminearum*	*Triticum aestivum,* Wheat	ADC, ODC	Higher accumulation of Put	Gardiner et al., 2010
Fungus, *Blumeria graminis* f. sp. *hordei*	*Hordeum vulgare* L., Barley	ADC, ODC, SAMDC, PAO, DAO	Higher accumulation of free Put and Spm/ Higher accumulation of conjugated Put, Spd,Spm	Cowley and Walters, 2002
Tobbaco Musaic Virus (TMV)	*Nicotiana tabacum, Tobacco*	ADC, ODC, DAO	Higher accumulation of free and conjugated Put and Spd/ Higher accumulation of apoplastic Spm	Negrel et al., 1984; Torrigiani et al.,1997; Marini et al., 2001; Yamakawa et al., 1998
Fungus powdery mildew, *Erysiphe graminis*	*Hordeum vulgare* L., Barley	ADC, ODC, PAO, SAMDC	Higher accumulation of Put, Spd, Spm	Walters and Wylie, 1986; Coghlan and Walters, 1990; Walters et al., 1985
Compatible Interaction	**Plant Species**	**Induction of Polyamine biosynthesis gene**	**Additional note**	**Reference**
Fungus Puccinia graminis f. sp. *tritici*	*Triticum aestivum,* Wheat	ODC	Higher accumulation of Put,Spd,Spm	Foster and Walters, 1992
Pseudomonas syringae DC3000	Solanum lycopersicum, Tomato	ADC1, ODC, SAMDC	Higher accumulation of Put	Vilas et al., 2018

Despite of the fact that polyamines metabolism increases in both compatible and incompatible plant-pathogen interactions, this response differs in resistant and susceptible cultivars. Resistant cultivars exhibit higher polyamines content compared to susceptible ones (Asthir et al., 2004; Cowley & Walters, 2002). However, in response to smut fungus *Ustilago scitaminea,* susceptible sugarcane buds exhibits higher level of conjugated polyamines while decreased free polyamine levels (Legaz et al., 1998).

Consistent with this, variation of polyamines levels in plant-pathogen interactions alters gene expression involved in phytohormone signaling, such as salicylic acid. The active contribution

of salicylic acid to initiate defense responses against biotrophic and hemi-biotrophic is well documented (Qi et al., 2018). Although some works have addressed the correlation of polyamine metabolism and SA signaling, the SA-PA interaction still remained elusive.

Objectives

To face with diverse environmental conditions, such as a vast variety of pathogenic microbes, plants possess two branches of pathogen recognition. PAMPs-triggered immunity (PTI) relies on cell surface recognition of Microbial- or Pathogen-Molecular Associated Patterns (MAMPs or PAMPs) such as flagellin, by transmembrane pattern recognition receptor (PRRs). In addition, recognition of pathogen effectors by polymorphic NB-LRR protein products encoded by *Resistance* (*R*) genes, results in activation of Effector-Triggered Immunity (ETI), which is often associated with hypersensitive response (HR). A successful pathogen can disrupt PTI and cause Effector-Triggered Susceptibility (ETS) by deploying effectors.

During plant- pathogen interactions, notable changes occur in polyamine metabolism that usually result in the accumulation of most abundant PAs putrescine (Put), spermidine (Spd) and spermine (Spm). However, little is known about signaling pathways which modulate polyamine metabolism during defense. In addition, the responses of polyamines to the PTI and ETI branches have not been studied in depth.

In addition to known polyamine metabolism genes, other genes might condition polyamine homeostasis. The identification of new genes can be performed through genetics screens based on the study of natural variation through GWAS mapping. The biosynthesis of higher polyamines (Spd and Spm) requires S-adenosylmethionine (SAM) as universal methyl donor. As an important metabolite, SAM participates in essential metabolic pathways in plants by entering in polyamines and ethylene biosynthesis and plays a critical role in response to environmental stresses. However, little is known about the competition for SAM between polyamines and ethylene biosynthesis, as well as the limitations of polyamine biosynthesis by SAM availability.

Based on this, we propose the following objectives:

I- To investigate the alteration of polyamines metabolism in response to PTI and ETI branches of plant defense, using bacteria inoculations (*Pseudomonas syringae*) and pathogen-free systems.

II- To study the contribution of the salicylic acid (SA) pathway to polyamine metabolism during defense by using the loss-of-function mutants of the SA biosynthesis and signaling pathways.

III- To study the contribution of polyamines to cell death, and the effect of the co-treatment of polyamines with known PAMPs (flg22) in cell death responses.

IV- To investigate the role of Spm and thermospermine (tSpm) to plant defense against *Pseudomonas syringae* and the establishment of systemic acquired resistance by using *spms* and *acl5* loss-of-function mutants.

V- To identify new genes involved in the modulation of polyamine metabolism by GWAS mapping.

VI- With a focus on SAM metabolism derived from GWAS mapping, to study the competition between polyamine and ethylene biosynthesis via SAM metabolism, the influence of SAM metabolism on polyamine levels and defense against *Pseudomonas syringae.*

Material and Methods

1. Plant material and growth conditions

1.1. Plant growth on soil

For plant growth on soil, seeds from different genotypes were vernalized for 3 days on a wet filter paper at 4º C and directly sown on a mixture containing perlite (10%), peat moss (40%) and vermiculite (50%). Plants were grown at 20-22 ºC under 8 h light (8:00 a.m. to 4:00 p.m.)/16 hr dark cycles at 100–125 µmol photons $m^{-2} s^{-1}$ of light intensity and 60–70 % relative humidity. Seeds from auto-immune mutants were grown at 16 ºC and 28 ºC under the same light conditions.

1.2. Plant in vitro culture

For in vitro culture, seeds were sterilized using the chlorine (Cl_2) method for three hours. Seeds were sown on growth media [1/2 Murashige and Skoog salts (MS) supplemented with vitamins (Duchefa Biochemie), 1% sucrose, 0.5% plant agar (Duchefa Bio- chemie) and 0.05% MES adjusted to pH 5.7 with 1 M KOH]. To synchronize germination, seeds were stratified in the dark at 4º C for 2–3 days. Plates were incubated in 12 hr light/ 12 hr dark cycles at 20-22 ºC at 100–125 µmol photons m−2 s−1 of light intensity.

1.3. Modified MS growth media

To avoid the suppression of autoimmunity phenotype in in-vitro condition, we applied modified MS media to perform the experiment (**Table 5**).

Table 5. MS and modified MS media

Chemical compounds		0.5* MS	0.5 * modified MS
Macro elements	NH_4NO_3	10.31 mM	1 mM
	KNO_3	9.40 mM	9.40 mM
	$CaCl_2$	1.50 mM	1.50 mM
	KI	2.5 µM	2.5 µM
	$MgSO_4.7\ H_2O$	750 µM	750 µM
	KH_2PO_4	630 µM	630 µM
Micro elements	Fe-EDDHA	50 µM	50 µM
	H_3BO_3	50.14 µM	50.14 µM
	$MnSO_4.H_2O$	50 µM	50 µM
	$ZnSO_4.7\ H_2O$	14.96 µM	14.96 µM
	$CuSO_4$	0.05 µM	0.05 µM
	$CoCl_2$	0.06 µM	0.06 µM
	$Na_2MoO_3.\ 2H_2O$	0.52 µM	0.52 µM

1.4. Germplasm information

The *npr1-1* mutant (Cao et al., 1997) was kindly provided by Prof. Xinnian Dong (Duke University, USA). The *esd1-2* mutant was kindly provided by Jane Parker. The *acl5* mutant was previously reported by (Hanzawa et al., 1997). The *Arabidopsis thaliana* accession Kashmir (Kas-2) and NIL and cNIL lines were previously reported (Alcázar et al., 2010).The mutants of SAM pathway were obtained from the Nottingham Arabidopsis Stock Center (NASC, UK) are listed in **Table 6**.

Table 6. The list of SAM pathway genes and mutants

Gene		Mutants	ABRC stock number
Methionine SAM synthase	At1*g02500* / SAM1	*N662619, N672705*	SALK_059210C/ SALK_073599C
	At*4g01850/ SAM2*	*N676306*	SALK_097197C
	At*2g36880/ MAT3*	*N519375, N631793*	SALK_019375/ SALK_131793
	At*3g17390/ MAT4*	*N658683, N511935*	SALK_052289C/ SALK_011935
SAMDC	At*3g02470/ SAMDC1*	*N520362, N531967*	SALK_020362/ SALK_031967
	At*5g15950/ SAMDC2*	*N734813-N734827, N542743*	SALK_042743/ GK-492B08
	At*5g18930/ SAMDC4*	*N2046765-N2046776*	GK-156H11.01/ GK-156H11.12
SAM dependent methyl transferase	*At1g63855*	*N2011935, N2011602*	GK-911F08.03/ GK-911F08.12
	At4g26420/ GAMT1	*N659188, N677684*	SALK_088960C/ SALK_035597C
	At4g26460	*N542506, N656413, N657887*	SALK_042506/ SALK_042505C/ SALK_021186C
	At4g26600	*N671936, N686434*	SALK_117497C/ SALK_084427C
	At4g26730	*N537987, N695454*	SALK_037987/ SALK_206047C

The information of other mutants utilized in this research is listed in **Table 7**. These seeds were obtained from the Nottingham Arabidopsis Stock Center (NASC, UK).

Table 7. The list of other seeds applied in this research

Gene	Mutant	ABRC stock number	Gene	Mutant	ABRC stock number
At1G74710	sid2-1	-----	At3g51770	eto1	CS3072
At3g52430	pad4	CS3806	At3g49700	eto3	CS8060
At5G53120	spms	SALK_059355	At5g03730	ctr1	CS8057
At5G19530	acl5	-----	At4g01370	mpk4	CS5205
At4G16890	snc1	CS69908			

1.5. Isolation of homozygous mutants

The mutants obtained from NASC were tested for homozygosity by antibiotic resistance and PCR genotyping. For antibiotic screening, seeds were sterilized (see 1.2.) and sown on growth media supplemented with Kanamycin 50 µg/ml. The DNA of kanamycin resistant seedlings was extracted using the CTAB method (**Figure 9**).

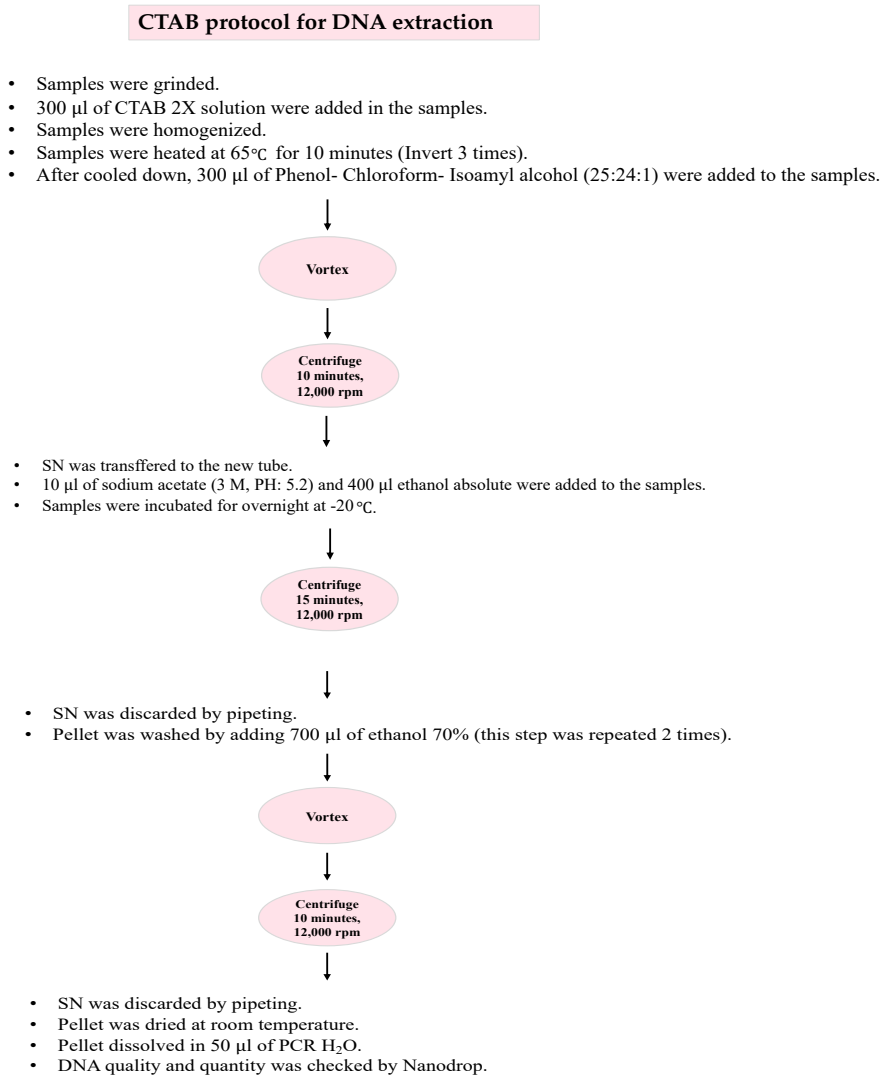


Figure 9. CTAB protocol for DNA extraction of *Arabidopsis thaliana*. 2X CTAB solution is consist of CTAB (2 g), NaCl (8.182 g), Tris 1 M/ PH:8 (10 ml), EDTA 0.5 M (4 ml), PVP 40 (1 g) and H_2O added up to 100 ml.

Each mutant was PCR tested by two pairs of primers, to amplify the corresponding gene (primers Re and Fw) and to determine T-DNA insertion (primers Re and LB 1.3). The list of the primers used for each mutant is shown in **Table 8**.

Table 8. Primers used for the genotyping of homozygous mutants

Gene	ABRC stock number	Primer (forward)	TM (°C)	Primer (Reverse)	TM (°C)
At1*g02500* / SAM1	SALK_059210C	CTT GAA GAA GTG CCA TCA AGC	59	CAG AAA GAA TGG TAC TTG CGC	59
	SALK_073599C	TTA TCT CCG TTA CGA CGG TTG	59	AGC CAT TGT CTG TCT TTG TGG	59
At*4g01850*/ *SAM2*	SALK_097197C	CTT GCC TCA CGA TGT AAG CTC	61	AGC CAT AAA TGA GCC TTC CTC	59
At*2g36880*/ *MAT3*	SALK_019375	TGA GGC CTG ATG GTA AGA CAC	61	TAA AGG GAC ATC GAC AAG TGC	59
	SALK_131793	CCT CAG AAT CAC GAC GAA CTC	59	CTT TGC TCT ATC ATC GCC ATC	59
At*3g17390*/ *MAT4*	SALK_052289C	ACA TGA ACT TCG CAT ATT GGC	57	AAT CTC ACG GCA TGT TTT ACG	57
	SALK_011935	AAT CTC ACG GCA TGT TTT ACG	60	ACA TGA ACT TCG CAT ATT GGC	60
At*3g02470*/ *SAMDC1*	SALK_020362	TAT GAT GGA GTC GAA AGG TGG	59	CCA GTC TTG TCA GCT TCA TCC	61
	SALK_031967	CGT GTT GGA TAA CCG TTT GAC	60	GCG AAC TCA TAC AAG CCA GAG	60
At*5g15950*/ *SAMDC2*	SALK_042743	TTG AAT TTC TTG ATT CCA CCG	55	GCA GTT TTT GTG TGG CTT AGC	59
At*5g18930*/ *SAMDC4*	GK-156H11.01 GK-156H11.12	AAA CTT GAT GCA TTG TGA CCC	57	AAA TGT TTA CTC GGA CAG GGG	59
At1g63855	GK-911F08.03 GK-911F08.12	AGT CCC CAT TTT ACC ATC AGG	59	CTT GTA GCG TTA TCC TCG CTG	61
At4g26420/ *GAMT1*	SALK_088960C	TGA GAA TTT CTT TGT CGG CAG	57	AGA GCT CCA TGT CGT TGT GAC	61
	SALK_035597C	CAC GTT TAG CCT TGA GCA AAC	59	AAG GGA CGT TTT GTC AAT TCC	57
At4g26460	SALK_042505C	GAT CCT TCT ATC CAC GCC TTC	61	CAA AAG GCC CCT TAA ATT TTG	55
	SALK_021186C	CTA TGG CAT CAA AAT TCC TCG	57	TTG ACA AAT TTA GCC TAA GGT CG	59
At4g26600	SALK_117497C	CTA AAA TTA TGG GGC TGG AGG	59	GAG ACA AGC ACG AGA GGA ATG	61
	SALK_084427C	AAC ATC CTC AAG CAT GTA CCG	59	TCT TCT GGC AGA TGT TTC CTG	59
At4g26730	SALK_037987	GGC CAT TTT CGT AAT TTC TCC	57	TGT TGC CTT GGT CTC TCT TTG	59
	SALK_206047C	CTA TGG CAT CAA AAT TCC TCG	57	TTG ACA AAT TTA GCC TAA GGT CG	59

The PCR runs were performed in Peltier thermocycler (PTC-225) by using 10X buffer (Peq Lab), Taq DNA polymerase (Peq Lab), dNTPs (1.25 mM) (Peq Lab), each primer 100 µM and PCR H_2O up to the final volume (20 µl). The thermal cycle was as follows: 5 min at 95 °C for DNA polymerase activation, 15 sec at 95 °C for DNA denaturation, 45 sec at 55 °C or 48 °C (gene amplification) and 50 °C or 48 °C (inserted T-DNA amplification) for annealing, elongation for 2 min at 72 °C, 35 cycles of DNA denaturation (15 sec at 95 °C); and final elongation step for 10 min at 72 °C.

2. Polyamine analyses

2.1. Chemicals

The chemical products were used in polyamines extraction are listed in **Table 9**.

2.2. Solutions

1. Saturated Na_2CO_3 or $CaCO_3$ solution.

2. Polyamine stock solution; Fresh polyamine stock solutions were prepared at 100 mM concentration in water and sterilized by filtration.

Table 9. The list of chemical products for polyamine extraction

Chemical product	CAS number	Company
Putrescine dihydrochloride	110-60-1	Sigma- Aldrich
Spermidine	124-20-9	Sigma- Aldrich
Spermine	71-44-3	Sigma- Aldrich
Dandyl chloride	605-65-2	Sigma- Aldrich
1,7 Diaminoheptane (HTD)	646-19-5	Sigma- Aldrich
Toluene	108-88-3	Sigma- Aldrich
Perchloric acid (PCA)	7601-90-3	Sigma- Aldrich
Acetonitrile (ACN)	75-05-8	Sigma- Aldrich
Acetone	67-64-1	Merck

2.3. Polyamine extraction

The protocol of polyamines extraction from *Arabidopsis thaliana* used in this research is described in **Figures 10 and 11**.

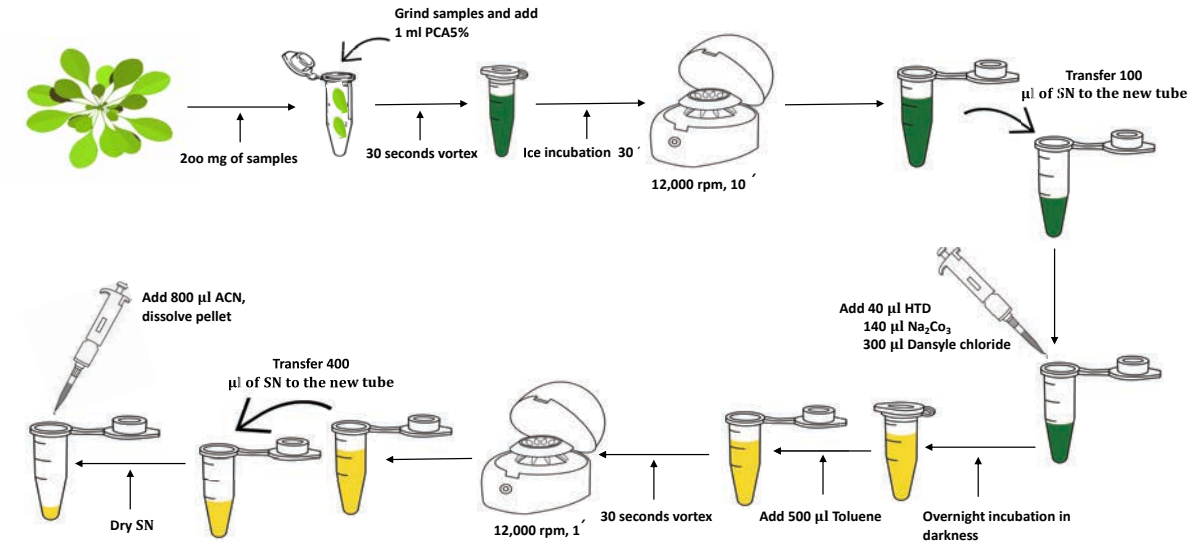


Figure 10. Schematic of polyamines extraction process

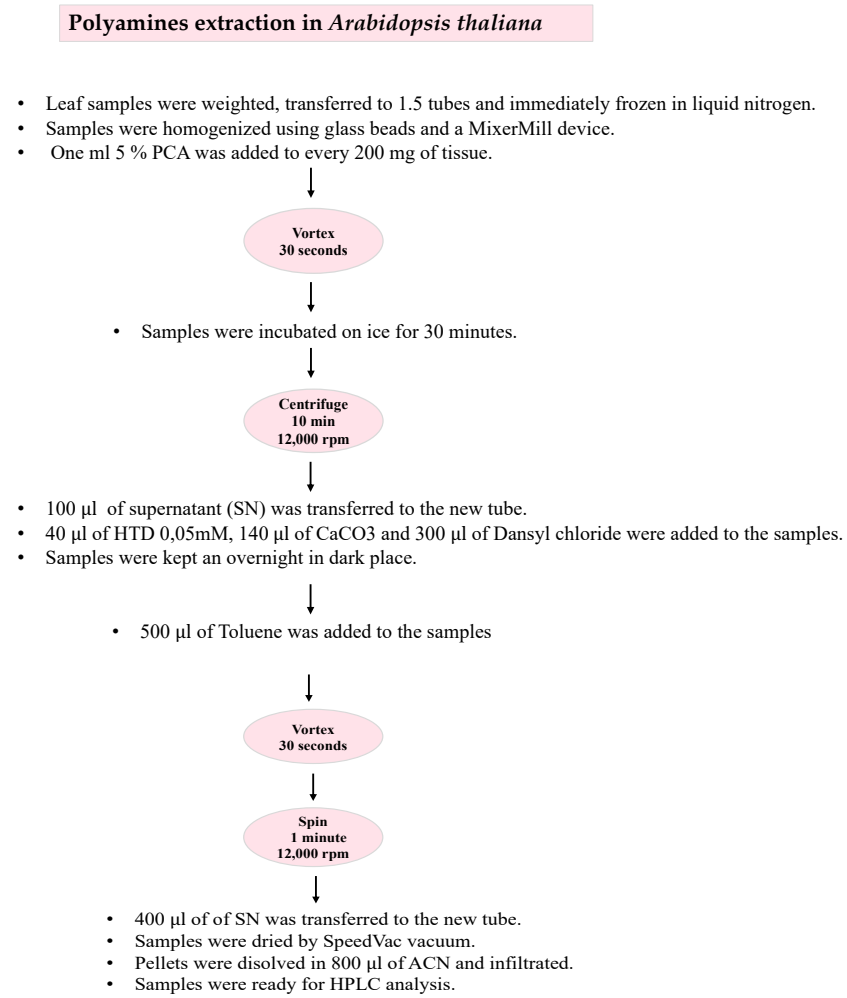


Figure 11. The protocol of polyamines extraction in *Arabidopsis thaliana*

2.4. Polyamine levels determination

Polyamines were analyzed by high-performance liquid chromatography (HPLC) separation of dansyl chloride- derivatized polyamines. Polyamines were injected into the column (BRISA-LC2, C18) that was previously eluted with 100% acetonitrile and water. The gradient used is shown in **Figure 12**. The initial condition was 70% acetonitrile and 30% water for 4 minutes (Perkin-Elmer 200). At minute 4, the concentration of acetonitrile was elevated up to 100 % for 10 minutes. High concentration of acetonitrile is optimized for Spd and Spm elution, which are strongly retained on the column. This concentration of acetonitrile returned to the initial condition at minute 10 (**Figure 12**). The column was re-equilibrated during the remaining time (Marcé et al., 1995).

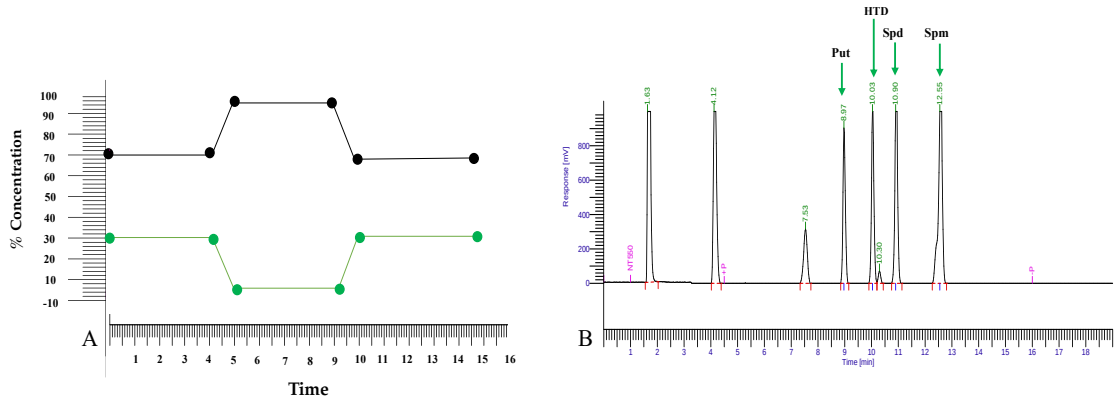


Figure 12. Schematic of HPLC analysis. A. HPLC gradient for polyamine analysis (Adapted from Marce et al., 1995). B. Chromatograph of HPLC analysis.

2.5. Polyamine levels calculation

According to the data derived from HPLC chromatographs, polyamine values were calculated as follows:

Putrescine → nmol/g PF: 44,3085. Area Put. (Area HTD)$^{-1}$

Spermidine → nmol/g PF: 27,4505. Area Put. (Area HTD)$^{-1}$

Spermine → nmol/g PF: 22,9089. Area Put. (Area HTD)$^{-1}$

1,3DAP → nmol/g PF: 51,2205. Area Put. (Area HTD)$^{-1}$

3. Pathoassays

3.1. Bacterial inoculation

Pseudomonas syringae pv. *tomato* DC3000 (*Pst* DC3000) strains (**Table 10**) were streaked on solid NYGA medium (3 gr/L yeast extract, 5 gr/L bacto peptone, 20 ml/L glycerol, 15 g/ L bacto agar in case of solid medium) containing corresponding antibiotics (**Table 10**). The day of inoculation, bacteria were suspended on 10 mM $MgCl_2$ to the required OD_{600} (**Table 10**), Silwet L-77 was added to a final concentration of 0.04% (v/v) before spraying. Samples were harvested at 0h, 24h and 72h for polyamine analysis.

Table 10. Bacterial strains and growth conditions

Pseudomonas syringae pv tomato	Antibiotic	Incubation temprature
Pst DC3000	Rifampicin / 50 µg/ml	28º C
Pst DC3000 *AvrRpm1*	Rifampicin / 50 µg/ml and kanamycin / 25 µg/ml	28º C
Pst DC3000 *AvrRps4*	Rifampicin / 50 µg/ml and kanamycin / 25 µg/ml	28º C
Pst DC3000 COR -	Rifampicin / 50 µg/ml	28º C
Pst DC3000 boiled extract	Rifampicin / 50 µg/ml	28º C

3.2. *Arabidopsis* bacterial growth curve

Pseudomonas syringae pv tomato DC3000 (*Pst* DC3000) was grown on solid NYGA medium supplemented with 25 µg/ml rifampicin for an overnight at 28º C. Bacteria was collected from the plate and resuspended in 10 mM $MgCl_2$ to OD_{600} = 0.1. Silwet L-77 was added to a final concentration of 0.04% (v/v) before spray inoculation of 4-week-old *Arabidopsis* plants. Leaves were harvested at 3 h and 72 h of pathogen inoculation for the determination of bacterial growth as described in Alcázar et al. (2010). At least three biological replicates were determined for each time point of analysis.

3.3. *Arabidopsis* bacterial growth curve by infiltration

The bacterial propagation method was described above (see 3.2). Bacteria collected from the plate and resuspended in 10 mM $MgCl_2$ to OD_{600} = 0.001. Leaves were harvested at 72 h of inoculation. At least three biological replicates were determined for the analysis. The details are shown in **Figure 13**.

4. Ion leakage

The leaves of 4-weeks-old *Arabidopsis* soil-grown plants were cut and rinsed with distilled H_2O. Leaf discs were incubated with different solutions (Put and Spm (100 µM), β-estradiol (10 µM), flg22 (100 nM) and mock (distilled H_2O)) and vacuum infiltrated for 5 minutes. Leaf

discs were then rinsed by distilled H_2O and distributed in 10 ml falcon tubes (6 leaf discs per replicate). Ion conductivity was measured over time (0, 1h, 2h, 4h, 8h, 16h, 24h and 72h).

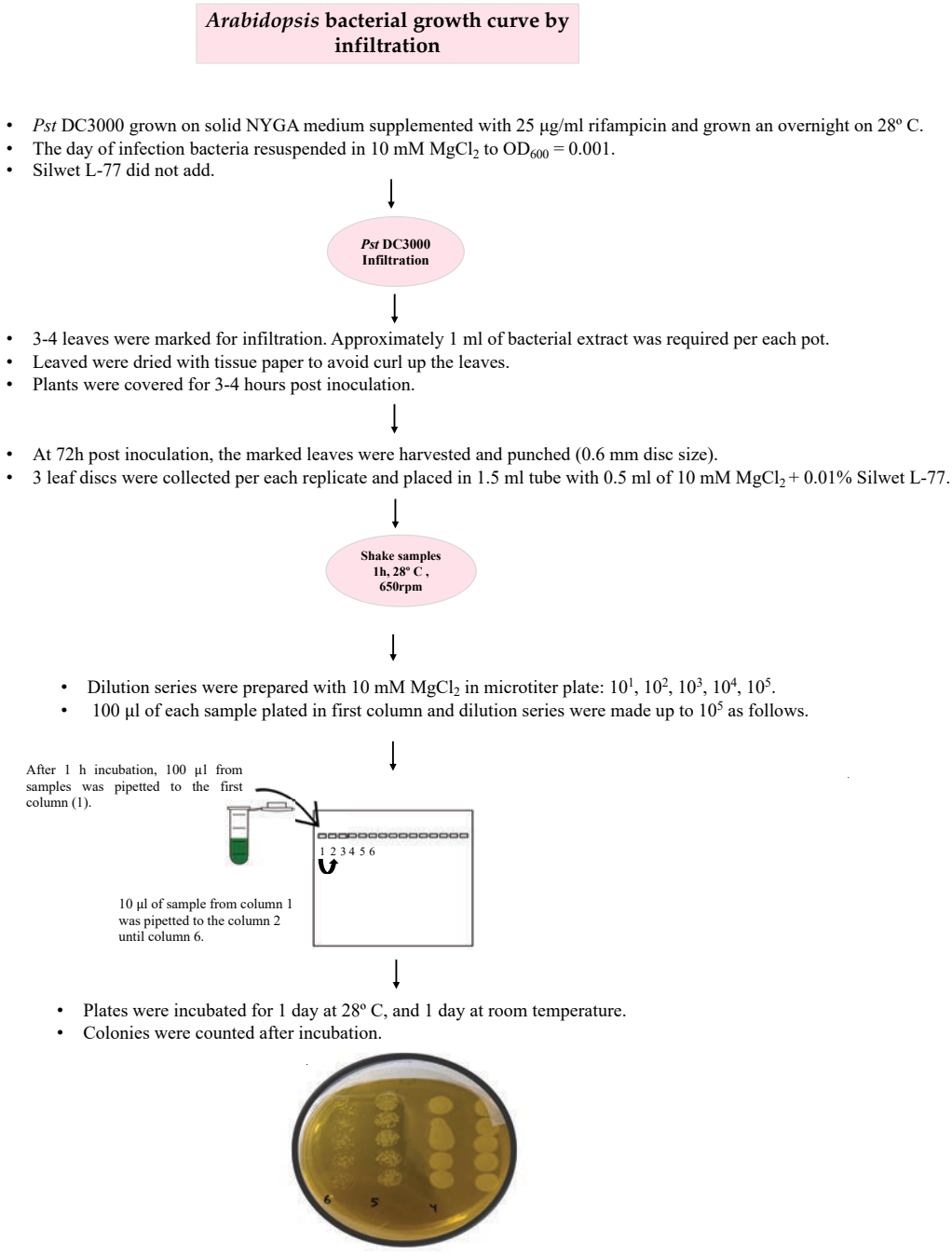


Figure 13. Bacterial growth curve of *Arabidopsis thaliana* by infiltration.

5. Trypan blue staining

Leaves of 3-weeks-old *Arabidopsis* soil-grown plants were cut and immersed in Trypan Blue 1:2 (v/v) with ethanol 96%. Stock solution of Trypan Blue consists of phenol (10 ml), glycerol (10 ml), lactic acid (10 ml), H_2O (10 ml) and trypan blue (0.02 g). Samples were boiled for 3-5 minutes, and then immersed in glycerol-ethanol (1/4) (v/v) solution for microscope visualization.

6. Salt stress

Sterilized seed were sown on 1/2 MS media and stratified in the dark at 4º C for 2–3 days. Plates were incubated in 12 hr light/ 12 hr dark cycles at 20-22 ºC at 100–125 μmol photons m−2 s−1 of light intensity. After germination, seedlings were transferred to new MS media supplemented with NaCl (0 mM, 100 mM and 150 mM). Samples were harvested for trypan blue staining when salt stress symptoms appeared.

Results

1- Polyamine metabolism in response to bacteria-triggered ETI, PTI, and ETS.

During plant- pathogen interactions, notable changes occur regarding to polyamine biosynthesis and catabolism to maintain the homeostasis balance. Several works demonstrated the modification of PA metabolism in response to biotic stresses, that usually result in the accumulation of most abundant PAs such as Putrescine, Spermidine, and Spermine (Jiménez-bremont et al., 2014; Walters, 2000; Walters, 2003). These data suggest the contribution of PA metabolism to the defense response (Broetto et al., 2005; Romero et al., 2018). Many reports have shown that overexpression of PA biosynthesis genes is an effective tool to improve stress tolerance (Liu et al., 2015). In addition, enhanced levels of PAs result in plant tolerance to biotic stresses, whereas plant susceptibility correlates with the suppression of PA metabolism gene expression and reduction in PA contents (Fernández-Crespo et al., 2015; Marini et al., 2001; Mo et al., 2015; Mo et al., 2015).

Another line of evidence demonstrates that increased endogenous levels of PAs in *Arabidopsis* alters the expression of genes involved in signaling and biosynthesis of various plant hormones and secondary metabolites, such as salicylic acid (SA), auxins, abscisic acid (ABA), gibberellins (GAs), ethylene and jasmonic acid (JA). The overall picture points to a role for PAs in the crosstalk between signaling pathways (Alcázar, Altabella, et al., 2010; Marco et al., 2011). Consistent with this, changes in PA levels are important for fine-tuning PA signaling, which affects hormonal balance that mediates plant defense response (Szalai et al., 2017).

Salicylic acid mediates the defense response against biotrophic pathogens to establish local defense and SAR (Glazebrook, 2005; Hernández et al., 2017). The investigation of the relationship between SA and PAs suggests a positive feedback loop between them in some species. Many studies revealed that the synthesis and/or catabolism of PAs is influenced by exogenous SA application (Hassannejad et al., 2012; Németh et al., 2002; Szepesi et al., 2011). However, there are few reports on the effect of PA treatment on SA content under optimum conditions (Radhakrishnan & Lee, 2013; Rahdari & Hoseini, 2013; Szalai et al., 2017; Liu et al. 2020).

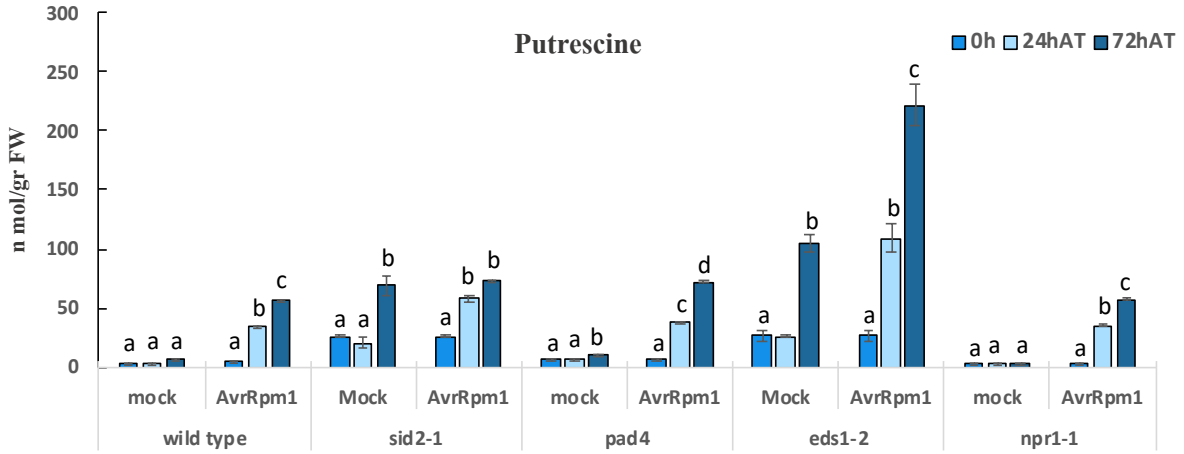
In order to study the involvement of polyamine metabolism in plant defense, and the contribution of SA pathway in such responses, the present study includes SA-related defense loss-of-function mutants and *Pseudomonas syringae* pv. *tomato (Pst)* DC3000 strains that trigger different immune responses (**Table 11**). In addition, temperature-dependent auto-immune hybrids between wild accessions have been tested.

Table 11. Bacteria-triggered defense response in *Arabidopsis thaliana*

Pseudomonas syringae pv tomato	Stress treatment	Defense response
Pst DC3000	In vitro, 15 days old seedlings treated with bacteria OD_{600}: 0.1/ 0.01	PTI+ETS
Pst DC3000 *AvrRpm1*	In vitro, 15 days old seedlings treated with bacteria OD_{600}: 0.1	ETI+PTI
Pst DC3000 *AvrRps4*	In vitro, 15 days old seedlings treated with bacteria OD_{600}: 0.1	ETI+PTI
Pst DC3000 COR -	In vitro, 15 days old seedlings treated with bacteria OD_{600}: 0.01	PTI+ETS
Pst DC3000 boiled extract	In vitro, 15 days old seedlings treated with bacteria OD_{600}: 0.01	PTI

1-1 Polyamine levels in response to *Pst* DC3000 carrying the AvrRpm1 effector

Previously, it has been demonstrated that the delivery of Avr effectors into the plant cell is unavoidably accompanied with PAMP perception, which results in co-activation of ETI and PTI (Hatsugai et al., 2017; Tsuda et al., 2009). ETI seems to halt pathogen progression through potentiation of PTI responses, likely involving ROS production, callose deposition and PTI-related gene expression (Ngou et al., 2020). To investigate how ETI+PTI co-activation affects PA metabolism, we inoculated wild-type, *eds1-2, sid2-1, npr1-1* and *pad4* mutants with *P. syringae* pv. *tomato (Pst)* DC3000 carrying the *AvrRpm1* effector *(Pst AvrRPM1*) and monitored the levels of free Put, Spd and Spm during three days (**Figure 14**).



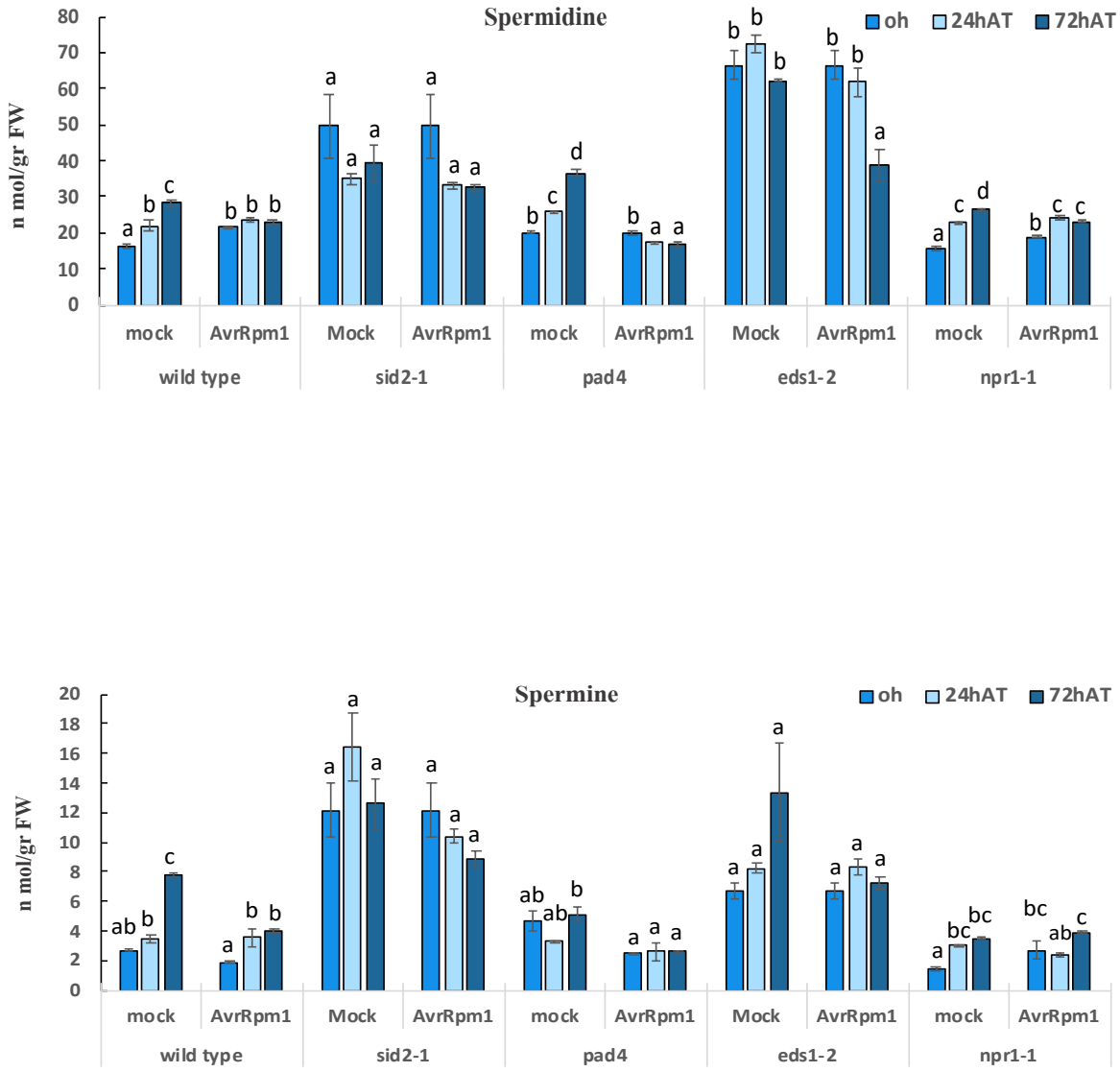


Figure 14. Polyamine levels in response to *Pst AvrRpm1*. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 15-day-old wild-type, *sid2-1*, *pad4*, *eds1-2* and *npr1-1 Arabidopsis* seedlings treated with *Pst AvrRpm1*. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst AvrRpm1* (OD_{600}= 0.1). Samples were harvested at 0 h, 24 h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

One-day post inoculation, the Put content in *sid2-1*, *eds1-2*, *pad4* and *npr1-1*mutants enhanced between 2- to 10-fold higher in plants inoculated with *Pst AvrRpm1* than in mock (10 mM $MgCl_2$) - inoculated plants (**Figure 14**). The result was also evidenced at three-days post inoculation (**Figure 14**). Conversely, Spd and Spm levels did not exhibit significant changes

in response to *Pst AvrRpm1*, except for *eds1-2*, in which Spd levels dropped almost by half after 72 h of treatment, with no concomitant increases in Spm (**Figure 14**).

We further analyzed the level of 1,3 Diaminopropane (DAP), which is the byproduct of terminal oxidation of higher PA (Spd and Spm) (Tavladoraki et al., 2016) (**Figure 15**). Significant increase of DAP level was evidenced in *eds1-2* mutant compared to mock (**Figure 15**). These results suggest that higher PA (Spd and Spm) oxidation occurs in *eds1-2* mutant inoculated with *Pst AvrRpm1*.

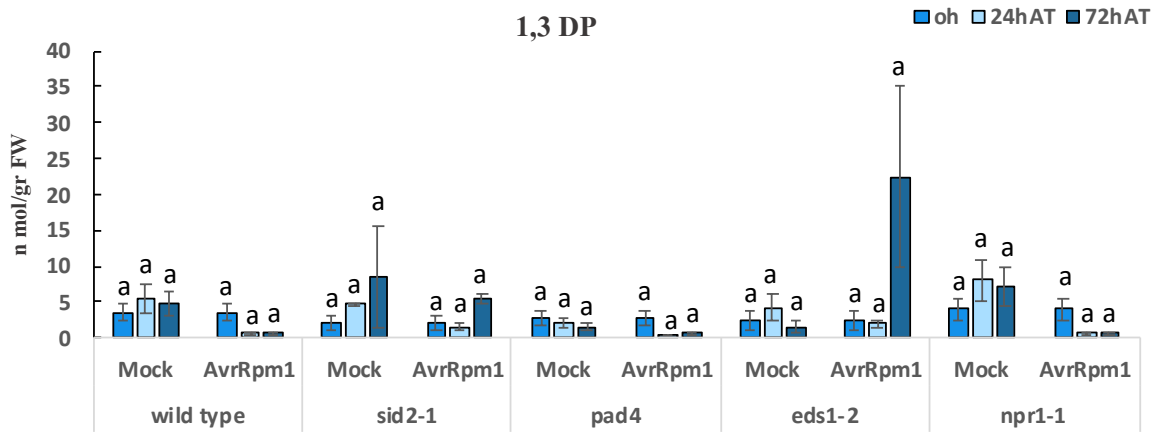
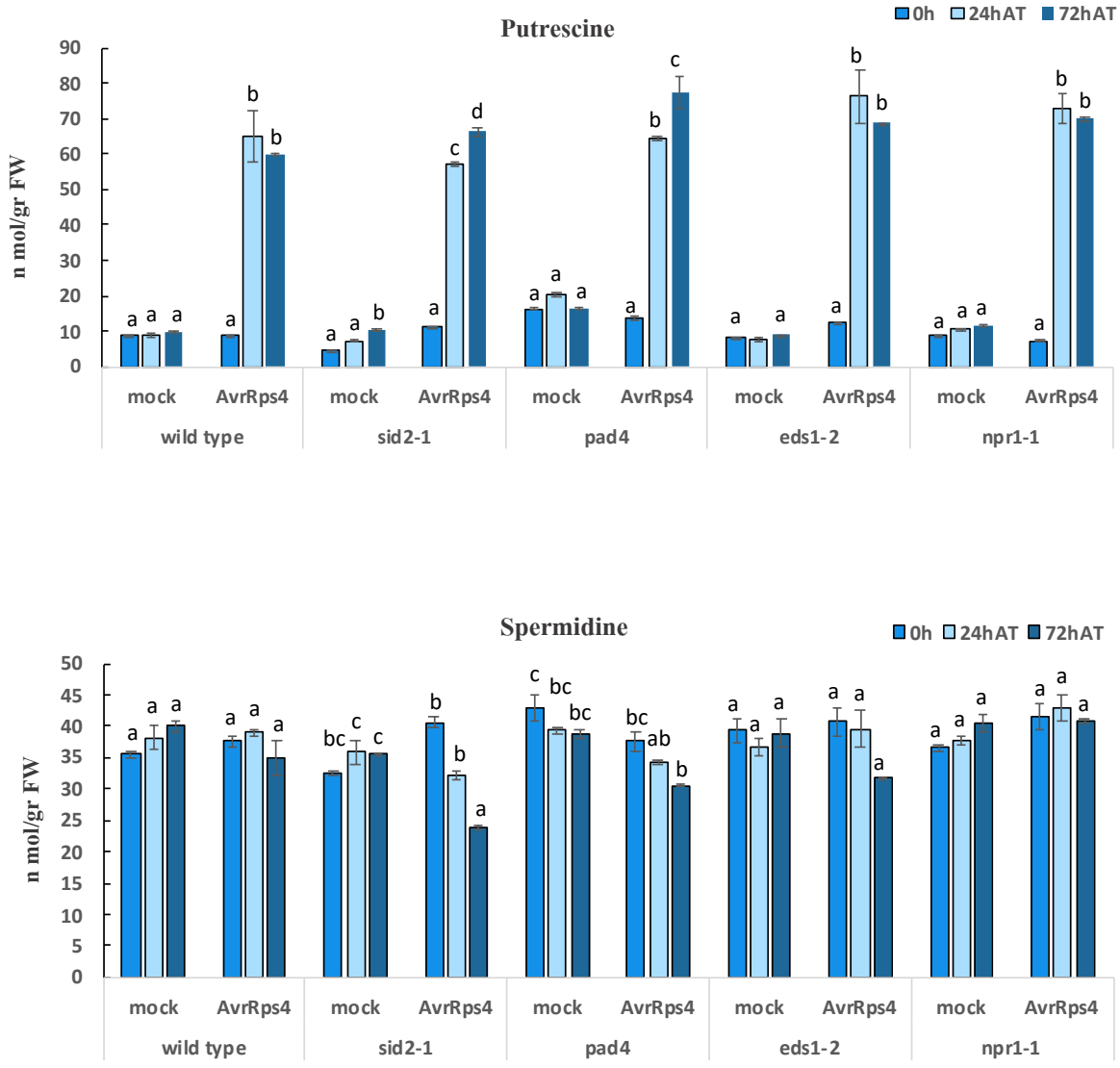


Figure 15. 1,3 Diaminopropane (DAP) levels in response to *Pst AvrRpm1*. 15-day-old wild-type, *sid2-1*, *pad4*, *eds1-2* and *npr1-1 Arabidopsis* seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst AvrRpm1* (OD_{600}= 0.1). Samples were harvested at 0 h, 24 h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

Overall, the data indicate that co-activation of ETI+PTI triggered by *Pst AvrRpm1* inoculation mainly results in Put accumulation, which is independent of salicylic acid (*SID2*), *PAD4*, *EDS1* and *NPR1*. The highest Put accumulation in response to *Pst AvrRpm1* was detected in the *eds1-2* mutant. This might be related to the higher bacteria growth supported in this immune-deficient mutant (Bhandari et al., 2018; Sun et al., 2020). However, this pattern was not observed in other mutants also exhibiting compromised defense responses (*sid2-1*, *pad4* and *npr1-1*). It remains to be determined the molecular mechanism underlying such differential metabolic response between immune compromised mutants.

1-2 Polyamine levels in response to *Pst* DC3000 carrying the *AvrRps4* effector

The *Pseudomonas syringae* pv. tomato carrying the *AvrRps4* virulence gene *(Pst AvrRps4)* (Hinsch & Staskawicz, 1996), also induces PTI+ETI response by delivering the AvrRps4 effector into the plant host cell (Halane et al., 2018). In order to determine whether alteration in polyamines metabolism induced by PTI+ETI was influenced by diverse Avr proteins, we determined Put, Spd and Spm contents in wild-type *sid2-1*, *eds1-2*, *pad4* and *npr1-1* mutants inoculated with *Pst AvrRps4*. Polyamines levels were quantified at 0 h, 24 h and 72 h after treatment (**Figure 16**).



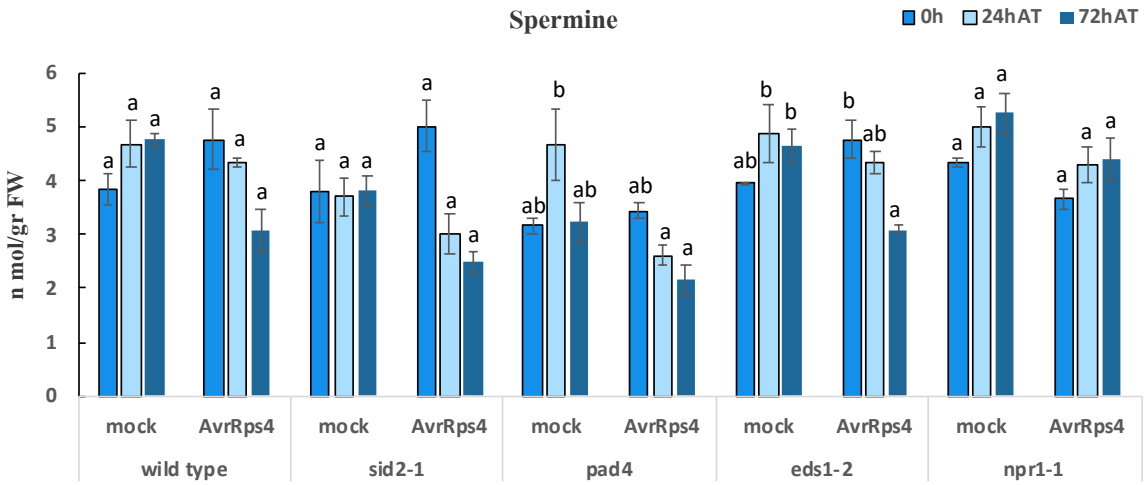


Figure 16. Polyamine levels in response to *Pst AvrRps4*. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 15-day-old wild-type, *sid2-1*, *pad4*, *eds1-2* and *npr1-1 Arabidopsis* seedlings inoculated with *Pst AvrRps4*. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst AvrRps4* (OD_{600}= 0.1). Samples were harvested at 0 h, 24 h, and 72 h after inoculation for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

In loss-of-function mutants *sid2-1*, *eds1-2*, *pad4* and *npr1-1,* the level of Put in response to *Pst AvrRps4,* increased significantly at 24 h and 72 h post-inoculation compared to mock (10 mM $MgCl_2$) treatment (**Figure 16**). The level of Put did not change remarkably after 3 h of treatment (data not shown), which indicated that Put accumulation triggered by *Pst AvrRps4* is not an early response. The level of Spd and Spm in seedlings treated with *Pst AvrRps4* did not show significant changes except for Spd levels in *sid2-1* and *pad4*, and Spm levels in *eds1-2*, which were reduced after 72 h of *Pst AvrRps4* inoculation (**Figure 16**). In addition, we analyzed the level of DAP in response to *Pst AvrRps4* (**Figure 17**), and we observed the accumulation of DAP in *sid2-1*, *eds1-2, pad4* and *npr1-1* mutants (**Figure 17**), which suggests the occurrence of terminal catabolism during the defense response. Similarly, to AvrRpm1-triggered ETI, *Pst AvrRps4* did not favor the synthesis or accumulation of Spd and/or Spm. These data indicated that Put accumulation in response to *Pst AvrRps4* is independent of SA (*SID2*), *PAD4*, *EDS1* and *NPR1*. Except for *EDS1*, Put content in response to *Pst AvrRps4* inoculation increased to a similar extent as *Pst AvrRpm1*, although the effectors are recognized by a different set of receptors and involve different signaling components.

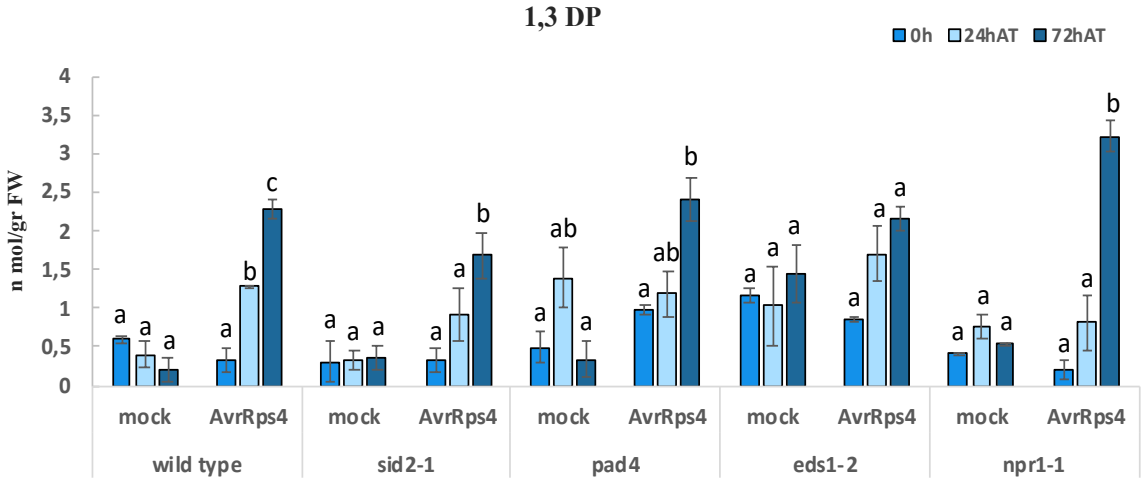


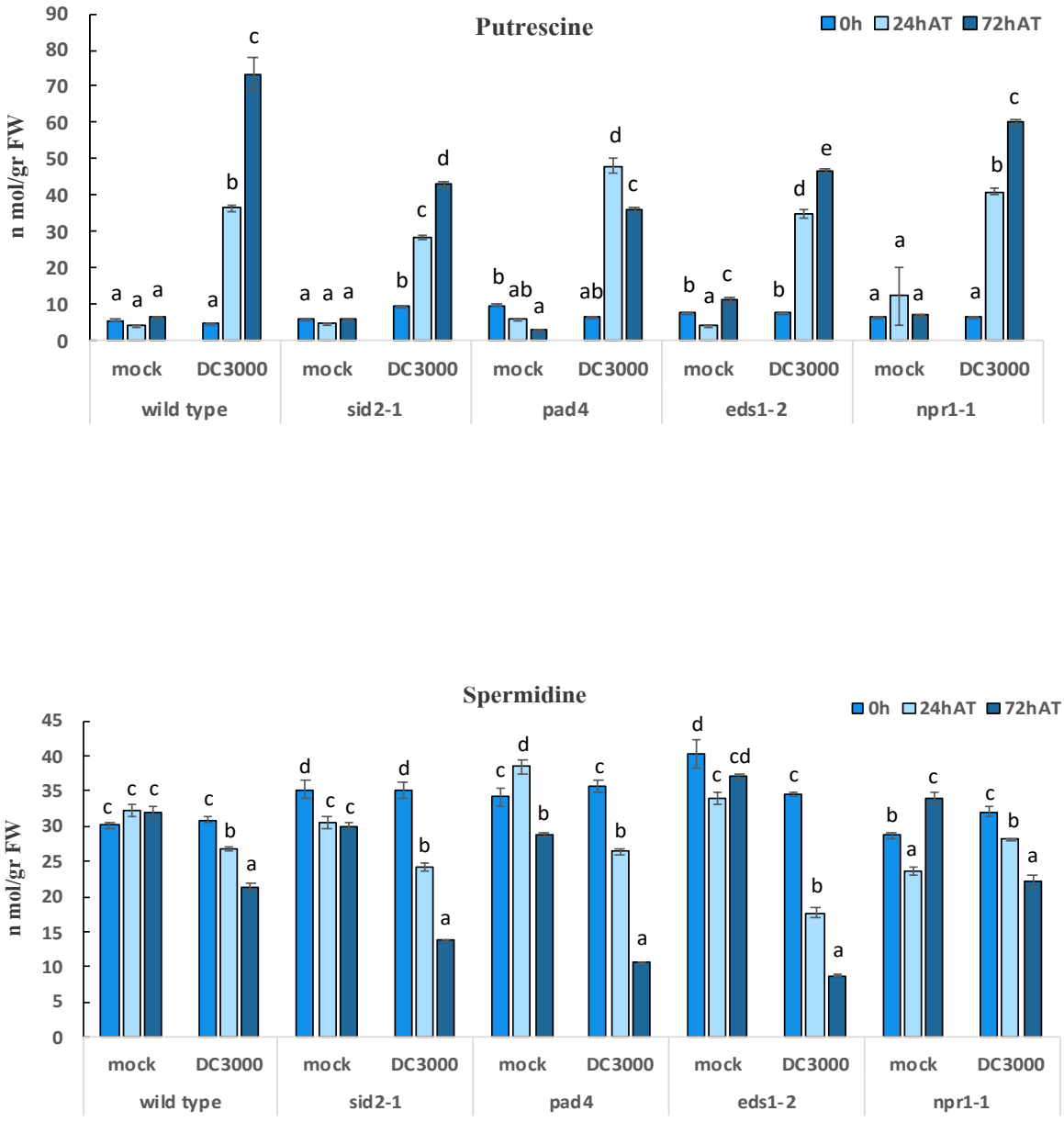
Figure 17. 1,3 Diaminopropane (DAP) levels in response to *Pst AvrRps4*. 15-day-old wild-type, *sid2-1*, *pad4*, *eds1-2* and *npr1-1 Arabidopsis* seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst AvrRs4* (OD_{600}= 0.1). Samples were harvested at 0 h, 24 h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

1-3 Polyamine levels in response to virulent *Pst* DC3000 bacteria

The *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) carries multiple potential virulence factors that initiate the defense response, particularly PTI (Boller & Felix, 2009). As discussed earlier, PTI is often suppressed by Type III effectors from *Pst* DC3000 and other *P. syringae* strains and results in Effector-Triggered Susceptibility (ETS) (Jones & Dangl, 2006). Although PTI signaling has been studied in depth, the relationship between effector recognition and polyamine metabolism during PTI and PTI+ETS is not completely established. In order to study the involvement of polyamines during PTI induced by *Pst* DC3000 and to determine whether type III effector proteins might suppress the changes in polyamine metabolism, we monitored polyamines level in wild type and *sid2-1*, *eds1-2*, *pad4* and *npr1-1* at 0 h, 24 h and 72 h after *Pst* DC3000 inoculation (**Figure 18**).

The level of Put in *sid2-1*, *eds1-2*, *pad4* and *npr1-1* mutant increased up to 6- to 8- fold higher at 24 h of *Pst* DC3000 treatment compared to mock (10 mM $MgCl_2$) inoculated plants (**Figure 18**). As infection progressed, Put content elevated in all loss-of-function mutants at 72 h after treatment, however, at this time point of analysis, the absolute Put levels (nmol/g FW) in SA-pathway compromised mutants were significantly lower than in wild-type (**Figure 18**). The

Spd levels were significantly reduced in all genotypes after 72 h of treatment. In addition, the Spm levels were found to be reduced in *pad4* and *eds1-2* mutants at the same time point of analysis (**Figure 18**).



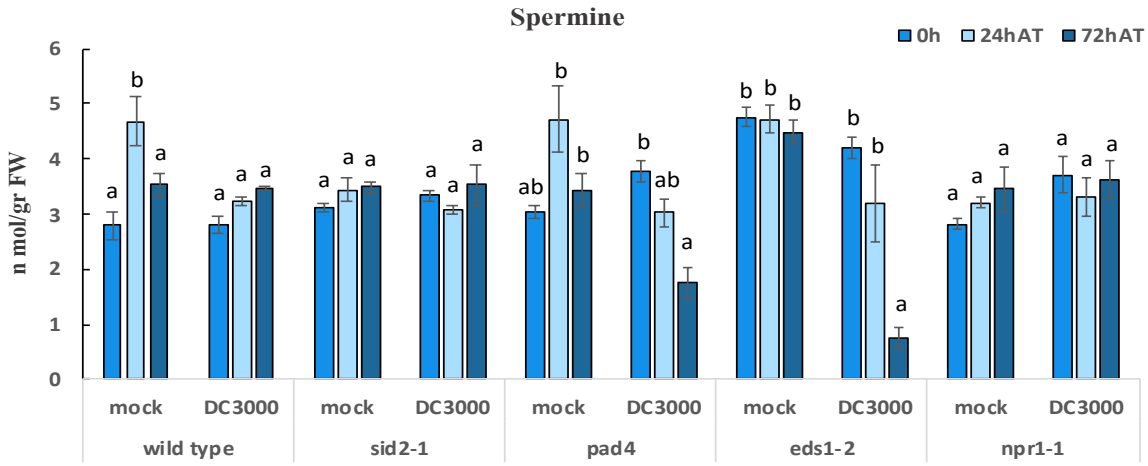


Figure 18. Polyamines level in response to *Pst* DC3000. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 15-day-old wild-type, *sid2-1*, *pad4*, *eds1-2* and *npr1-1 Arabidopsis* seedlings treated with *Pst* DC3000. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst* DC3000 (OD_{600}: 0.1). Samples were harvested at 0 h, 24 h, and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

Further, we analyzed DAP content in response to *Pst* DC3000 (**Figure 19**), and we observed the accumulation of DAP at 72 h after inoculation in *sid2-1* and *npr1-1* mutants (3.8- and 7.6-fold) compared to the wild type mock (**Figure 19**), although no significant increment of DAP was evidenced in *pad4* and *eds1-2*.

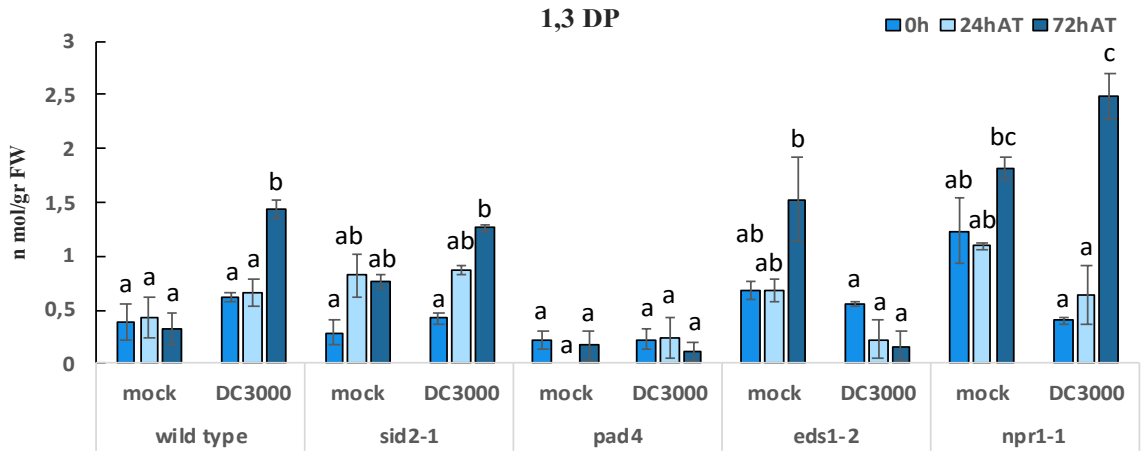
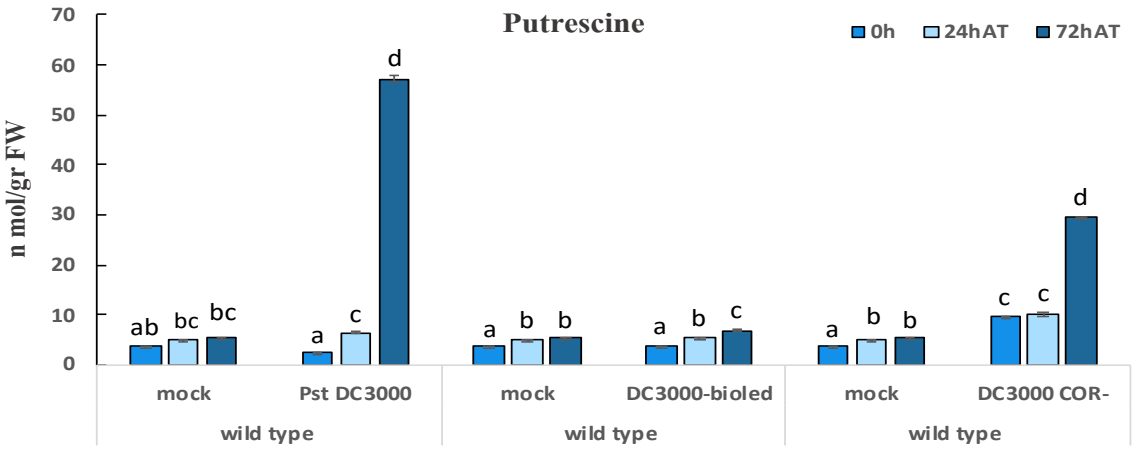


Figure 19. 1,3 Diaminopropane (DAP) levels in response to *Pst* DC3000. 15-day-old wild-type, *sid2-1*, *pad4*, *eds1-2* and *npr1-1 Arabidopsis* seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst* DC3000 (OD_{600}= 0.1). Samples were harvested at 0 h, 24 h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

These results indicated that significant reduction of Spd level in *sid2-1*, *npr1-1* and wild type (**Figure 18**) resulted from terminal oxidation of Spd (**Figure 19**). We concluded that *Pst* DC3000 inoculation triggers Put accumulation and Spd depletion independently of SA (*SID2), EDS1, PAD4* and *NPR1*. The lower Spm levels were only detected in *pad4* and *eds1-2* mutants. These results indicated that Put accumulates during PTI + ETS activation and type III effectors delivered by *Pst* DC3000 do not suppress the increases in Put levels. It is intriguing to know whether *Pst* DC3000 effectors underlie the reduction in Spd levels observed after 72 h of treatment. The increases in Put triggered by *Pst* DC3000 might be related to recognition of PAMPs, ETS or other virulence factors such as the phytotoxin coronatine (COR). To address this question, we further examined polyamine levels in response to COR deficient *Pst* DC3000 bacteria (see next section).

1-4 Polyamine levels in response to coronatine deficient *Pst* DC3000

The phytotoxin coronatine (COR) is synthesized by *P. syringae* pv. *tomato (Pst)* as an important virulence factor for bacterial pathogenicity. COR has remarkable structural homology to methyl jasmonate (MeJA), an endogenous plant regulator involved in the defense response (Bender et al., 1999) . *Pst* DC3000 produces COR to suppress stomatal defense by inhibition of ABA- and flg22-triggered NADPH oxidase-dependent ROS production in guard cells (Toum et al., 2016). COR mimics JA, interfering SA signaling through SA–JA antagonistic crosstalk (Kloek et al., 2001). The effect of *Pst* DC3000 COR$^-$ inoculation on polyamine levels was studied in wild type *Col-0* (**Figure 20**). In addition, boiled bacteria extract of *Pst* DC3000 were tested for their polyamine inducing activity (**Figure 20**).



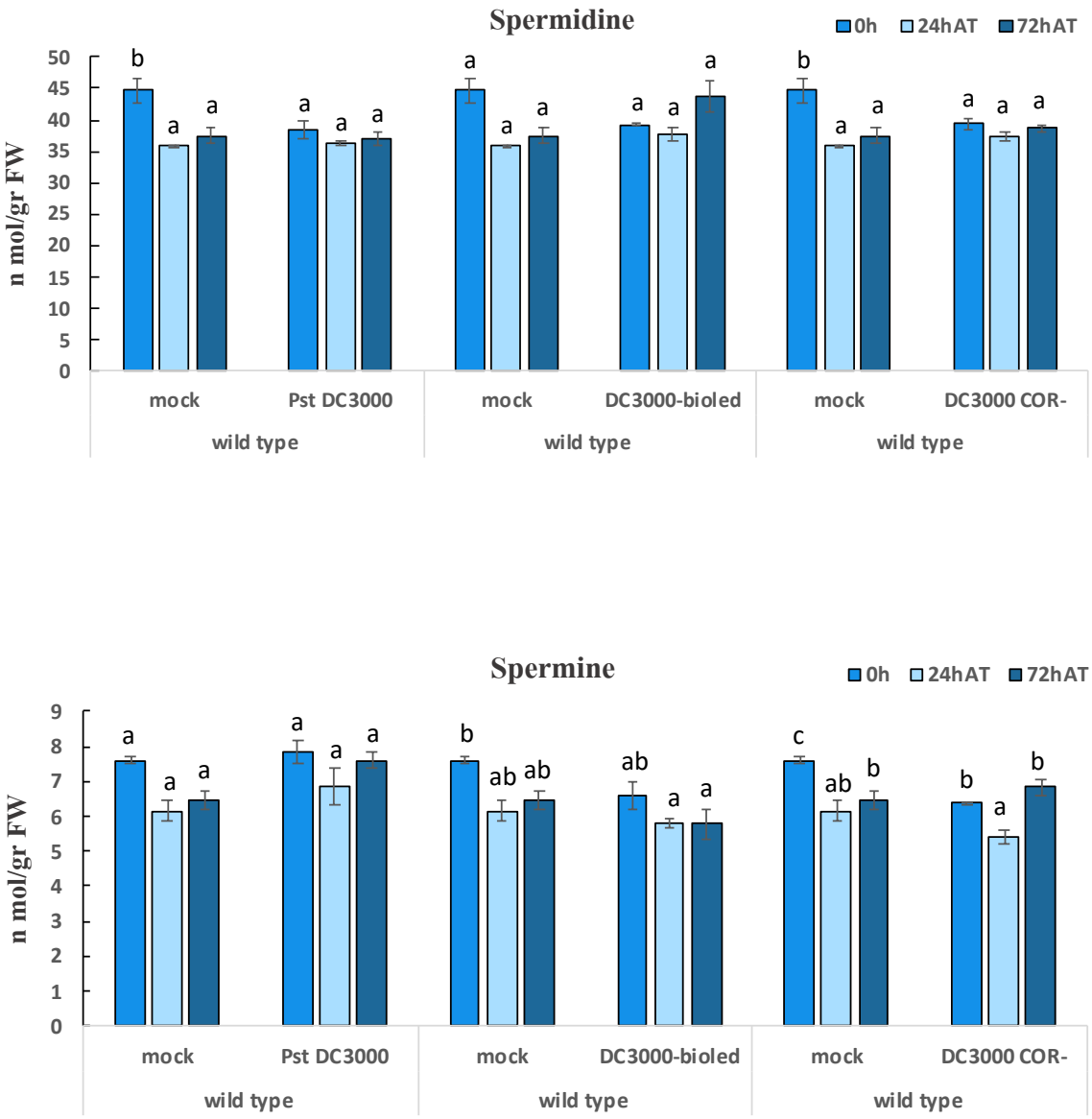


Figure 20. Polyamines level in response to *Pst* DC3000, DC3000 COR⁻ and DC3000 boiled extract. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 15-day-old wild-type *Arabidopsis* seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst* DC3000, DC3000 COR⁻ and DC3000 boiled extract (OD_{600}: 0.01). Samples were harvested at 0 h, 24 h, and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

The level of Put enhanced significantly (10.6- fold) in wild type plants at 72 h post inoculation with *Pst* DC3000 compared to mock (10 mM $MgCl_2$) treatment (**Figure 20**). In wild type plants Put accumulated significantly less (5.4-fold) in response to *Pst* DC3000 COR⁻ inoculation, however, Put content in wild type plant treated with boiled extract did not exhibited notable

changes (**Figure 20**). In this experiment, Spd and Spm levels did not change significantly in contrast to a previous analysis (**Figures 16 and 18**). No significant change in Spd level in wild type inoculated plants with *Pst* DC3000 compared to previous analysis (**Figure 18**) might result from the bacterial inoculation used (OD_{600}: 0.01) in this experiment, which differs from the previous one (OD_{600}: 0.1). In addition, we analyzed the DAP level of in wild type *Col-0* in response to *Pst* DC3000, *Pst* DC3000 COR$^-$ and *Pst* DC3000 boiled extract (**Figure 21**).

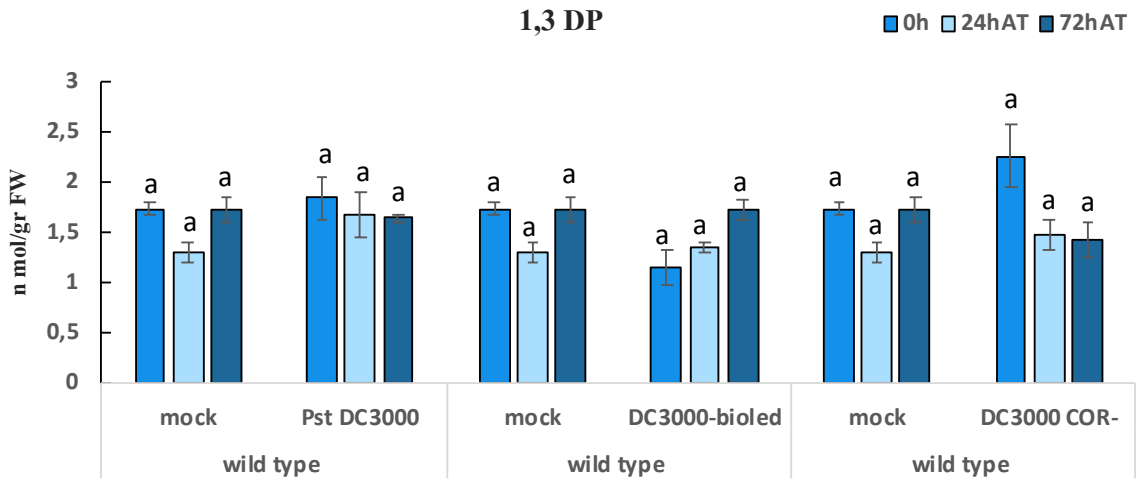


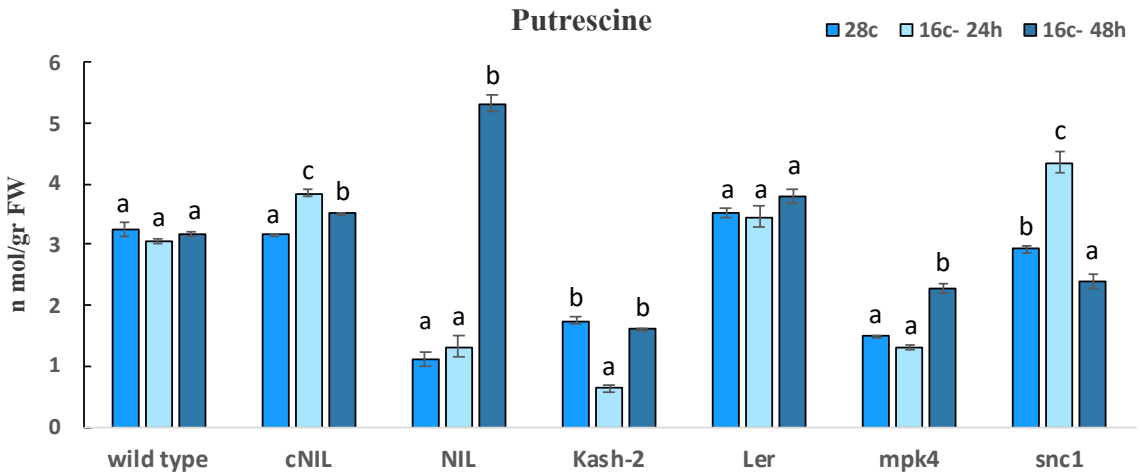
Figure 21. 1,3 Diaminopropane (DAP) levels in response to *Pst* DC3000, DC3000 COR$^-$ and DC3000 boiled extract. 15-day-old wild-type, *sid2-1*, *pad4*, *eds1-2* and *npr1-1 Arabidopsis* seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst* DC3000, DC3000 COR$^-$ and DC3000 boiled extract (OD_{600}= 0.1). Samples were harvested at 0 h, 24 h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

No significant accumulation of DAP was observed in the wild type *Col-0* (**Figure 21**). This data is consistent with our observation of Spd and Spm level in this experiment (**Figure 20**). These data indicated that in the absence of coronatine, Put accumulation is reduced, which suggests the contribution of coronatine to Put accumulation. Alternatively, this limited response can be associated with lower bacterial colonization due to stomatal defense activation in the absence of coronatine. Indeed, previous studies in *Arabidopsis* revealed that the DC3000 COR$^-$ mutant is severely reduced in growth rate and virulence, (Brooks et al., 2004; Kloek et al., 2001; Mittal & Davis, 1995). This view is supported by the fact that boiled extracts of *Pst* DC3000 did not trigger Put accumulation.

1-5 Polyamine metabolism in temperature-dependent autoimmune mutants (*snc1, mpk4*) and hybrids (Ler/Kas-2)

Permanent activation of the immune system is costly for plant, and to avoid this, plant immune receptors are retained in an 'off' state, until pathogen perception. By utilizing genetic screens, several autoimmune mutants have been identified that unravel how NLRs receptors switch from inactive to active states. Gain and loss-of-function mutants in plant immune receptors such as *snc1*, which leads to an autoimmune phenotype, have been reported in the last years (Wersch et al., 2016). In this work, we used autoimmune mutants (*snc1, mpk4*) and an incompatible hybrid line (Ler/Kas-2 near isogenic line, NIL) (Alcázar & Parker, 2011) to study the changes in polyamine metabolism in plants exhibiting constitutive activation of defense (ETI) in the absence of pathogens. Autoimmune hybrids are sensitive to temperature (Alcázar & Parker, 2011), and the severe stunning phenotype of Ler/Kas-2 NIL plants shown at low temperature (16 °C) is suppressed at higher temperature (20 °C) (Alcázar et al., 2009).

In this regard, we analyzed polyamines level in the parental Ler and Kas-2 accessions, the autoimmune hybrid Ler/Kas-2 NIL, and a complemented NIL line (cNIL) which suppresses autoimmunity. The data was complemented with the use of autoimmune mutants *snc1* and *mpk4*. Wild type plants (*Col-0*) Ler, Kas-2, NIL, cNIL, *snc1* and *mpk4* were grown at 28 °C for 3 weeks and then transferred to 16 °C to activate autoimmunity. Polyamine levels were determined at 24 h and 48 h of low temperature treatment (**Figure 22**).



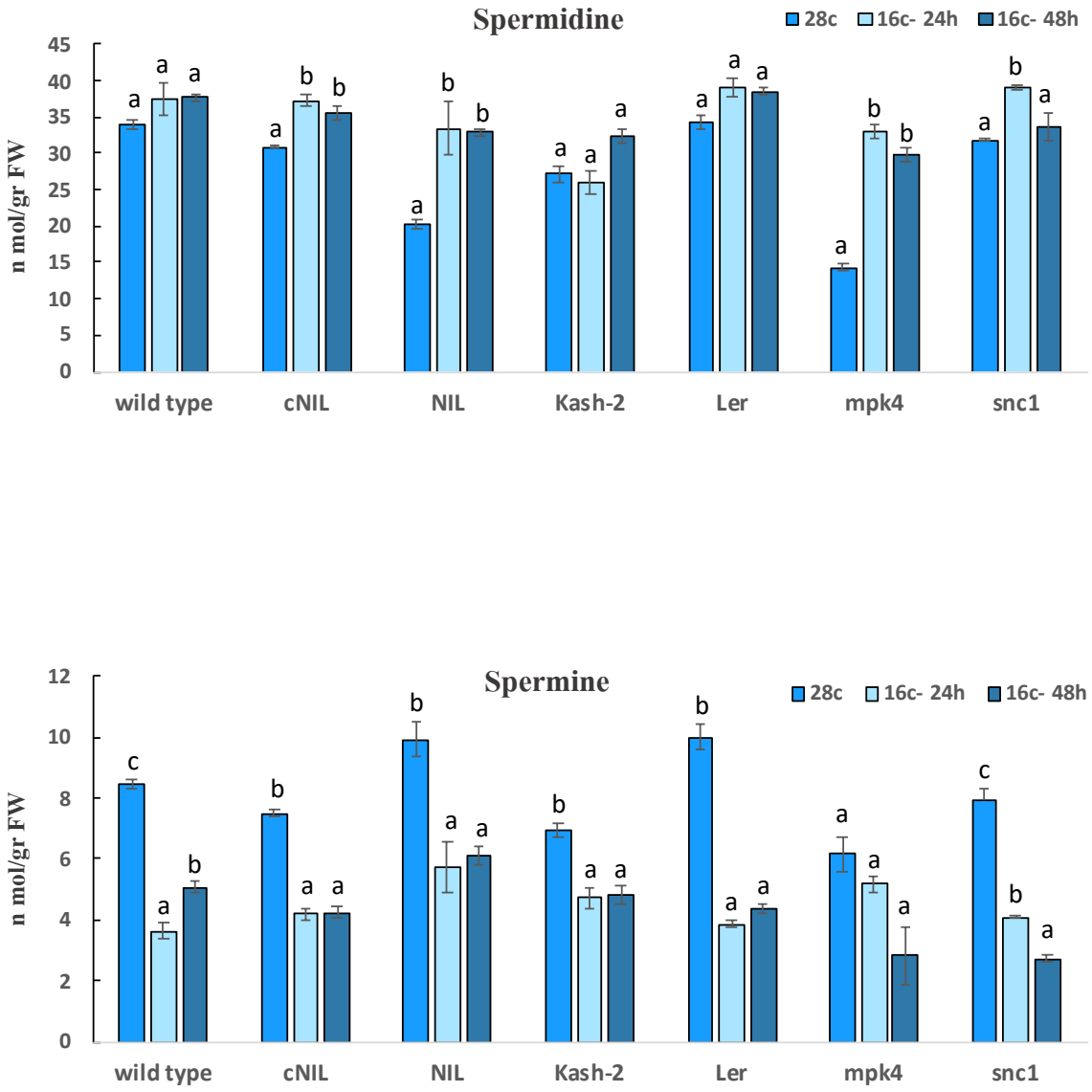


Figure 22. Polyamines level in response to auto-immune activation. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 3-weeks old *Arabidopsis* autoimmune mutants (*snc1* and *mpk4*), incompatible lines (NIL and cNIL) and natural accessions (Ler and Kas-2) were grown in 28 °C and treated with low temperature (16 °C). Samples were harvested at 0 h, 24 h, and 48 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

In wild type plants, low temperature treatment (16 °C) did not affect Put and Spd levels. However, Spm content was reduced to half at 24 h after temperature shift (**Figure 22**). Conversely, in NIL plants Put accumulated significantly at 48 h of the temperature shift, even so, a slight increment of Spd was observed at 24 h (**Figure 22**). This increase was not evidenced in Spm level - which was reduced similarly to the wild-type. It has been reported that autoimmunity in NIL incompatible lines is triggered by TIR-NB-LRR proteins and is strongly

conditioned by EDS1 and SA-pathway activation (Alcázar et al., 2009). This result demonstrated that ETI activation in the absence of pathogens also triggered Put accumulation. In response to temperature shift, cNIL incompatible plants exhibited slight accumulation of Put and Spd at 24 h of treatment, although, Spm level was found to be reduced notably (**Figure 22**). We further examined the two parental accessions, Ler and Kas-2, to determine whether low temperature influences polyamine levels. Put or Spd levels were not increased in Ler and Kas-2, whereas Spm levels were reduced (**Figure 22**).

To further investigate the polyamine metabolism in autoimmune mutants, we analyzed polyamine levels in *snc1* and *mpk4* plants after temperature shift from 28 °C to 16 °C. In response to low temperature, Put content did not increase in *mpk4* mutants, however enhanced level of Spd was observed at 24 h of temperature shift (**Figure 22**). In *snc1* mutants slight increase of Put was observed at 24 h after treatment. This mutant did not exhibit significant change in Spd level, although reduction of Spm was noticed at 24 h of temperature shift (**Figure 22**).

These data indicated that Put accumulates to different degrees in autoimmune mutants upon temperature shift that activates defense responses, independently of the presence of the pathogen. The most responding line was the Ler/Kas-2 NIL, which involves the Kas-2 background. This response was not observed in wild-type genotypes (*Col-0*, Ler and Kas-2). Conversely, the reduction in Spm levels is observed in all genotypes tested, which indicates that this is a response to low temperature treatment. Indeed, lower Spm levels have also been documented in wild-type plants exposed to cold (4 ºC) (Cuevas et al. 2008).

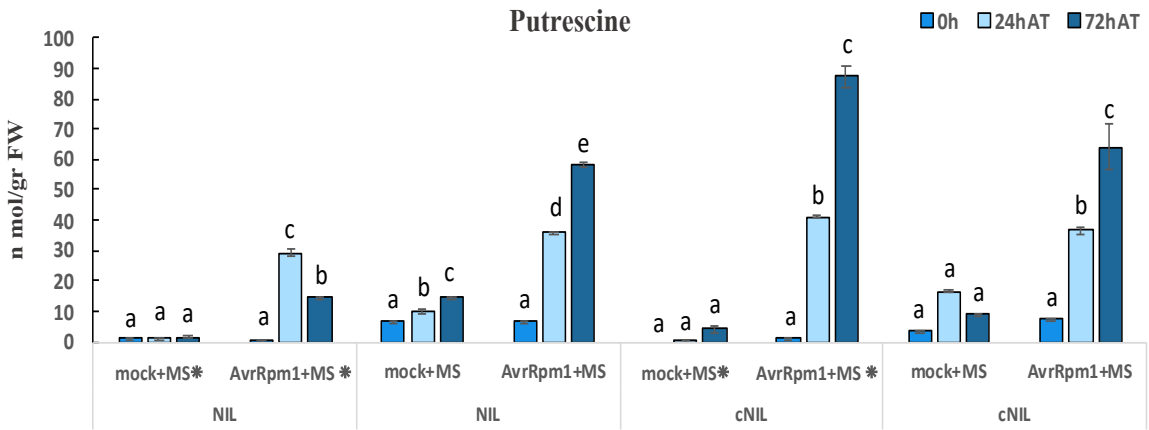
1-6 Polyamine metabolism in L*er*/Kas-2 autoimmune hybrids inoculated with *Pst* DC3000 *AvrRpm1*

In order to reveal more about polyamine metabolism during ETI and to investigate whether autoimmune activation affects polyamine level in response to pathogens, we inoculated NIL and cNIL plants with *Pst AvrRpm1* and quantified polyamines level at 0 h, 24 h and 72 h after treatment. NIL and cNIL seedlings were grown on 1/2 Murashige and Skoog (MS) media at 16 °C for two weeks. To avoid the suppression of the autoimmune NIL phenotype due to in vitro growth (Alcázar & Parker, 2011), we used a modified MS media (MS*) which contains lower concentration of ammonium (NH_4NO_3) (**Figure 23**). This media reconstitutes

autoimmunity in Ler/Kas-2 NIL plants grown in vitro (Atanasov, 2019). Polyamine contents were determined in response to *Pst AvrRpm1* in MS and low ammonium MS media (**Figure 24**).

Figure 23. *Arabidopsis* NIL phenotype with severe growth defect. 15-day-old NIL and cNIL Arabidopsis seedlings were grown *in vitro* on a nylon mesh in 1/2 Murashige and Skoog low ammonium MS media. 1. NIL seedlings, 2. cNIL seedlings.

In response to *Pst AvrRpm1,* NIL seedlings grown on MS*, exhibited significant accumulation of Put (21.6- fold) at 24 h after treatment, however, stronger response was observed in NIL grown on MS with higher accumulated Put (36- fold) at 72h after inoculation compared to mock (10 mM $MgCl_2$) plants (**Figure 24**). This might be result from the interplay between polyamines and nitrogen (N) assimilation in plant stress conditions, reviewed by Paschalidis et al. (2019).



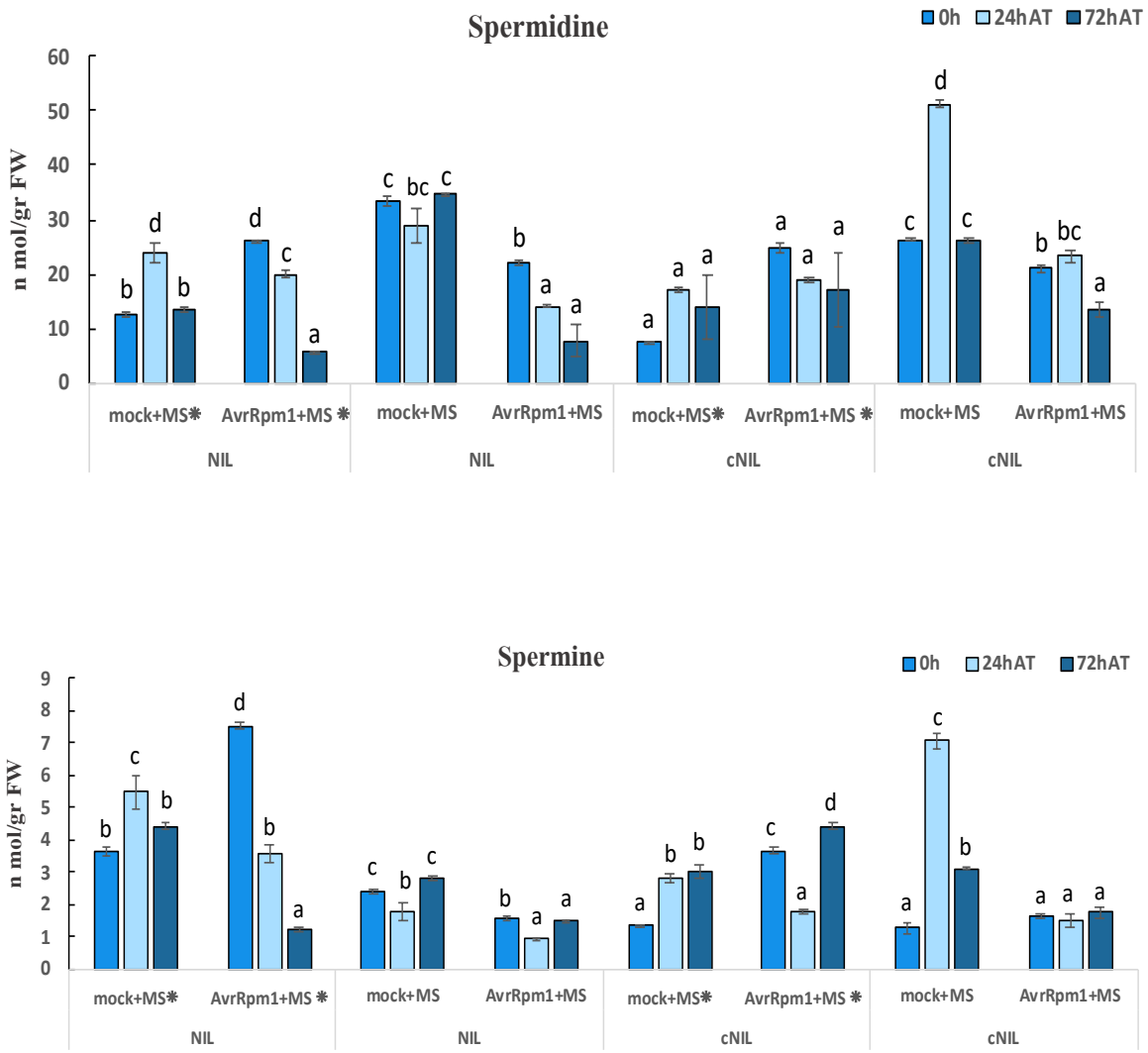


Figure 24. Polyamines level in response to *Pst AvrRPM1*. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 15-day-old seedlings of *Arabidopsis* incompatible lines (NIL and cNIL) were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media (MS) and reconstitute media (MS*) and inoculated by spraying with *Pst AvrRPM1* (OD_{600}: 0.1). Samples were harvested at 0 h, 24 h, and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05

Nitrogen as an essential nutrients elements, plays a crucial role in plant development and stress responses (Majumdar et al., 2016; P. N. Moschou et al., 2012; Serapiglia et al., 2008; Skopelitis et al., 2006). As it mentioned above, MS* media has lower level of ammonium compared to MS, that might be results in less available nitrogen for polyamine biosynthesis.

This data suggests a competition between autoimmunity and polyamines for nitrogen resources. In NIL plants grown in both media, slight decrease of Spd was observed in response to *Pst AvrRpm1,* although, Spm level dropped significantly at 24h after inoculation (**Figure 24**). The reduction of Spd and Spm level in NIL plants grown in MS* media might the result of polyamine oxidation to keep polyamine homeostasis in the low level of ammonium.

These results indicated that constitutive activation of defense (ETI) in NIL hybrid line amplifies plant response to *Pst AvrRpm1* in favor of Put accumulation. Although NIL phenotype is suppressed in vitro (MS media), a robust defense response was observed in response to pathogen attack (**Figure 24**). Altogether, these data determined that ETI activation triggered by autoimmunity amplifies plant response to pathogen effectors, and this response is independent of incompatibles phenotypes.

In cNIL plants grown in both media, significant accumulation of Put (30- to 54- fold) was evidenced in response to *Pst AvrRpm1*(**Figure 24**). Noteworthy to say that the highest level of Put was quantified at 72 h of treatment in cNIL plants grown in MS* media. No notable changes were noticed in Spd and Spm levels in of cNIL plants, except for a slight decrease in Spd content in cNIL plant grown in MS (**Figure 24**).

These data revealed that auto- immune activation amplifies plant response to pathogen attack, and Put accumulation is a crucial part of this defense response. Moreover, autoimmunity increases the consumption of nitrogen which can be a matter of competition with polyamine biosynthesis in lower ammonium context. Lack of autoimmune phenotype in cNIL plants might be the reason of higher accumulated Put in response to pathogen attack.

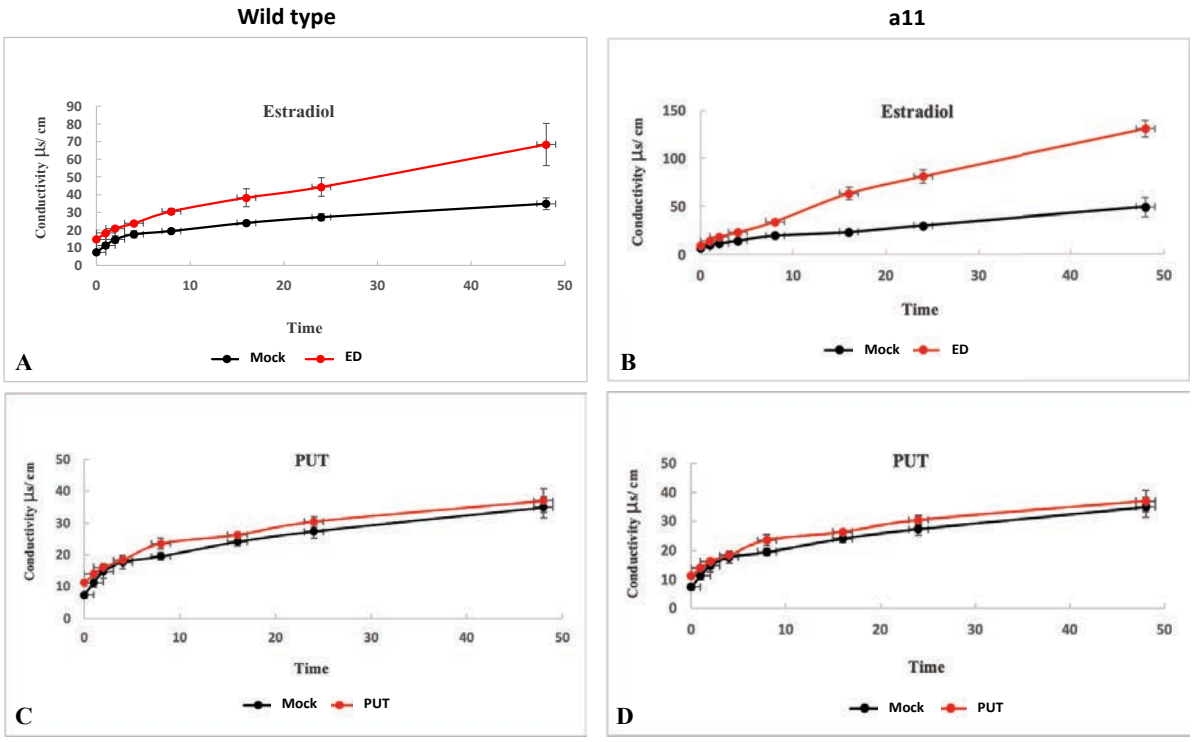
1-7 Polyamines involvement in Programmed Cell Death (PCD)

Defense responses triggered by pathogen recognition are often associated with HR, a form of programmed cell death that contributes to pathogen restriction (Coll et al., 2011; Mur et al., 2008). Accumulation of hydrogen peroxide (H_2O_2) and superoxide (O_2^-) is an early event to initiate HR (Delledonne et al., 2001), although the burst production of ROS and NO is necessary to stimulate HR. Plant polyamine accumulation is involved in PCD. PCD might be induced by direct action of polyamines through their effects on K^+ and Ca^{2+} influxes (Lecourieux et al., 2006; Zepeda-Jazo et al., 2011) or indirectly by yielding hydrogen peroxide (H_2O_2) (Cohen, 1998), as a part of PCD inducers (Clément et al., 1998).

1.7.1 Polyamines in ETI, PTI or both?

We performed an additional analysis to have an overall view about the contribution of polyamines in plant immunity. In this regard, we induced ETI, PTI and ETI+PTI separately, and studied the involvement of polyamines in the different layers of pathogen recognition. To stimulate ETI response we used homozygous *a11* transgenic line, that produces a strong *avrRpm1- RPM1* dependent response in the presence of β-estradiol (ED) (Tornero et al., 2002). From this perspective, *Arabidopsis* wild type *Col-0* and *a11* transgenic line were vacuum infiltrated with β-estradiol (10 µM), flg22 (100 nM), Put (100 µM) and mock (distilled H_20) and electrolyte leakage was monitored over time at 0 h, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h and 48 h after treatment (**Figure 25**).

In ETI response induced by β-estradiol, *a11* plants exhibited strong ion leakage compared to the control (**Figure 25-A, B**). A clear induction of membrane damage was notably found after 8 h (**Figure 25-B**). Conversely, ion leakage in *a11* plants treated with Put did not significantly change (**Figure 25-D**). This result was also evidenced in the wild type (**Figure 25-C**). Although exogenous application of Put stimulated a defense response similar to PTI (Liu et al., 2019), the data also indicated that cell death is not a part of Put- triggered PTI response, in agreement with Liu et al. (2019).



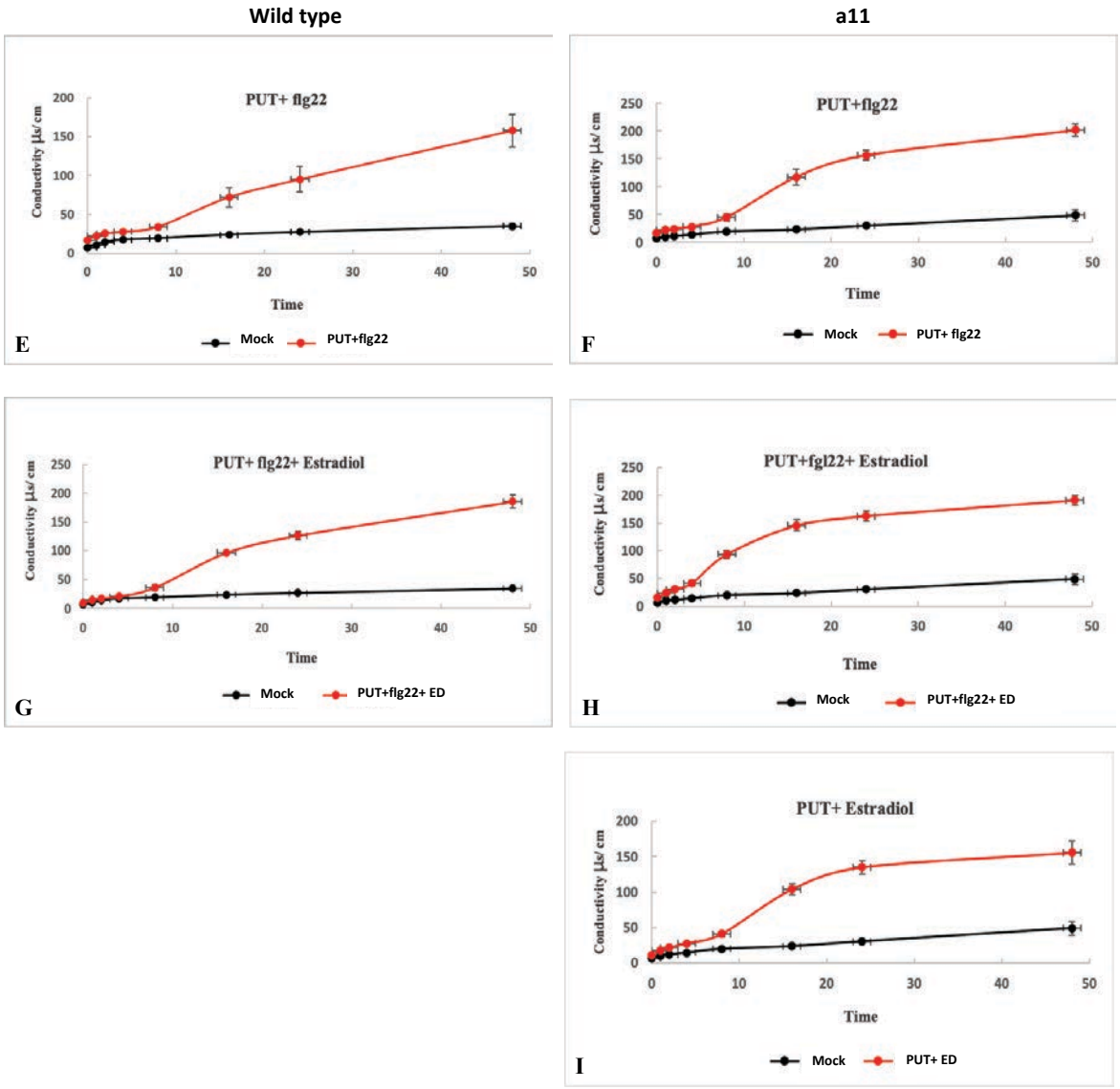


Figure 25. Involvement of Polyamines in cell death during plant defense responses. Four-weeks old *Arabidopsis* wild type and *a11* transgenic line were vacuum infiltrated with β-estradiol (10 µM), flg22 (10 nM), Put (100 µM) and mock (distilled H_20). Cell death was measured at 0 h,1 h, 2 h, 4 h, 8 h, 16 h, 24 h and 48 h post- infiltration by electrolyte leakage. Results are the mean of three biological replicates ± SD (standard deviation).

It is well known that recognition of the peptide flagellin 22 (flg22) results in PTI activation (Felix et al., 1999). To study the role of Put during PTI, we treated wild type *Col-0* and *a11* plants with flg22+Put and analyzed ion leakage during HR (**Figure 25-E, F**). We observed that ion leakage was increased after 8 h in *a11* plants in response to flg22+ Put (**Figure 25-F**), similar to the control (**Figure 25-E**). This increase might be induced by the high production of ROS due to flg22-triggered RBOHD activity (Torres et al., 2002, 2005). Regarding to our previous study (**Figures 14 and 16**), which indicated that co-function of ETI+PTI boosts plant

immunity in a favor of Put accumulation, we performed the analysis to investigate HR response triggered by ETI+PTI. We did so by vacuum infiltration of *a11* plants with Put, flg22 and β-estradiol and determination of ion leakage over the same period (**Figure 25-H**). Ion leakage was increased significantly after co-infiltration of Put+flg22+ β-estradiol and exhibited a kinetics response seemingly stronger than Put+ flg22 infiltration. Moreover, a clear induction of membrane damage was observed after 4 h, that is an earlier response compared to the control (**Figure 25-G**). Further, we quantified cell death induction in response to ETI and exogenous application of Put (**Figure 25-I**). Compared to mock or estradiol induction (**Figure 25-B**) the induction of cell death was quicker and increased markedly after 8 h in response to ETI+Put. This result suggested that Put modifies cell death kinetics to an earlier, but not stronger, ETI response. Overall, Put treatment led to an earlier PTI and ETI response. We further examined cell death in response to Put and Spm in SA-defense signaling mutants. In this regard, we vacuum infiltrated *sid2-1, pad4, eds1-2* and *npr1-1* mutants with 100 µM of Put, Spm and mock (distilled H_2O). Electrolyte leakage was monitored over time at 0 h, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h and 48 h post- treatment (**Figure 26**).

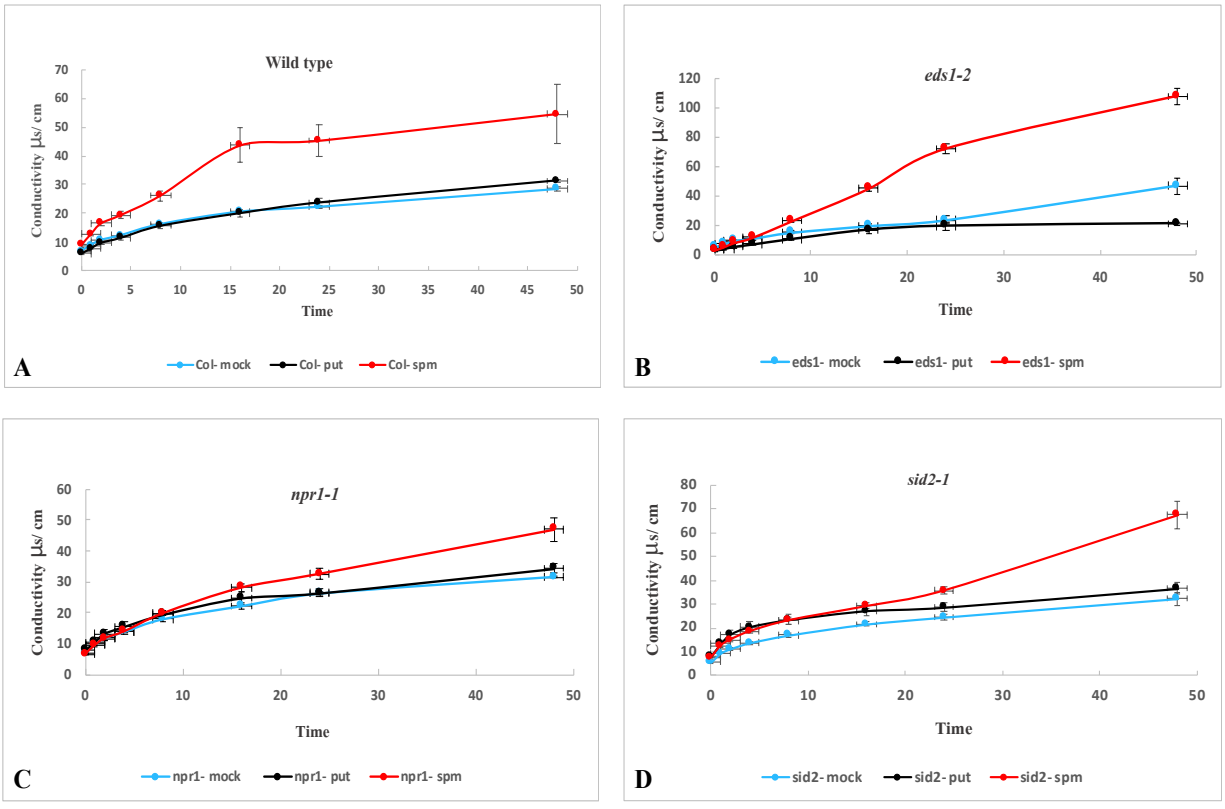


Figure 26. Involvement of Polyamines in Cell Death. Four-weeks old *Arabidopsis* loss-of-function mutants, *sid2-1*, *pad4*, *eds1-2* and *npr-1-1* were infiltrated with 100 µM Put, Spm and mock (distilled H_20). Cell death was measured at 0 h,1 h, 2 h, 4 h, 8 h, 16 h, 24 h and 48 h after infiltration by electrolyte leakage assay. Results are means of three biological replicates ± SD (standard deviation). wild type Col-0 (21-A), eds1-2 (21-B), npr1-1 (21-C), sid2-1 (21-D).

Although, Put treatment in wild type plants did not induce cell death (**Figures 25-C, 26-A**), exogenous application of Spm stimulated cell death indicated by increment of ion leakage (**Figure 26, A**). In the wild-type, cell death induced by Spm was observed as early as 1 h post-infiltration. However, more evident cell death was noted after 8 h. In *eds1-2* and *sid2-1* mutants the cell death was induced by Spm infiltration (**Figure 26-B, D**), even so, this response was not evidenced in Put treatment. These results indicated that exogenous applied Spm provokes cell death independently of *EDS1* and SA in *Arabidopsis*. Overall, these data evidenced the role of Spm inducing HR (Sagor et al., 2009), which might be related to Spm catabolism and PAOs activity (Yoda et al., 2003).

1.8 Involvement of Spermine in the defense response

Activation of HR by exogenous Spm led us to analyze its potential role in the response to biotic stress. In this regard, we first monitored cell death induced by Spm and its structural isomer tSPM at different concentrations (100 µM and 500 µM) by trypan blue staining at 24 h after treatment (**Figure 27**).

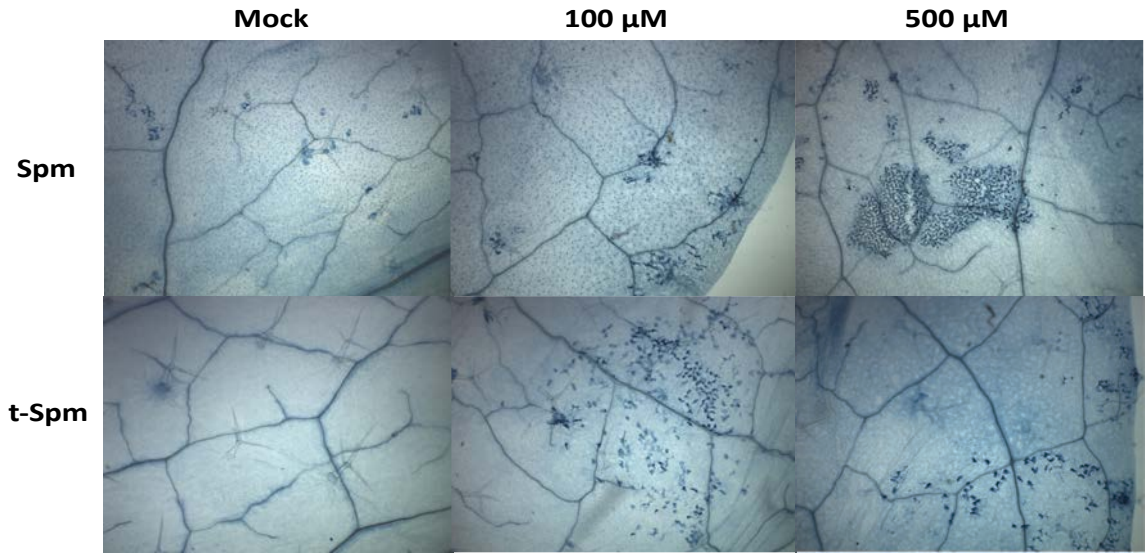


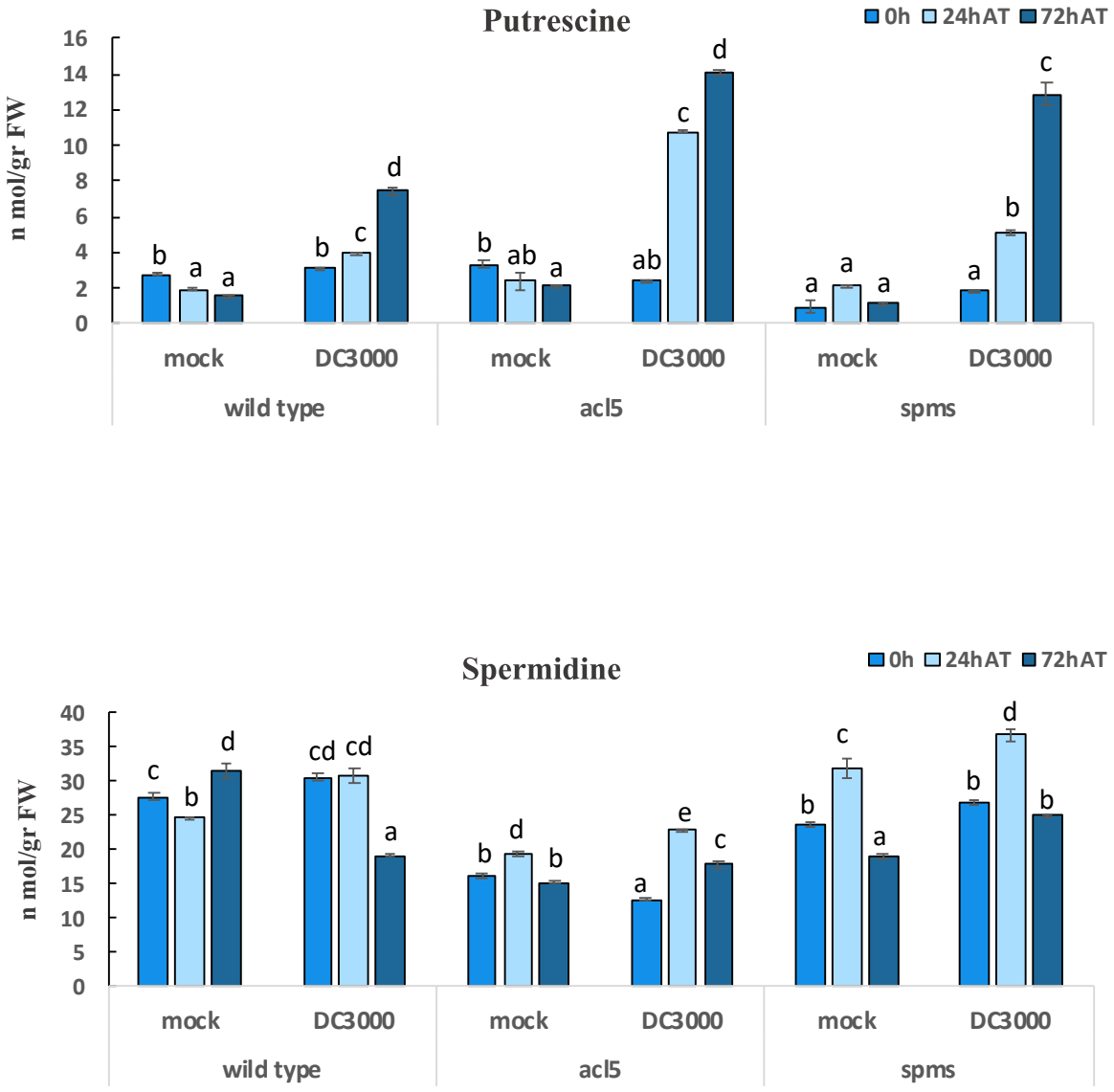
Figure 27. Cell death in response to Spm and t-Spm. Trypan blue staining of 4 weeks-old *Arabidopsis* wild- type *Col-0* infiltrated by Spm and t-Spm (100 µM and 500 µM) and mock (HPLC H_20). Samples were harvested at 24 h after treatment.

One-day post infiltration, wild-type leaves treated with 100 µM Spm and t-Spm exhibited cell death (**Figure 27**), although this response was more clearly evident at 500 µM Spm.

Application of t-Spm, induced less cell death than Spm at the higher concentration, and no clear differences were found between 100 µM and 500 µM t-Spm. These data demonstrated that the optimal concentration of Spm to induce cell death was 500 µM and we used this concentration for further experiments.

1.8.1 Polyamine metabolism in *spms* and *acl5* in response to *Pst* DC3000

To study the potential contribution of Spm metabolism to bacterial disease resistance, we challenged *Arabidopsis* wild-type, *spms* and *acl5* loss-of-function mutants with *Pst* DC3000 and quantified polyamine levels at 0 h, 24 h and 72 h post-treatment (**Figure 28**).



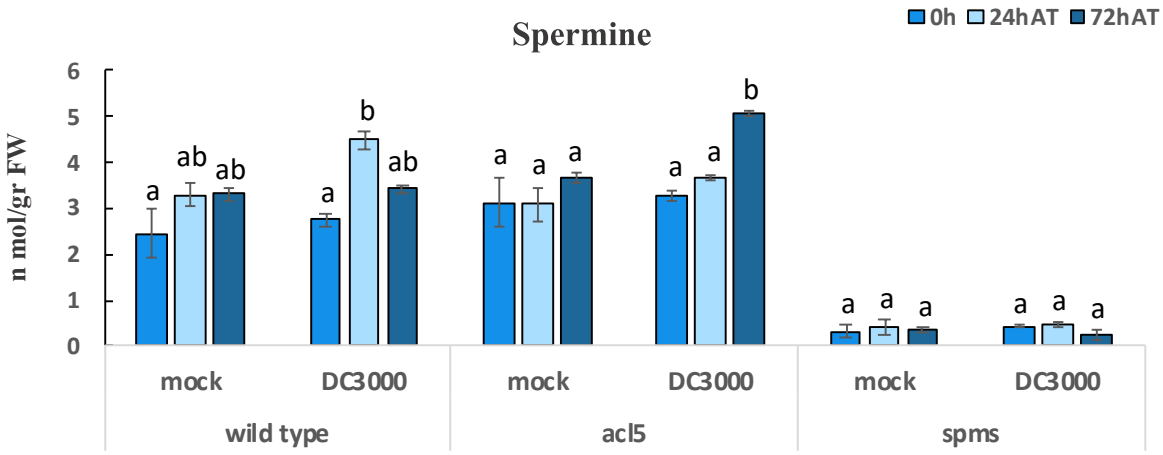


Figure 28. Polyamines level in response to *Pst* DC3000 Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 3 weeks-old, soil-grown *Arabidopsis* wild-type *Col-0* and *spms* and *acl5* loss-of-function mutants sprayed by *Pst* DC3000 (OD_{600}: 0.1) and mock (10 mM $MgCL_2$+ Silwet L-77). Samples were harvested at 0 h, 24 h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

Similar to our previous results in **Figure 18**, accumulation of Put in wild type in response to *Pst* DC3000 was also evidenced here (**Figure 28**). Interestingly, in *spms* and *acl5* mutants, higher Put accumulation was detected in response to *Pst* DC3000 compared to the wild type. Put content increased significantly (between 4.5- to 11- fold) in *acl5* and *spms* mutant at 24 h and 72 h after treatment (**Figure 28**). Nevertheless, total Put levels were similar in either genotype at 72 h post-inoculation and about 1.8-fold higher than the wild-type. These results indicated that Put accumulates more in *spms* and *acl5* loss-of-function mutants in response to *Pst* DC3000. The data might suggest the occurrence of a Put to Spm canalization in response to *Pst* DC3000, which is impaired in *spms* or *acl5* mutants. Alternatively, the higher Put levels might be related to increased susceptibility, considering Put as a marker of stress. Spd levels were slightly and transiently increased at 24 h post-inoculation in *spms* and *acl5* mutants, although their levels did not increase after 72 h of treatment (**Figure 28**). *acl5* mutants exhibited very small increment of Spm level at 72 h post-inoculation with *Pst* DC3000. As expected, no remarkable change was evidenced in Spm content of *spms* mutants compared to mock (**Figure 28**). Noteworthy to mention that defense response to *Pst* DC3000 is accompanied by Put accumulation and Spd depletion in wild type plants (**Figures 18, 28**), and this response seems to be related to bacterial dilutions. The data demonstrated that *Pst* DC3000

mainly leads to changes in Put, however, and this response is stronger in *spms* and *acl5* mutants. Conversely, Spd does not accumulate, even though it is the direct substrate of SPMS and ACL5 enzymes. The possibility of Spd oxidation cannot be excluded. In order to investigate whether Spd oxidation occurred, we analyzed the levels of 1,3-diaminopropane (DAP) (**Figure 29**).

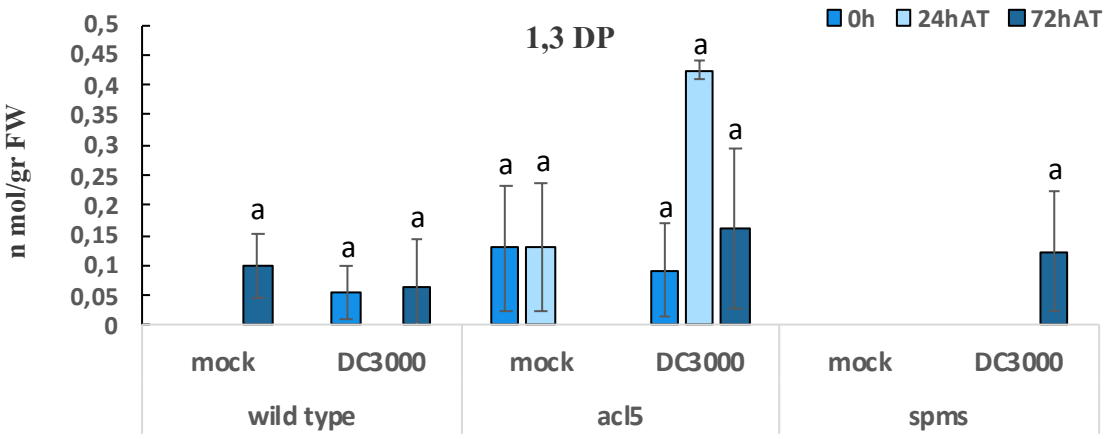


Figure 29. 1,3-diaminopropane (DAP) level in response to *Pst* DC3000. Three weeks-old, soil-grown *Arabidopsis* wild-type *Col-0* and *spms* and *acl5* loss-of-function mutants sprayed by *Pst* DC3000 (OD_{600}: 0.1) and mock (10 mM $MgCL_2$+ Silwet L-77). Samples were harvested at 0 h, 24 h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

Consistent with its Spm accumulation, DAP also accumulated up to 3.2-fold in *acl5* mutant inoculated with *Pst* DC3000. However, the detection of DAP was very variable between replicates. Hence, a Put to Spd/Spm metabolic flow seems to occur in *acl5* which is not so evident in the wild-type or spms mutant (Put to Spd metabolic flow, in this case). The increases in Spm levels may be buffered by the oxidation of Spm or its precursor, although this question still remains to be determined what causes the metabolic imbalance of *acl5* in response to *Pst* DC3000.

1.8.2 Disease resistance to *P. syringae* pv. tomato DC3000 in *spms* and *acl5*

In order to determine whether the deficiency in Spm or t-Spm affects bacterial disease resistance, we performed pathoassays in *Arabidopsis* wild-type plants, *spms* and *acl5* mutants infiltrated with *Pst* DC3000.

Figure 30. Macroscopic symptoms in response to *Pst* DC3000. *Arabidopsis* wild-type and *spms* and *acl5* loss-of-function mutants after infiltration with *Pst* DC3000 (OD_{600}: 0.001) at 72 h post inoculation.

As shown in **Figure 31**, no statistically significant differences were detected in the growth of *Pst* DC3000 in *spms* and *acl5* loss-of-function mutants compared to wild type. As expected, the *eds1* mutant enabled higher bacteria growth (Bhandari et al., 2018). We concluded that, although Spm plays a critical role in response to abiotic stresses such as high salinity and drought (Kusano et al., 2007), disease resistance in response to hemi-biotrophic bacteria *Pst* DC3000 does not seem to be strikingly affected by Spm-deficiency.

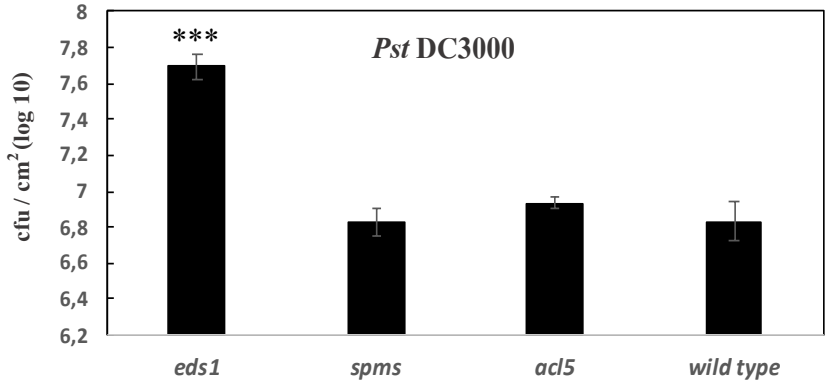


Figure 31. Growth of *Pst* DC3000 on *spms* and *acl5* mutants. Growth of *Pseudomonas syringae* pv. tomato DC3000 in *Arabidopsis* wild-type and *spms* and *acl5* loss-of-function mutants. Four-week-old plants were infiltrated with *Pst* DC3000 (OD_{600}: 0.001). Bacterial counting was performed at 3 days post-inoculation. Results are the mean of five replicates ± SD (standard deviation). Asterisks indicate values that are significantly different according to T-test P value <0.05.

1.8.3 Involvement of Spm in the systemic response

Local defenses trigger systemic responses leading to Systemic- Acquired Resistance (SAR) , which establishment is tightly dependent on SA and other defense-related metabolites (Q. M. Gao et al., 2015; Wildermuth et al., 2002). Liu et al., 2019 reported the contribution of Put in the establishment of SAR. To investigate the potential participation of Spm on SAR establishment, we pre-infiltrated local (1°) leaves of the wild-type *Col-0* and *sid2-1*, *pad4*, *eds1-2* and *npr1-1* mutants with 500 μM Spm and mock (H_2O). After 24 h, systemic (2°) leaves were inoculated with *Pst* DC3000 and bacterial titers analyzed at 72 h.

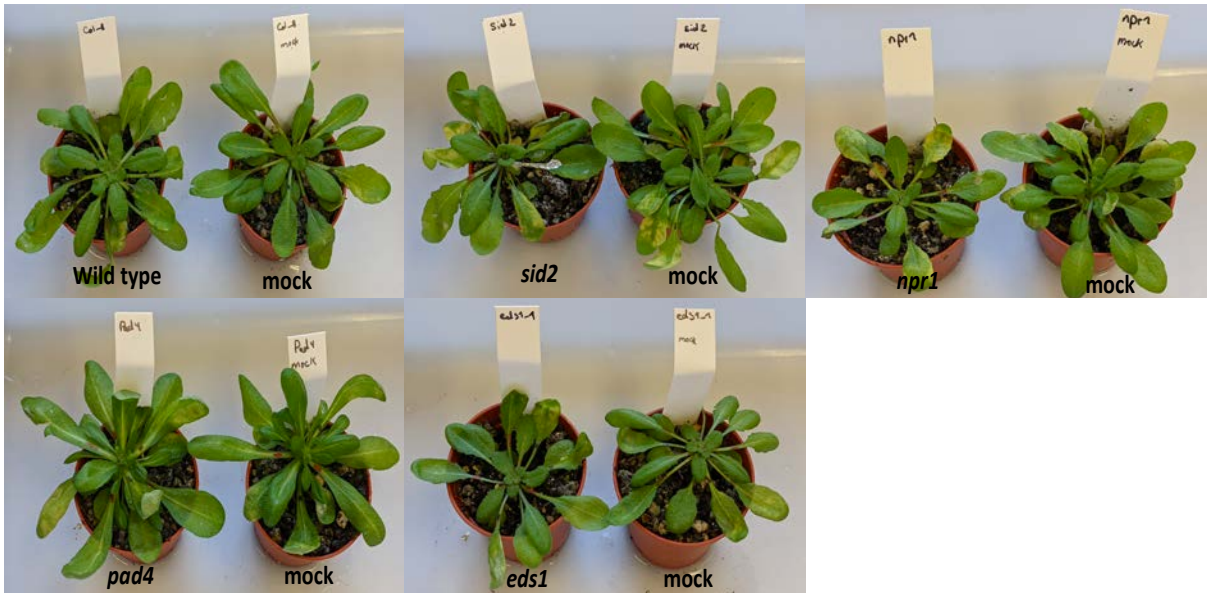


Figure 32. Macroscopic symptoms in response to *Pseudomonas syringae* pv. tomato DC3000. *Arabidopsis* wild-type and *sid2-1*, *pad4*, *eds1-2* and *npr1-1* mutants pre-infiltrated with Spm 500 μM and mock (H_2O), and after 24 h were inoculated with *Pst* DC3000 (OD_{600}: 0.001).

Spm pre-treatment did not inhibit growth of *Pst* DC3000 in 2° leaves of wild type compared to mock inoculated plants (**Figure 33**). This result indicated that Spm treatment does not initiate defense response leading to SAR establishment. Moreover, no significant resistance was observed in *sid2-1*, *pad4* and *npr1-1* mutants in comparison with mocks after pre-treatment of Spm. However, these loss-of-function mutants exhibited more susceptibility in response to pathogen inoculation compared to the wild-type, which is consistent with the requirement of SA during defense response.

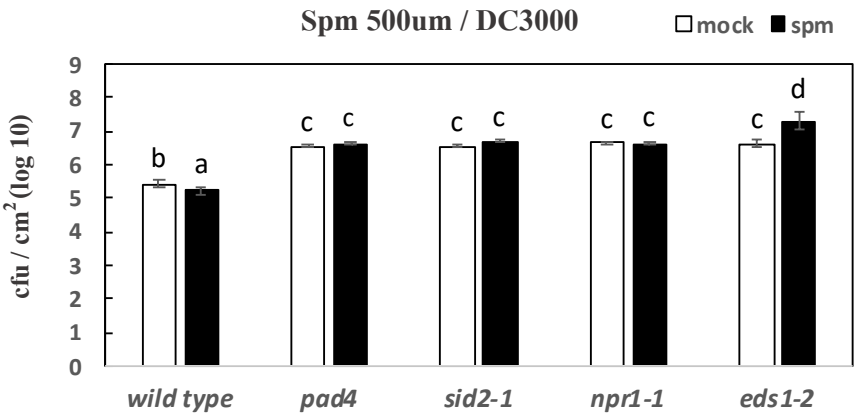


Figure 33. Analysis of Spm contribution in SAR response in SA pathway. Four-week-old soil-grown *Arabidopsis* wild-type and *sid2-1*, *pad4*, *eds1-2* and *npr1-1* mutants pre-treated in 1° leaves with Spm 500 μM. At 24 h post-infiltration, 2° leaves inoculated with *Pst* DC3000 (OD_{600}: 0.001). Bacterial counting was performed at 3 days post-inoculation. Results are the mean of five replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

1.8.4 Spm-deficient mutants in response to abiotic stress

Protective roles of plant polyamines in response to abiotic stresses are well reviewed (Alcázar et al., 2020). Given that Spm and t-Spm triggered cell death when infiltrated in leaves, we wondered whether abiotic stresses leading to cell death would be attenuated in *spms* or *acl5* mutant. For this, we exposed *spms* and *acl5* mutants to different NaCl concentrations and determined cell death by trypan blue staining (**Figure 34**).

Sporadic cell death was observed in the wild type, *spms* and *acl5* seedlings grown in MS media + 100 mM NaCl. At 150 mM NaCl, stronger cell death was detected in the *spms* mutant, whereas *acl5* and wild type did not show differences between them, or with the 100 mM NaCl treatment. These data are in agreement with a protective role for Spm during salinity stress. Huh et al. (2002) suggested that salt-triggered PCD is associated with ion disequilibrium, which is mainly controlled through Ca^{2+}-dependent signaling pathway (Huh et al., 2002). Here we report that high concentrations of Spm lead to cell death, whereas its absence also enhances cell death during salinity. A dual role for Spm is envisaged: ROS scavenger at low dosages (physiological concentrations) and ROS producer at higher dosages.

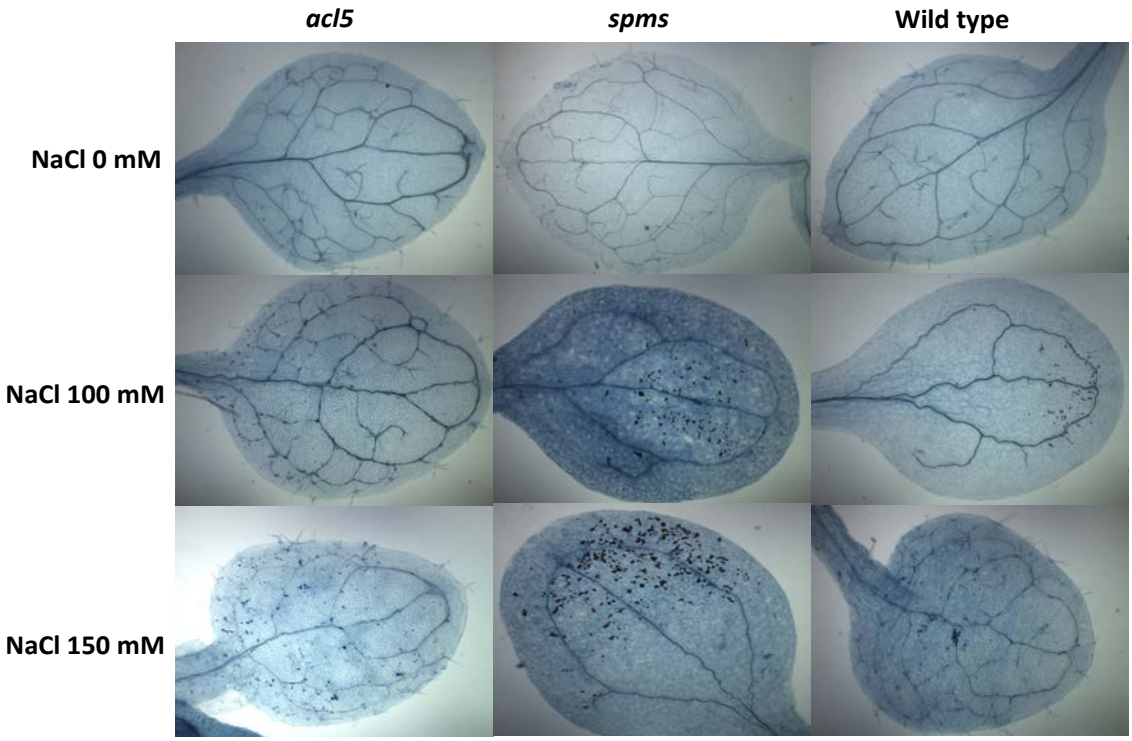


Figure 34. Analysis of cell death initiated by different concentration of NaCl. *Arabidopsis* wild type and *spms* and *acl5* seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media. 7-days-old seedlings were moved to new MS media supplemented with NaCl (0 mM, 100 mM, 150 mM). Samples were harvested once phenotypic symptoms revealed for cell death detection by trypan blue staining assay.

2. Identification of new genes regulating Polyamine metabolism by GWAS mapping

2.1 GWAS mapping of polyamine levels under basal conditions

Genome-Wide Association Study (GWAS) identifies genetic variation associated with specific traits and represents a powerful tool to dissect a vast variety of complex traits (Huang et al., 2011). This technology enabled scientists to reveal new genes involved in plant development such as growth, flowering time and plant defense responses (González et al., 2020; Kooke et al., 2016). In the current study GWAS was applied to *Arabidopsis thaliana* to identify new genes modulating polyamine metabolism.

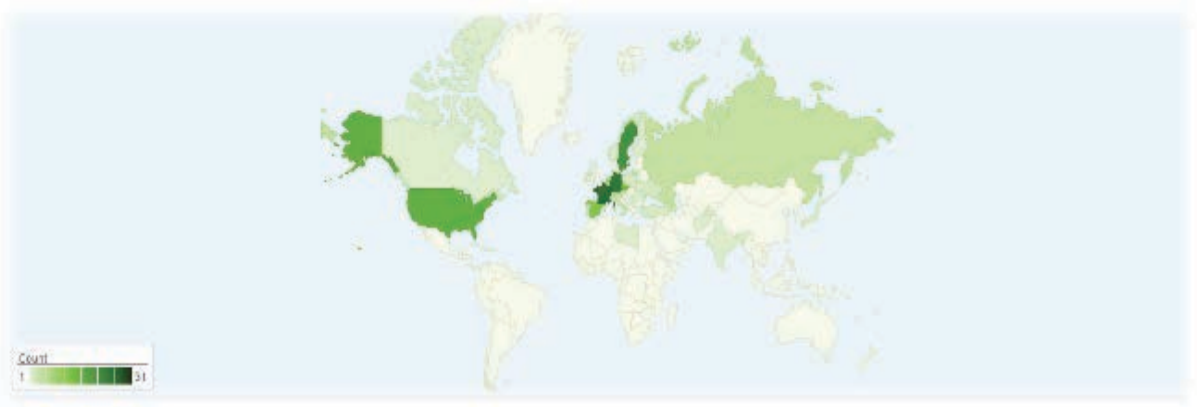


Figure 35. Geographic distribution of *Arabidopsis thaliana* natural accessions used in this study.

A GWAS analysis previously performed in our lab used 204 *Arabidopsis* natural accessions from worldwide (**Figure 35)**, with some overrepresentation of Central European and North European accessions, but with limited population structure. Polyamine levels were determined under non-stressed basal conditions at 14-16º C, which is the average temperature of *Arabidopsis* populations in the wild. The quantitative traits were used for GWAS mapping using the accelerated mixed model (AMM) **(Figure 36**).

Table 12. List of top-associated genes with polyamine levels variation identified by GWAS analysis

Gene		Description	Chromosome
At4g26420	GAMT1	Gibberellin methyltransferase 1	4
At4g26460	_	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	4
At4g26600	_	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	4
At4g26730	_	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	4
At4g23590	_	Tyrosine transaminase family protein	4

The analysis of data obtained from GWAS, allowed us to identify genes associated with free Put and Spm contents (**Table 12**). A cluster of SAM-dependent methyltransferases on chromosome 4 and the ACC-synthase At4g23590 were found associated with the variation of Put and Spm levels, respectively (**Figure 36**).

SAM is a universal methyl donor. When decarboxylated by SAM decarboxylases, dcSAM can only be used for polyamine (Spd and Spm) biosynthesis. SAM is also substrate for ethylene biosynthesis via ACC synthase. Competition for SAM in ethylene and polyamine biosynthesis has been a subject of debate in the last decades.

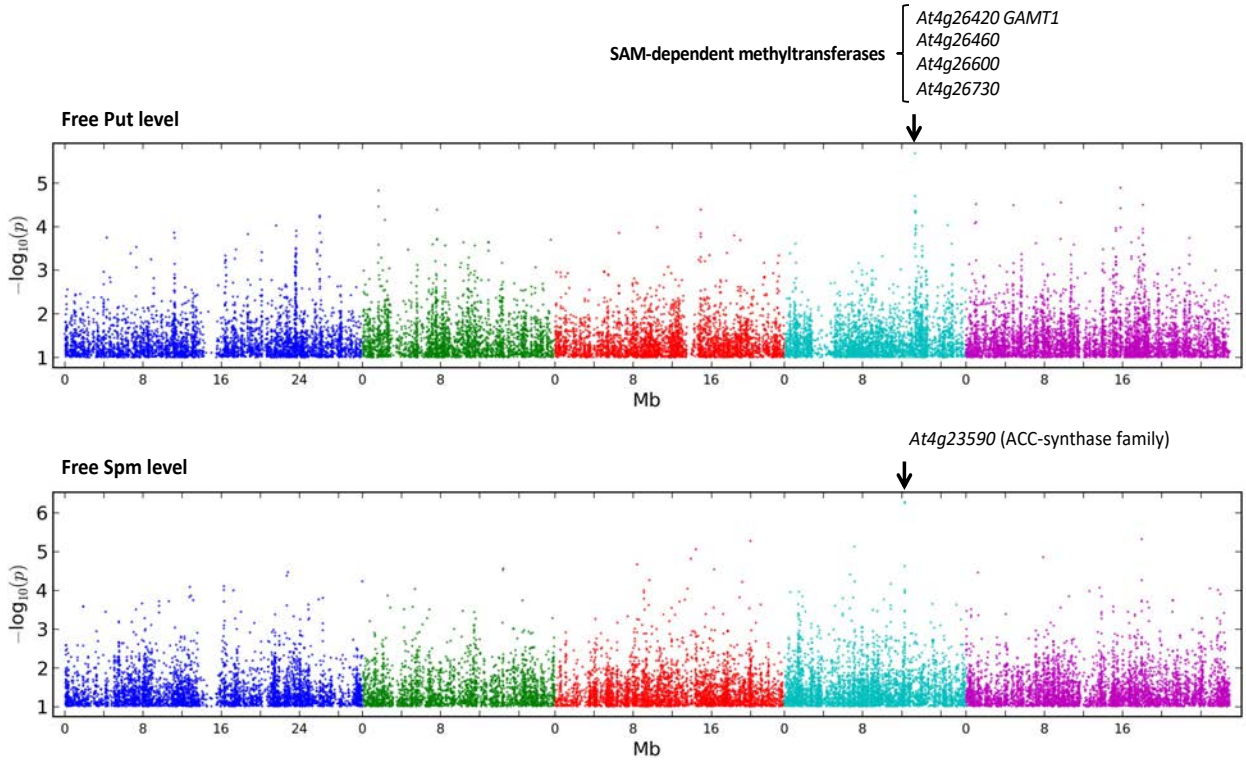


Figure 36. Manhattan plot of GWAS results for polyamine levels. The SNPs with significant differences (higher than the established threshold − logP > 5) were selected. Some SNPs which placed within the genes marked by arrows.

Our finding that Put and Spm levels are directly or indirectly modulated by SAM metabolism, prompted us to investigate the SAM-polyamines-ethylene interrelationship. For this, we selected a number of mutants impaired in either SAM biosynthesis (SAM synthases), as well as SAMDC, ethylene and SAM-methyltransferase mutants shown in (**Figure 37**).

2.2 Isolation of homozygous SAM synthase mutants

Mutants were ordered from the Nottingham *Arabidopsis* Stock Center (NASC, UK) (see material and methods, **Table 6**). All the mutants contained T-DNA insertion expected to disrupt the gene expression. In order to gain insight into the SAM pathway and its contribution to polyamine biosynthesis, primary we checked the homozygosity of mutants by antibiotic screening (MS medium+ kanamycin 50 µg/ml) and PCR method. The list of the homozygous mutants and their location in the SAM pathway is shown in **Figure 37** (For more information see material and method).

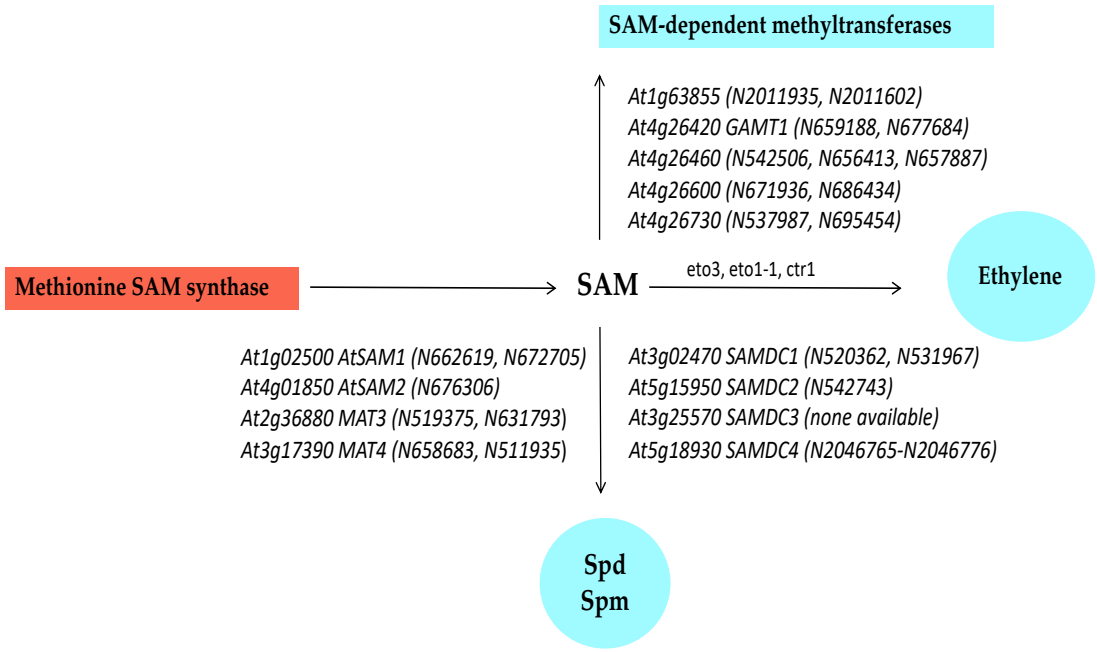
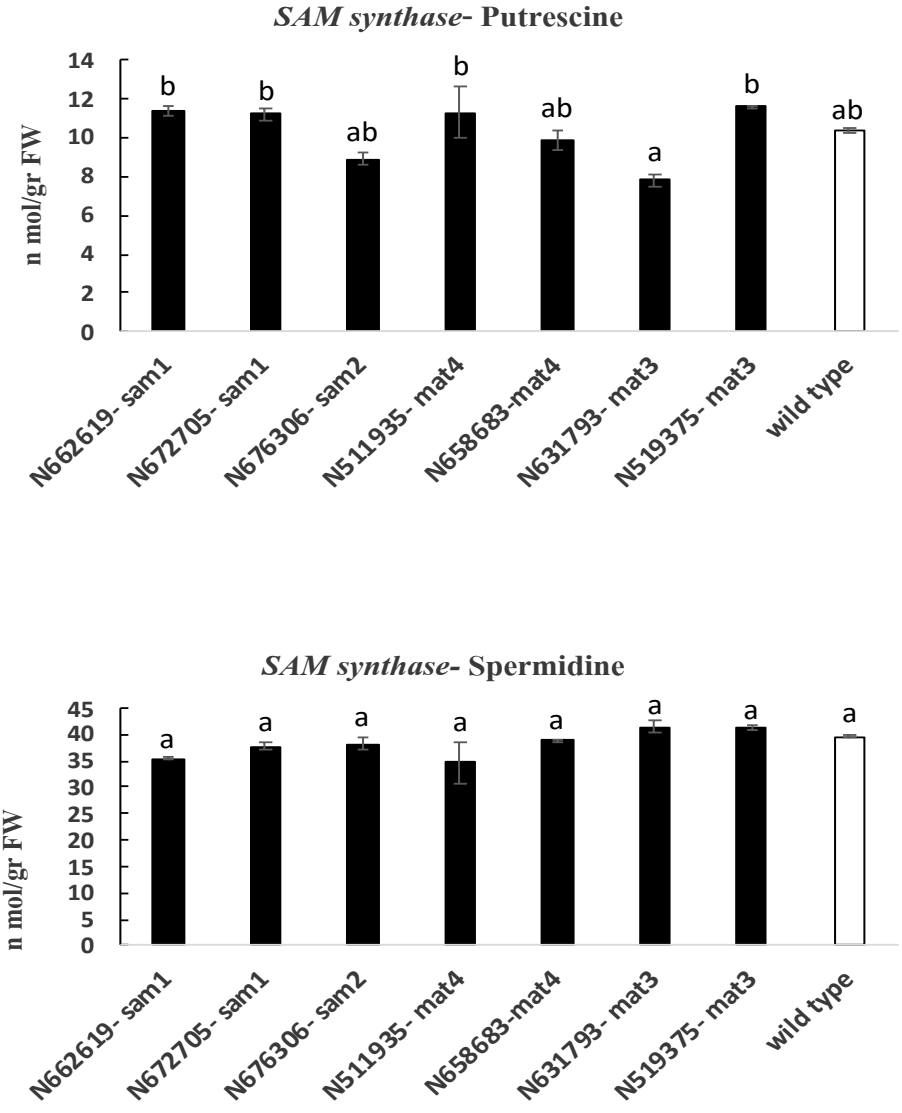


Figure 37. S-adenosylmethionine (SAM) pathway in *Arabidopsis thaliana*. The enzymic function of SAMDC, results in dcSAM that is the main aminopropyl group donor, which is exclusively used in higher polyamines (Spd and Spm) production. SAM is also a substrate for ethylene biosynthesis.

2.3 Polyamine levels in *SAM synthase* mutants

SAM is synthetized from methionine and ATP by the action of *SAM synthase (SAMS)*. As an important metabolite, SAM participates in essential metabolic pathways in plants by entering in polyamines and ethylene biosynthesis (Sauter et al., 2013), and plays a critical role in response to environmental stresses (Jang et al., 2012; Roje, 2006). Therefore, tight regulation of its metabolism is required for plant development and stress responses.

To have new insight into *SAMS* function in *Arabidopsis* and to identify whether polyamine levels of *SAM synthase* mutants show any difference under basal conditions, polyamine levels were determined in the mutants N672705, N662619 (At1g02500/ *SAM1* gene), N676306 (At4g01850/ *SAM2* gene), N519375, N631793 (At2g36880/ *MAT3* gene), N511935 and N658683 (At3g17390/ *MAT4* gene). (**Figure 38**).



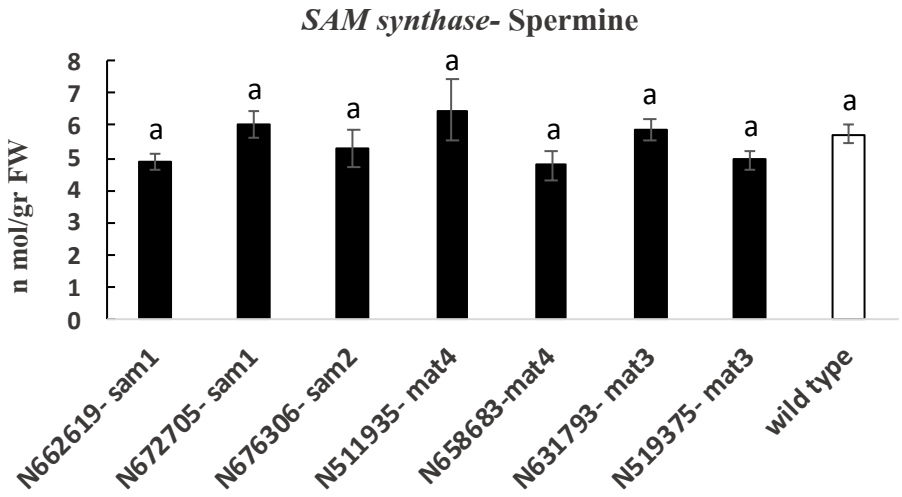


Figure 38. Basal polyamine levels of SAM synthase mutants. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 20-day-old *Arabidopsis* wild type *Col-0* and SAM synthase loss-of-function mutants in basal condition. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media. Samples were harvested and grinded with TissueLyser for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

Under non-stress conditions, the levels of Put did not exhibit significant (<2-fold) changes in *sam1* (N662619- N672705), *sam2* (N676306) and *mat4* (N511935- N658683) compared to the wild type (**Figure 38**), however, a slight reduction of Put content was observed in *mat3* (N631793) mutant. The basal level of Spd and Spm did not show any difference in all the *SAM synthase* mutants compared to the wild type (**Figure 38**). Even though the contribution of *SAMS* to polyamine metabolism is demonstrated in response to several environmental stresses (Gong et al., 2014; Z. Guo et al., 2014; Heidari et al., 2020; Ma et al., 2017; Y. C. Qi et al., 2010), our data indicated that compromised SAM synthetase does not affect polyamine metabolism under non-stress condition.

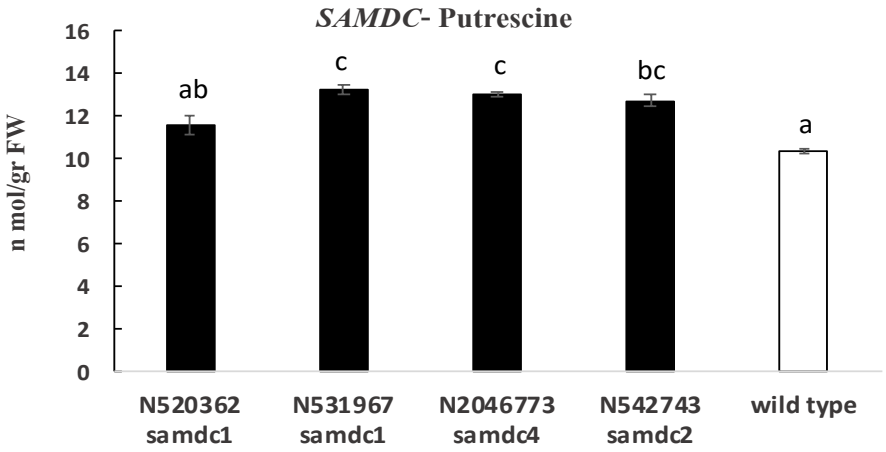
2.4.1 Polyamine levels in *SAMDC* mutants

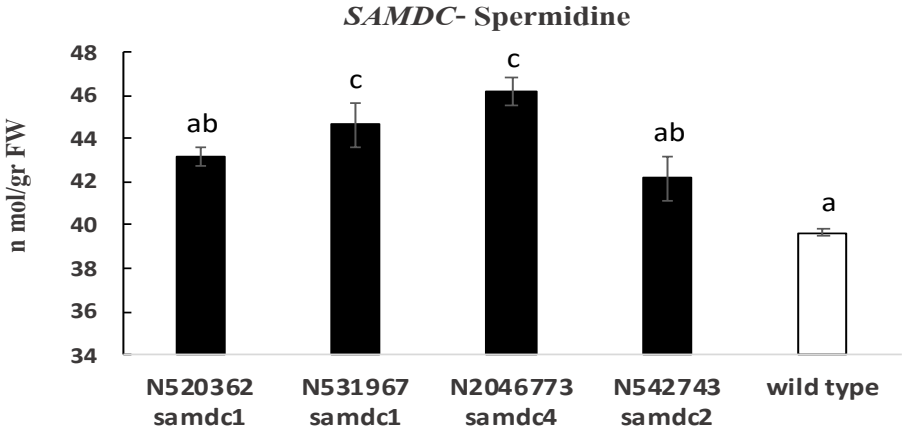
SAMDC a key enzyme in SAM cycle, is coded by multigene family and mainly function in response to plant stresses. Overexpression of *SAMDC* genes increase resistance to several (a)biotic stresses in *Arabidopsi*s, tomato and rice (Hazarika & Rajam, 2011; Marco et al., 2014; Roy & Wu, 2002; Wi et al., 2014). To further investigate *SAMDC* genes family function in

polyamine metabolism, we quantified the basal polyamine levels in *SAMDC* loss-of-function mutants (**Figure 39**).

Under basal conditions, polyamine metabolism did not show remarkable changes in *SAMDC* loss-of-function mutants compared to the wild type. However, small increments of Put and Spd contents (1.2- fold) were evidenced in *samdc1, samdc2* and *samdc4* mutants (**Figure 39**).

These results determined that under non-stress conditions, *SAMDC* genes seem redundant and single loss-of-function mutations do not affect polyamine metabolism significantly.





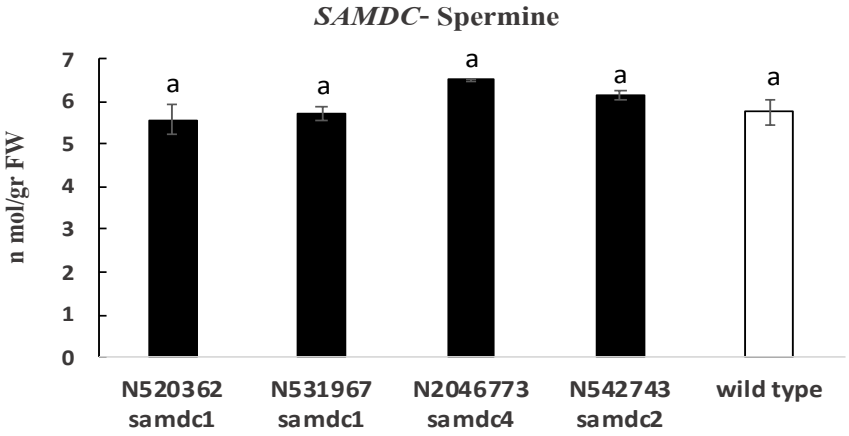
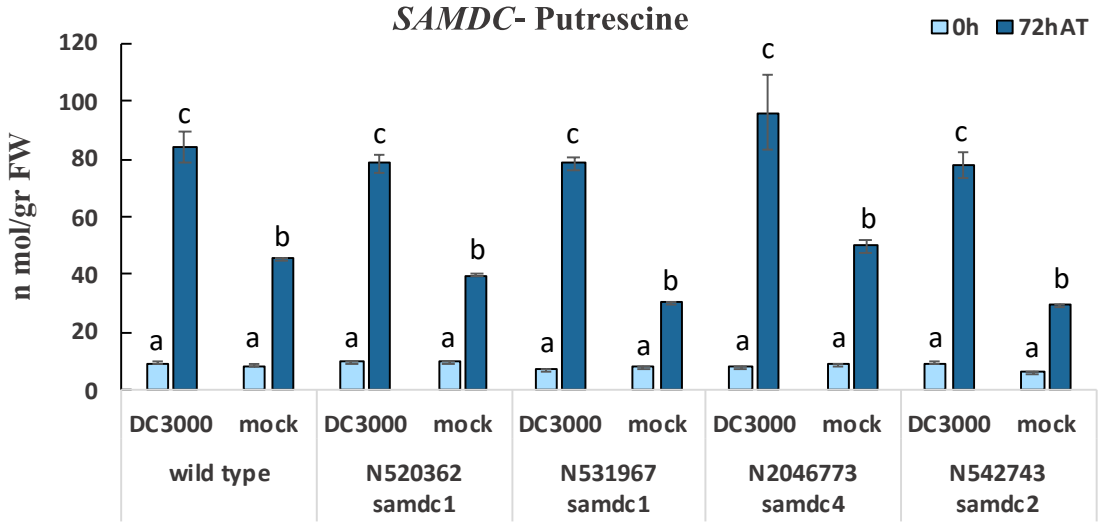


Figure 39. Basal polyamine levels of *SAMDC* mutants. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 20-day-old *Arabidopsis* wild type *Col-0* and *SAMDC* loss-of-function mutants under basal conditions. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media. Samples were harvested and dried with TissueLyser for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

2.4.2 Contribution of *SAMDC* genes to biotic stress in *Arabidopsis*

Up-regulation of *SAMDC*s in response to several abiotic stresses has been reported. Here we studied their contribution to polyamine biosynthesis in response to pathogens. For this we inoculated *Arabidopsis* wild type plants and *samdc loss*-of-function mutants with *Pst* DC3000 and determined polyamine levels at 0 h and 72 h post-inoculation (**Figure 40**).



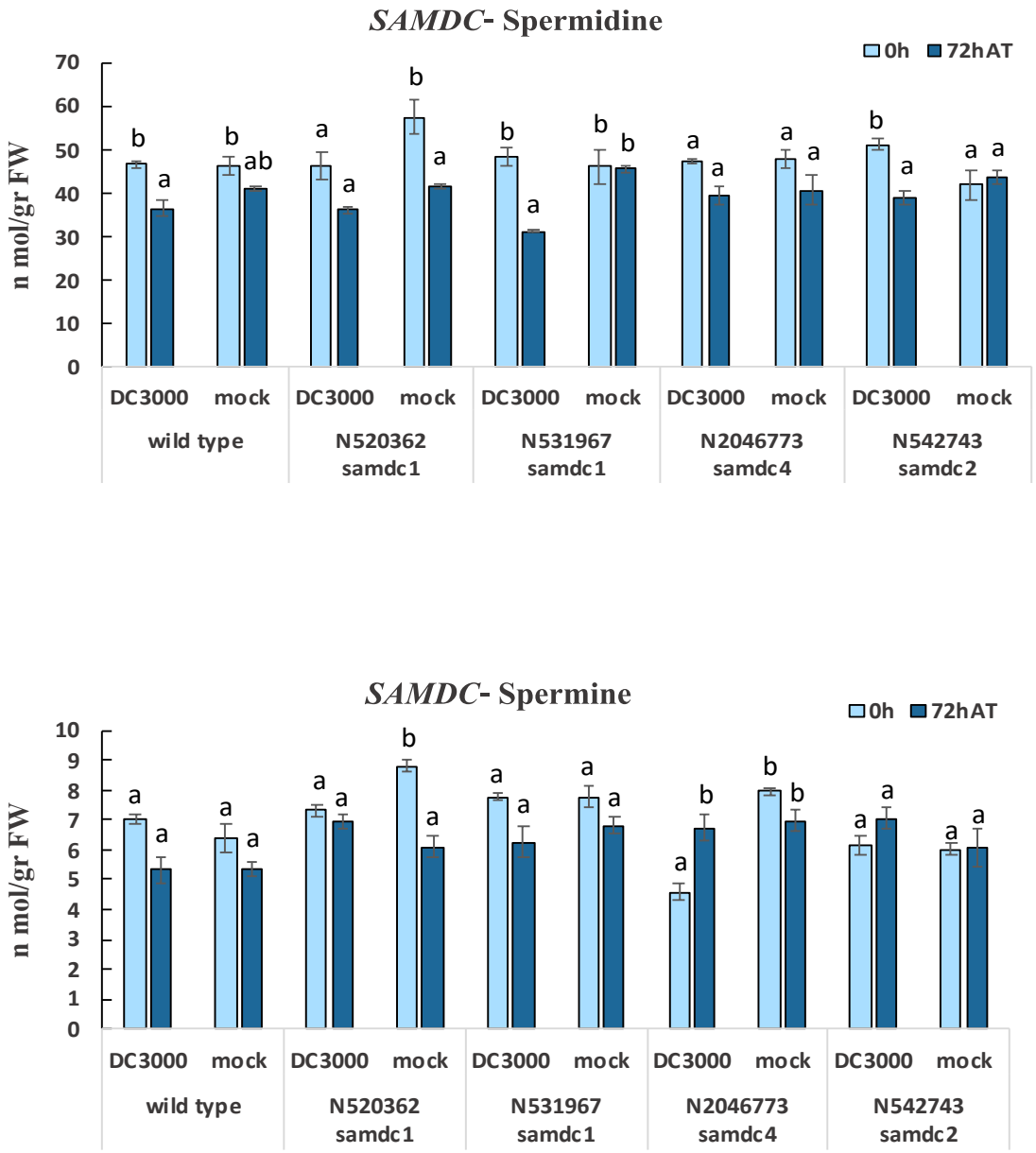


Figure 40. Polyamine levels in *samdc1*, *samdc2*, *mat3* and *mat4* mutants in response to *Pst* DC3000. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 15-day-old *Arabidopsis* wild-type *Col-0* and *SAMDC* loss-of-function mutants inoculated with *Pst* DC3000. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst* DC3000 (OD_{600}: 0.01) and mock (10 mM $MgCl_2$). Samples were harvested at 0 and 72 h post- treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

Three-days post inoculation Put accumulated significantly in wild type plants and *samdc* mutants in response to *Pst* DC3000 (**Figure 40**). Conversely, slight reduction of Spd level was observed in *samdc1* (N531967) and *samdc2* (N542743) mutants similar to the wild type (**Figure 40**). Although, this result was not evidenced in *samdc1* (N520362) and *samdc4*

(N2046773). The Spm level did not exhibit remarkable changes, except for *samdc4* (N2046773), that Spm content enhanced at 72h post inoculation (**Figure 40**). These data indicated that defense response triggered by *Pst* DC3000, results in Put accumulation and Spd reduction in wild type plants (**section I**, **Figure 18**). According to our previous results in section I, Put accumulates in response to hemi-biotrophic pathogens, whereas Spd and Spm levels do not increase. Several studies indicate that an increase in *SAMDC* activity leads to enhanced level of Spd (Pedros et al., 1999; Thu-Hang et al., 2002). Moreover, it has been demonstrated that Spd synthesis is mainly regulated by *SAMDC* rather than *SPDS* (Franceschetti et al., 2004; Gomez-Jimenez et al., 2010).

Altogether, this data indicated that *SAMDC* expression may not be required for polyamines metabolism in response to *Pst* DC3000, however, overexpression of *SAMDC*s increased resistance in response to fungal (Hazarika & Rajam, 2011) and bacterial pathogen (Marco et al., 2014). Moreover, it seems that defense response triggered by *Pst* DC3000 manipulates polyamine metabolism in favor of Put accumulation, which reinforces the view that Spd and Spm metabolism is tightly regulated.

We further analyzed 1,3-diaminopropane (DAP) content after *Pst* DC3000 inoculation (**Figure 41**) as proxy for polyamine oxidation in *SAMDC* mutants.

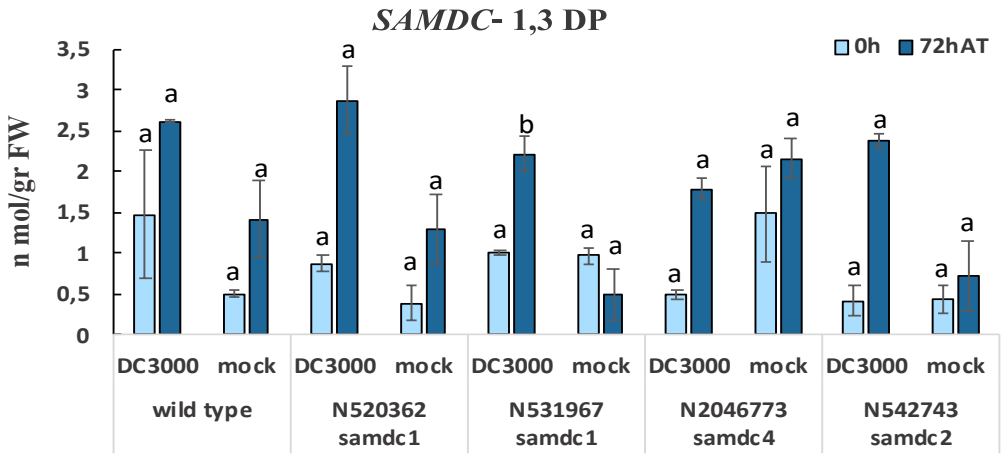


Figure 41. 1,3-diaminopropane (DAP) level in response to *Pst* DC3000. Level of DAP in 15-day-old, *Arabidopsis* wild-type *Col-0* and *SAMDC* loss-of-function treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

At 72h post- inoculation with *Pst* DC3000, *samdc* loss-of-function mutants accumulated DAP similar to the wild type (**Figure 41**). The data indicates that Spd or Spm oxidation is not significantly different between *samdc* mutants and the wild type. Thus, absence of Spd/Spm changes in response to *Pst* DC3000 in *samdc* mutants are not due to differences in catabolism. Overall, the data indicates a high level of redundancy between SAMDC members in pathogen responses.

2.4.3 *Pst* DC3000 pathoassays in *samdc* mutants

According to previous studies, down-regulation of *SAMDC* genes family reduces the tolerance to abiotic stresses (Chen et al., 2014; Panagiotis N Moschou et al., 2008). To investigate whether mutation in *samdc* would influence *Arabidopsis* tolerance to hemi-biotrophic pathogens, we inoculated wild type *Col-0* and *samdc* loss-of-function mutants with *Pst* DC3000 and determined bacteria growth at 3h and 72h post inoculation (**Figure 42**).

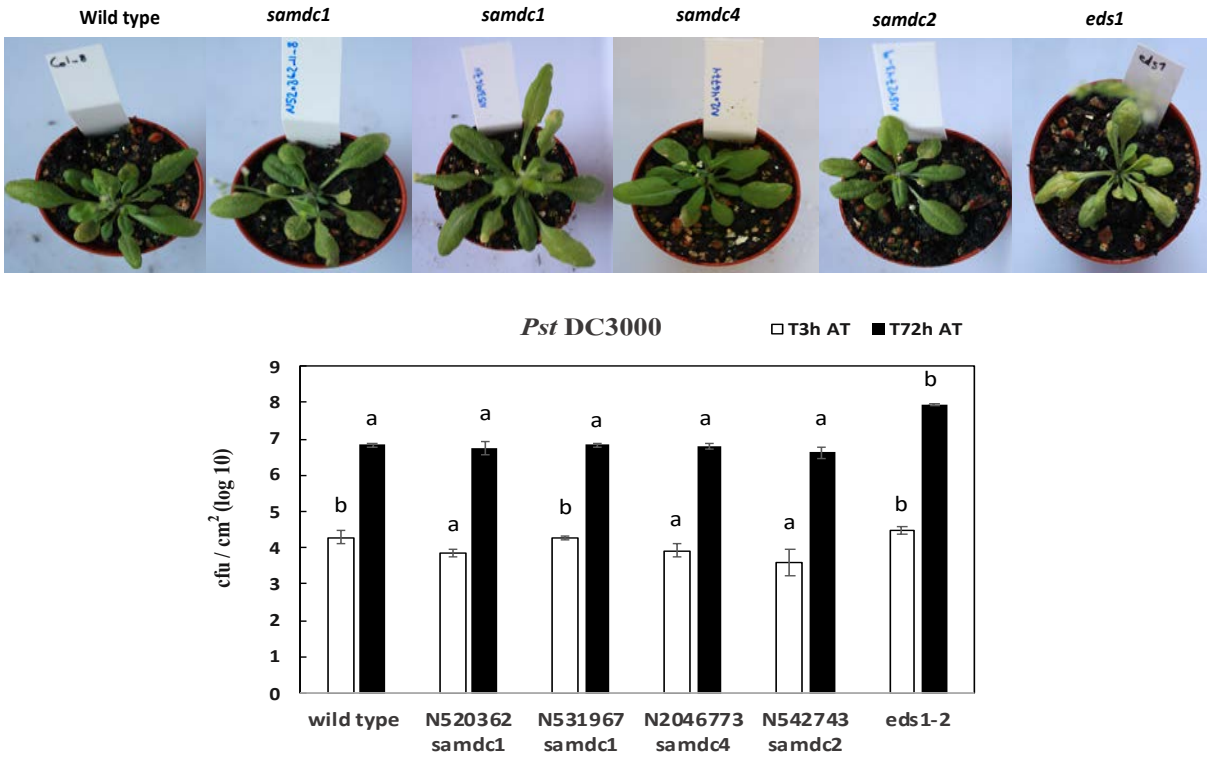


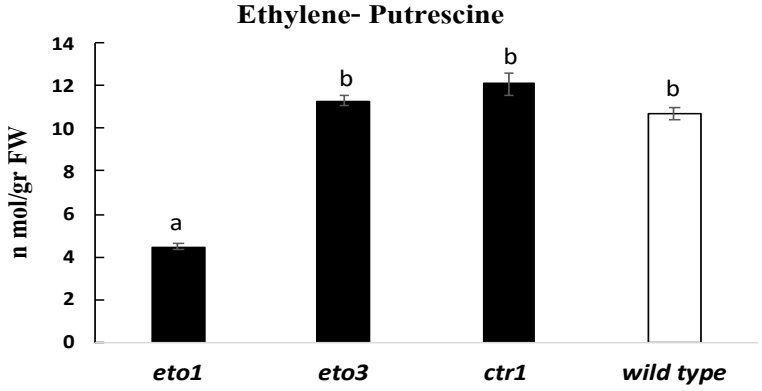
Figure 42. Growth of *Pseudomonas syringae* pv. tomato DC3000 on *Arabidopsis* wild type *Col-0* and *SAMDC* loss-of-function mutants. Four-week-old plants were spray inoculated with *Pst* DC3000 (OD_{600}: 0.2). Bacterial counting was performed at 3h and 72h post inoculation. Results are the mean of five replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05. Pictures were captured at 72h post-inoculation.

As shown in **Figure 42**, at 72h after inoculation with *Pst* DC3000, no differences were detected in bacterial growth in *samdc* mutants compared to the wild type. This result indicated that defense response activated by *Pst* DC3000 is not compromised by *SAMDC* dysfunction. Even though overexpression of *SAMDC* enhances tolerance to the biotic stress induced by *Fusarium oxysporum* and *Alternaria solani* in tomato (Hazarika & Rajam, 2011) and *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* in *Arabidopsis* (Marco et al., 2014), *SAMDC* dysfunction does affect resistance in response to *Pst* DC3000. The absence of polyamine differences between wild-type and *samdc* mutants inoculated with *Pst* DC3000 are in agreement with these results.

2.5 Polyamine-ethylene interrelationship

2.5.1 Polyamine levels in *eto1, eto3,* and *ctr1* mutants

In addition to the role of SAM in the biosynthesis of higher polyamines, it also acts as a substrate for ethylene biosynthesis through enzymatic activity of ACS (1-aminocyclopropane-1-carboxylic acid synthase) (Harpaz-Saad et al., 2012), which suggest a cross talk between ethylene and polyamine biosynthesis. Several studies revealed the inhibitory effect of ethylene in polyamine biosynthesis (Icekson et al., 1985; Roberts et al., 1984). Conversely, the modulation of ethylene biosynthesis by polyamines has also been discussed (Hyodo & Tanaka, 1986; Li et al., 1992). In order to gain inside into the interrelationship between ethylene and polyamines, we determined polyamine contents in ethylene overproducing mutants (*eto1* and *eto3*) and *constitutive triple response (ctr1)* under basal conditions (**Figure 43**) and in response to *Pst* DC3000 (**Figure 44**).



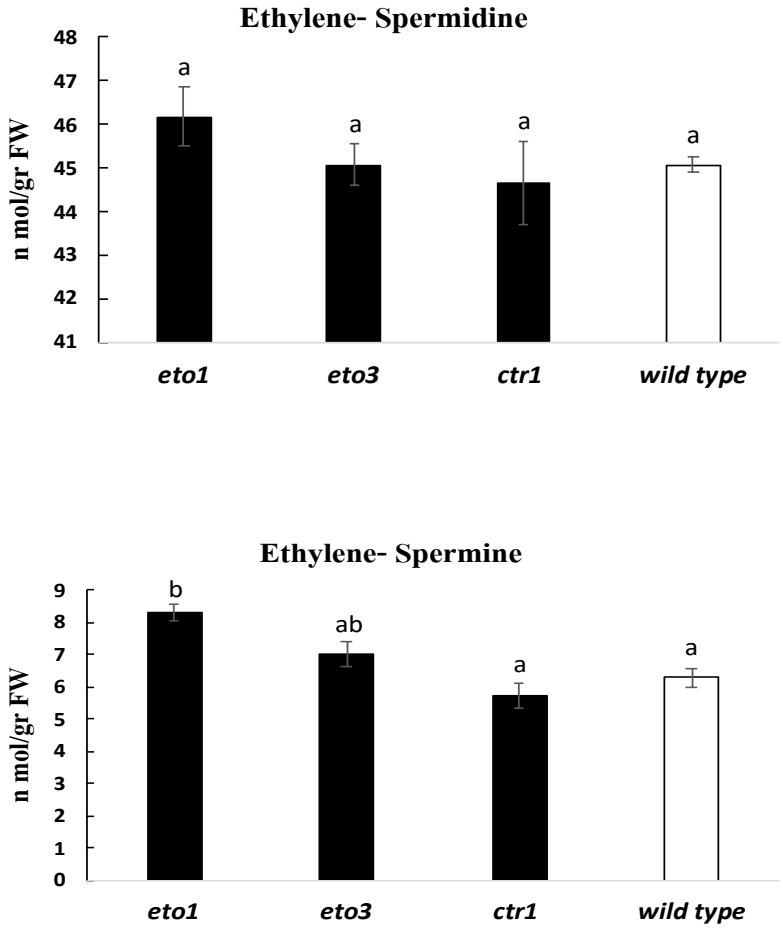


Figure 43. *Basal polyamine levels of ethylene mutants.* Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 20-day-old *Arabidopsis* wild type *Col-0* and ethylene overproducing mutants (*eto1* and *eto3*) and *ctr1*mutants in basal condition. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media. Samples were harvested and dried with TissueLyser for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

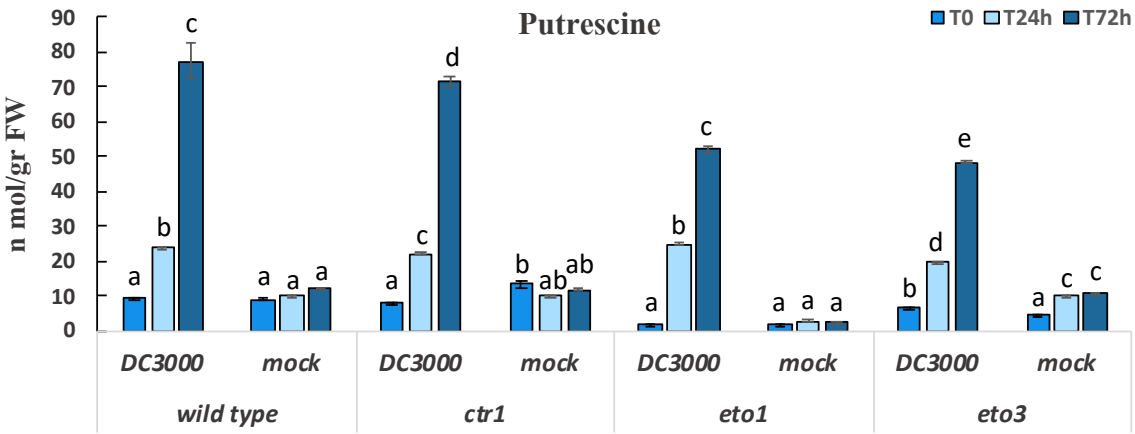
The basal level of Put did not exhibit significant changes in *eto3* and *ctr1* mutants. However, *eto1* mutant showed significant reduction in the basal Put level compared to the wild type (**Figure 43**). In *eto1, eto3,* and *ctr1*, the basal level of Spd did not show notable changes compared to the wild type (**Figure 43**). The basal level of Spm remained unchanged in *ctr1* and *eto3* mutants, even so, a slight increase in Spm level (1.3- fold) was observed in *eto1* mutants (**Figure 43**). The elevation of ethylene biosynthesis in *eto1* and *eto3* mutants results in the constitutive triple-response phenotype due to increased *ACS* activity (Woeste et al., 1999; Zhong & Chang, 2012), which is the rate-limiting for ethylene biosynthesis. Previously it has been reported that *eto1* recessive mutation increases ethylene production up to 10-fold

compared to the wild type, whereas, ethylene production enhanced 100-fold higher than the wild type in *eto3* (Kieber et al., 1993). The difference in Put content of *eto1* and *eto3* in non-stress condition might resulted from their endogenous level of ethylene. Thus, high ethylene biosynthesis is accompanied by high Put levels. The data argues against competition for SAM between polyamine-ethylene biosynthesis. Rather, high ethylene levels may activate a stress signaling response that leads to high Put levels.

2.5.3 Polyamine metabolism in ethylene overexpressor lines in response to pathogen

Myriad studies revealed the contribution of ethylene in response to pathogen attack (Argueso et al., 2007; Boller, 1991). Recognition of pathogen in plants induces ethylene biosynthesis as an early event. For instance, HR-triggered by incompatible plant-pathogen interactions is accompanied by a large burst of ethylene production (Van Loon et al., 2006). However, this response varies depending on the plant-pathogen interaction. It is supposed that enhanced ethylene production in response to pathogen contributes to stress depletion, although many pathogens can produce ethylene and interrupt plant responses. In *Arabidopsis*, *R* gene activation in response to *Pst avrRpt2* induced ethylene biosynthesis, indicating the involvement of ethylene in PTI and ETI (Guan et al., 2015). However, higher accumulation of ethylene was observed in ETI response due to the suppression of ethylene induction by PTI.

To get more insight into polyamine metabolism during defense activation and to investigate the role of ethylene in this regard, we tested *Arabidopsis* wild type *Col-0* and *eto1*, *eto3* and *ctr1* mutants with *Pst* DC3000 and quantified polyamine levels at 0h, 24h and 72h after inoculation (**Figure 44**).



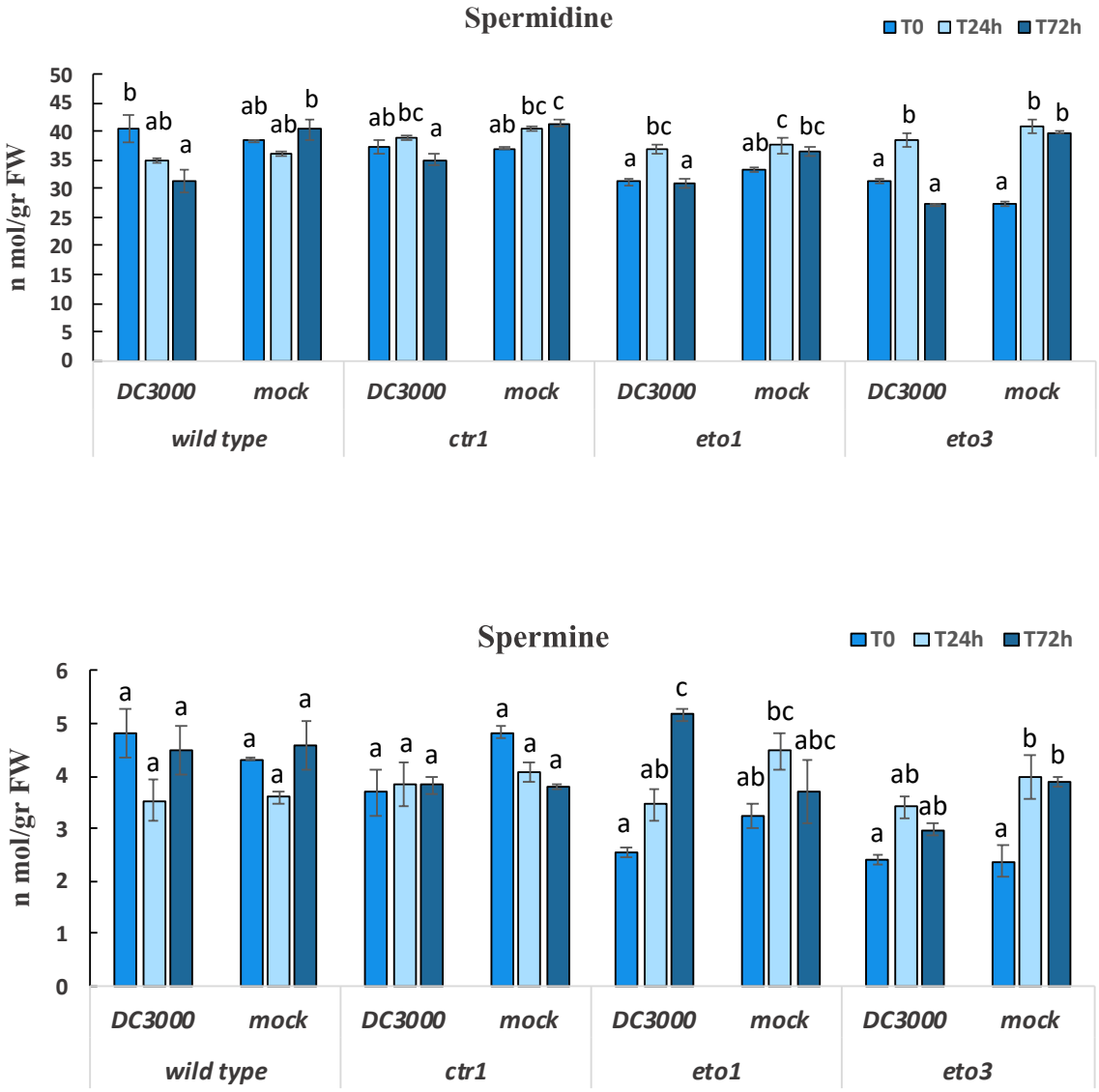


Figure 44. Polyamine levels in ethylene mutants in response to *Pst* DC3000. Levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) in 15-day-old *Arabidopsis* wild-type *Col-0* and ethylene overproducing mutants (*eto1* and *eto3*) and *ctr1*mutants treated with *Pst* DC3000. Seedlings were grown in vitro on a nylon mesh in 1/2 Murashige and Skoog media and inoculated by spraying with *Pst* DC3000 (OD_{600}: 0.01) and mock (10 mM $MgCl_2$). Samples were harvested at 0, 24h and 72 h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

One-day post inoculation, Put accumulated between 2- to 2.5- fold higher in *eto1*, *eto3, ctr1* and wild type in response to *Pst* DC3000 (**Figure 44**). However, significant increase in Put content was evidenced at 72h after inoculation. The level of Put enhanced up to 6- fold in *ctr1* mutants compared to mock inoculated plants (10 mM $MgCl_2$) (**Figure 44**). At 72h after inoculation of *eto1* and *eto3* mutants with *Pst* DC3000, Put accumulated 4- fold higher

compared to mocks (**Figure 44**). Three-day post inoculation, the slight decrease of Spd level was observed in wild type in response to *Pst* DC3000, although this response was not evidenced in *eto1*, *eto3* and *ctr1* mutants (**Figure 44**). In *eto1* mutants, Spm level increased remarkably at 72h post inoculation, the increment of Spm content was also observed in *eto3* mutants but not significant as *eto1* (**Figure 44**). These results determined that in compatible interaction between *Arabidopsis* and *Pst* DC3000, Put accumulates in *eto1*, *eto3* and *ctr1* mutants. However, when disease progressed, the accumulated Put in *eto1* and *eto3* mutants was lower compared to the wild type and *ctr1* (**Figure 44**). These data indicated that under stress conditions, over production of ethylene might suppress the accumulation of Put and induces biosynthesis of Spm.

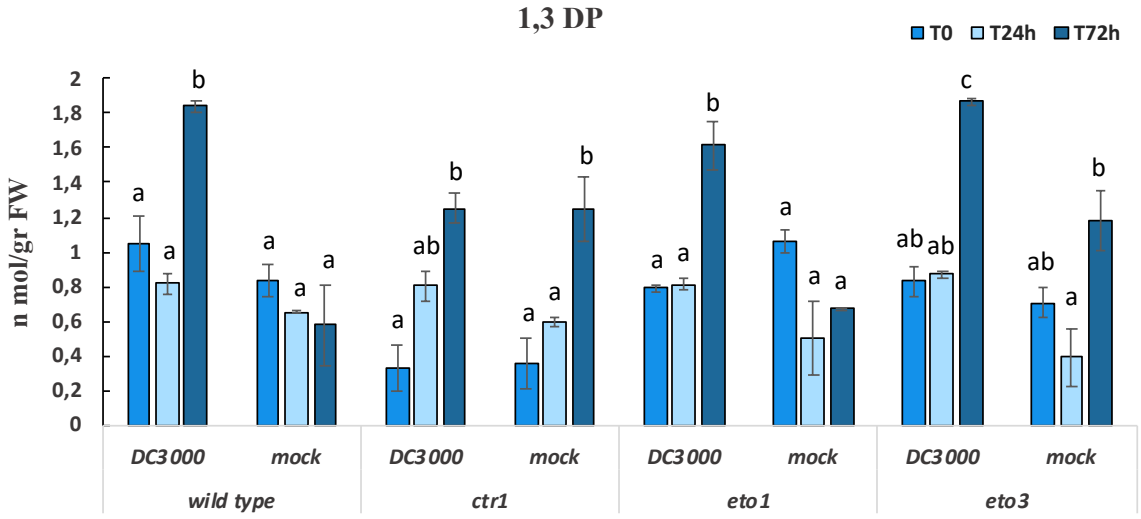


Figure 45. 1,3-diaminopropane (DAP) level in response to *Pst* DC3000. Level of DAP in 15-day-old, *Arabidopsis* wild-type *Col-0* and ethylene overproducing mutants (*eto1* and *eto3*) and *ctr1*mutants sprayed by *Pst* DC3000 (OD_{600}: 0.01) and mock (10 mM $MgCL_2$). Samples were harvested at 0, 24h and 72h after treatment for polyamine analyses. Results are mean of three biological replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05.

Further, we analyzed the level of 1,3 DAP in *eto1*, *eto3* and *ctr1* mutants in response to *Pst* DC3000 to study oxidation of polyamines in these mutants (**Figure 45**).

At 72h after inoculation with *Pst* DC3000, DAP accumulated up to 3- fold in *eto1*, *eto3* and *ctr1* mutant and the wild type compared to mock inoculated plants (10 mM $MgCl_2$) (**Figure 45**). Consistent with Spm accumulation in *eto1* mutant, the DAP content was also increased 2.7- fold higher compared to mock (**Figure 45**). This result demonstrated that in response to

hemi-biotrophic pathogen, ethylene over producer mutants (*eto1* and *eto3*) accumulate Put, however this response is lower compared to the wild type (**Figure 45**). In *eto1*, Spm accumulated significantly in response to *Pst* DC3000, although, increment of Spm in *eto3* was absent. As it mentioned above, *eto3* produces 10- fold higher ethylene compared to *eto1* (Kieber et al., 1993). We suggest that over production of ethylene results in Spm accumulation.

2.5.3 How ethylene modulates plant response to pathogen attack?

In order to indicate the role of ethylene in *Arabidopsis* resistance to hemi-biotrophic bacteria, we inoculated wild type, *eds1-2*, *eto1*, *eto3* and *ctr1* mutants with *Pst* DC3000 and bacterial titer was measured at 3h and 72h after infection (**Figure 46**).

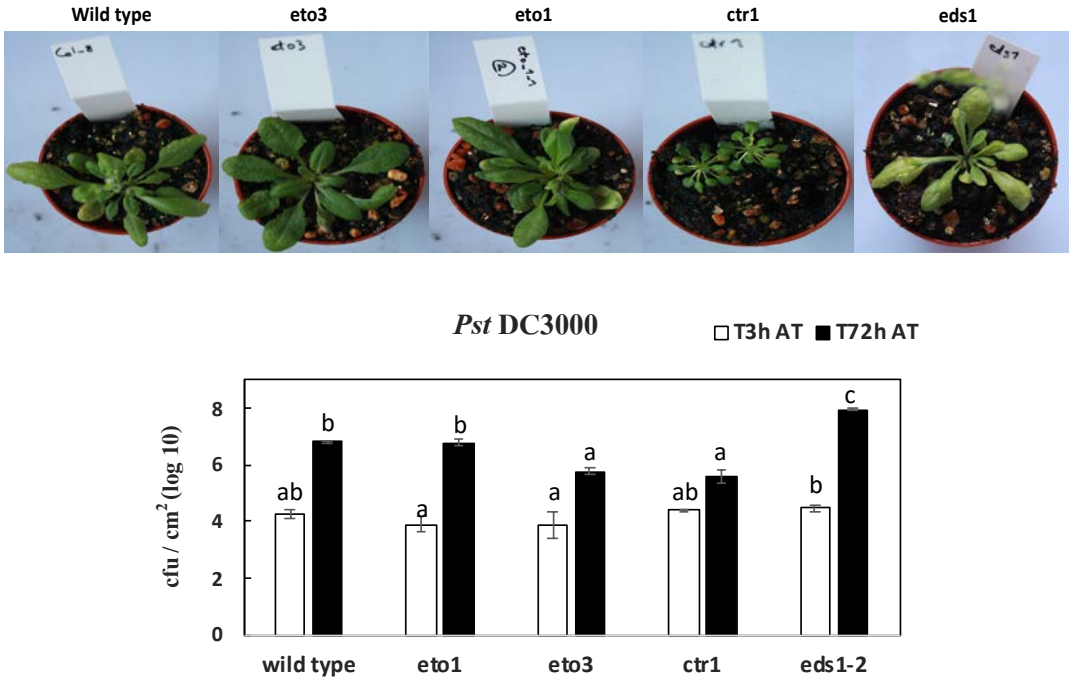


Figure 46. Growth of *Pseudomonas syringae* pv. tomato DC3000 on *Arabidopsis* wild type *Col-0* and ethylene overproducing mutants (*eto1* and *eto3*) and *ctr1*mutants. Four-week-old plants were spray inoculated with *Pst* DC3000 (OD_{600}: 0.2). Bacterial counting was performed at 3h and 72h post inoculation. Results are the mean of five replicates ± SD (standard deviation). Letters indicate values that are significantly different according to Student–Newman–Keuls test at P value <0.05. Pictures were captured at 72h post-inoculation.

As shown in **Figure 46**, at 72h after inoculation, ethylene over production lead to higher pathogen resistance in *eto3* mutants, however, the growth of *Pst* DC3000 in *eto1* did not show notable change compared to the wild type. This difference might be resulted from endogenous

level of ethylene in *eto1* and *eto3*. Previously the positive role of ethylene in pathogen resistance has been reported (Guan et al., 2015). The growth of *Pst* DC3000 was also limited in *ctr1* mutants (**Figure 46**), which determined that ethylene biosynthesis and signaling play role in defense response to hemi-biotrophic pathogen.

Discussion

ConclusionsDiscussion

Conclusions

BibliographyConclusionsDiscussion

ConclusionsDiscussion

The contribution of putrescine to different layers of plant immunity

Polyamines are small polycationic compounds with essential roles in plant growth and development. The participation of polyamines in fundamental physiological processes of plants has been studied in depth (Galston & Sawhney, 1990; Kusano et al., 2008; Martin-tanguy, 2001). In addition, contribution of polyamines in response to (a)biotic stresses has also been reported (Alcázar et al., 2006; Alcázar, Altabella, et al., 2010; Jiménez-bremont et al., 2014). Many studies have evidenced the alteration of polyamine metabolism in response to stress (Alet et al., 2011; Takahashi, 2016).

Plants are continuously exposed to a vast variety of microbes, and to combat with, several layers of immunity are utilized. Activation of PTI is initiated by recognition of Microbial- or Pathogen Associated Molecular Patterns (MAMPs or PAMPs) such as flagellin by PRRs (Zipfel & Felix, 2005). However, ETI is activated upon recognition of pathogen effectors by polymorphic NB-LRR protein products encoded by *Resistance* (*R*) genes (Dangl & Jones, 2001). Its noteworthy to mention that PTI response can be disrupted by pathogen effectors, which results in Effector-Triggered Susceptibility (ETS). Several works indicated that activation of defense response is associated with remarkable changes in polyamines contents and enzymatic activities involved in polyamine biosynthesis and oxidation (Romero et al., 2018). Moreover, alteration in polyamine content can affect the hormonal balance to mediate plant defense responses (Szalai et al., 2017) . In plant-pathogen interactions, salicylic acid (SA) plays an important role mediating the defense response against biotrophic and hemi-biotrophic pathogens (Hernández et al., 2017). Despite of the fact that many studies uncovered the relation of polyamines and SA during defense responses (Liu et al., 2020; Nagai et al., 2020; Pál et al., 2011; Rossi et al., 2020), the contribution of SA signaling to polyamine triggered defense responses is not fully understood. In this work we show that co-activation of PTI+ETI (Hatsugai et al., 2017) triggered by *Pst AvrRPM1* and *Pst AvrRPS4*, stimulate Put accumulation independently of SA-pathway (*SID2, EDS1, PAD4* and *NPR1*) (**Figures 14 and 16**). This data also suggests that diverse Avr proteins do not dampen polyamine metabolism during the defense response.

The involvement of Put in the defense response to incompatible and compatible plant-pathogen interactions has previously been documented. In the incompatible interaction between powdery mildew fungus *Blumeria graminis* f. sp. *hordei* and barley, the increment of Put and Spd content was evidenced (Walters et al., 1985). In another work, the incompatible interaction between the fungus *Fusarium graminearum* and wheat resulted in enhanced level of Put

(Gardiner et al., 2010; Rampersad, 2020). Remarkable changes in polyamine contents induced by pathogen attack are associated with alteration of biosynthesis and oxidative enzymatic activities. For instance, enhanced activity of ADC, ODC, PAO, DAO and SAMDC was reported in response to powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (Cowley & Walters, 2002). Moreover, up-regulation of ADC, ODC and DAO activity was detected in response to *Tobacco mosaic virus* (TMV) (Marini et al., 2001; Negrel et al., 1984). The compatible interaction between *Pseudomonas viridiflava* and *Arabidopsis* resulted in Spm accumulation (Gonzalez et al., 2011). More recently, Liu et al., 2019 revealed that the defense response triggered by *Pst DC3000* results in Put accumulation (Liu et al., 2019). We show that activation defense by *Pst DC3000* stimulates Put accumulation and Spd depletion independently of SA (*SID2, EDS1, PAD4* and *NPR1*) (**Figure 18**). Our data indicates that accumulation of Put is not suppressed by type III effectors from *Pst* DC3000, which is consistent with Liu et al., 2019. In addition, diminished level of Spd might be due to its oxidation or back-conversion into Put. It has been reported that *AvrBsT* effector from *Xanthomonas campestris* pv. *vesicatoria,* increases ADC1 activity (Kim et al., 2013). This result was also evidenced in ADC genes targeted by TALE-like effectors from the plant pathogen *Ralstonia solanacearum* (Wu et al., 2019).

The Put accumulation triggered by *Pst* DC3000 prompted us to investigate which virulence factors produced by *Pst* DC3000 induced this response. Interestingly, we found the partial contribution of the phytotoxin coronatine to Put accumulation (**Figure 20**). Coronatine as a multifunctional defense suppressor (Geng et al., 2012), can facilitate bacterial entry into the host cell though opening PAMP-closed stomata (McLachlan et al., 2014), and DC3000 Cor− mutant is significantly inhibited in bacterial virulence (Brooks et al., 2004).

To have an overall view of contribution of polyamines to plant immunity, we also employed the autoimmunity concept. We demonstrate that upon temperature shift of Ler/Kas-2 NIL plants to cold temperature (Alcázar & Parker, 2011), which leads to constitutive activation of defense (ETI) in the absence of pathogens, Put accumulates (**Figure 22**). Autoimmunity in NIL plants is triggered by TIR-NB-LRR proteins and requires SA signaling through EDS1 pathway (Alcázar et al., 2009). We also examined *mpk4* and *snc1* autoimmune mutants in response to temperature shift, and we concluded that constitutive activation of defense in the absence of pathogens results in different degrees of Put accumulation (**Figure 22**). Despite of the variation in Put content in autoimmune hybrid lines and mutants, the reduction of Spm was evidenced in all genotypes. In fact, lower levels of Spm were also observed under cold treatments (4 ºC)

in *Arabidopsis* wild type plants (Cuevas et al. 2008) and *Thellungiella salsuginea* (Lee et al., 2012), which indicates that lower Spm is a response to low temperature.

Autoactivation of plant immunity enhances resistance in incompatible hybrid lines and autoimmune mutants to virulent pathogens. Hybrid lines in *Arabidopsis* exhibited enhanced resistant to the biotrophic oomycete pathogen *Hyaloperonospora parasitica* (Alcázar et al., 2009). Li et al, demonstrated the increased resistance of *snc1* gain-of function mutants (Zhang et al., 2003) to *Pseudomonas syringae maculicola* ES4326 (Li et al., 2001). Growth of *Pst* DC3000 was also attenuated in *mpk4* loss-of function mutants (Brodersen et al., 2006). In this work we show that autoimmune activation (ETI) in NIL and cNIL hybrid lines amplifies metabolic response to *Pst AvrRpm1* in the favor of Put accumulation (**Figure 24**). Autoimmunity is conditioned by temperature and air moisture (Alcázar & Parker, 2011). However, the autoimmune phenotype can be reconstituted in vitro using a modified media with lower ammonium (NH_4NO_3) (AtanasovEvgeniev, 2019). Nitrogen (N) as one of the main nutrients in plants (Moschou et al., 2012), is commonly taken up as ammonium ions and nitrate from the soil. Several studies indicate that, due to the participation of nitrogen in important stress response metabolites, such as polyamines, its content affects plant productivity (Moschou et al., 2012). Elevated level of polyamines results in N consumption, which indicates the interaction between polyamines and N assimilation in stress responses (Paschalidis et al., 2019). Our observation in **Figure 24**, suggests a competition between polyamines and autoimmunity for nitrogen resources due to the lower Put content in NIL plants grown in media with lower amount of ammonium in response to pathogens.

Putrescine treatment leads to an earlier cell death response

Hypersensitive response (HR), is a rapid localized cell death, mostly accompanied by disease resistance (Mur et al., 2008). HR is initiated by a pathogen or metabolite, which leads to electrolyte leakage, as a result of cell membrane damage. HR response is mostly initiated in resistance-triggered by effectors. However, PTI response does not include HR (Balint-Kurti, 2019). Despite of ETI-triggered HR, other metabolites such as red maple hydroalcoholic (RME1) (Peghaire et al., 2020) and N-hydroxy-pipecolic acid (N-OH-Pip) (Chen et al., 2018) are describe as HR inducers. Several studies indicated the polyamines contribution as a source of H_2O_2 during HR. For instance, in response to *Tobacco mosaic virus* (TMV), production of

H_2O_2 through polyamines oxidation in apoplast, results in HR in tobacco (*Nicotiana tabacum*) leaves (Yoda et al., 2003). Moreover, the key role of polyamines oxidation to initiate oxidative burst has been discussed (Yoda et al., 2006). It has also been reported that lowering PAO activity suppresses H_2O_2 production and HR (Yoda et al., 2009). In ETI stimulated by β-estradiol in *a11* plants, a clear induction of cell death was evidenced (**Figure 25-B**). However, Put did not stimulate cell death (**Figure 25-C and 25-D**). Previously, Liu et al reported that exogenous Put application triggers callose deposition and increased expression of PTI related genes, with no induction of cell death (Liu et al., 2019). Other work also reported that Put treatment in *Arabidopsis* and rice (*Oryza sativa*) leaves do not trigger cell death (Yoda et al., 2009), which is an agreement with our data.

During PTI activation, a rapid burst of reactive oxygen species (ROS) occurs through NADPH oxidase (Averyanov, 2009; Lee et al., 2020), which in plants is also termed as respiratory oxidase homologs (RBOHs). The generation of ROS during the PTI response is initiated by RBOHD activity after PAMP recognition (Miller et al., 2009). The cell death increment observed in our result might be induced by the high production of ROS independently of RBOHD activity (Torres et al., 2002, 2005).

Previously, we observed that co-activation of ETI+PTI favors Put accumulation (**Figures 14 and 16**). Interestingly, this stronger response is accompanied by an earlier and stronger induction of cell death (**Figure 25-H)**. We also observed that exogenous application of Put likely promotes cell death (**Figure 25-I**).

Spermine and defense response

Yoda et al, reported that exogenous application of Spm in *Arabidopsis* and rice stimulates HR (Yoda et al., 2009). Furthermore, the role of Spm as a signaling molecule in defense response triggered by TMV and its contribution to trigger cell death has been proposed (Sagor et al., 2009). The relationship between HR and Spm was examined by Yoda et al mainly in tobacco, which demonstrates that polyamines oxidae activity results in hydrogen peroxide production in the apoplast, and this catalytic activity is substrate dependent (Yoda et al., 2003). Noteworthy to mention that apoplastic polyamine oxidases have more affinity for Spm than Spd. Here we show that exogenously applied Spm to *Arabidopsis* elicits cell death independently of *EDS1* and SA (**Figure 26**).

HR-triggered by *Pst* DC3000 *AvrRpm1, AvrRpt2* and *AvrRps4* was evidenced in *sid2* and *eds5* mutants (Dewdney et al., 2000; Nawrath & Métraux, 1999), which indicates that not all HR is SA dependent. Recently, a negative regulatory of SA in triggering cell death during ETI has been demonstrated (Radojičić et al., 2018). SA participates in the establishment of Systemic-Acquired Resistance (SAR) (Gao et al., 2015; Zhang et al., 2010). Recently, the contribution of Put to SAR has been reported (Liu et al., 2020). The role of Spm in plant defense against pathogens is mainly unveiled by utilizing *spms* mutant. For instance, infection of *Arabidopsis* plants with *Pseudomonas viridiflava*, results in Spm accumulation (Gonzalez et al., 2011; Marina et al., 2008). In addition, transgenic plants with overexpressing the *SPMS* gene exhibited more resistance to *P. viridiflava*. However, higher susceptibility was also evidenced in *spms* mutant (Gonzalez et al., 2011). In this regard, we found no evident enhanced susceptibility of *spms* and *acl5* mutants to *Pst* DC3000 infiltration (**Figure 31**).

Important to mention that infection of *Arabidopsis* with *P. viridiflava* results in Spm accumulation. In response to *Pst* DC3000, *Arabidopsis* wild type *Col-0* exhibited Put rather than Spm accumulation (**Figure 28**). Remarkable changes in Put content were also evidenced in *spms* and *acl5* mutants (**Figure 28**). The accumulation of precursor (Put) in *spms* and *acl5* suggests the main contribution of *SPMS* and *ACL5* to convert Spd to Spm and/or t-Spm during defense. With our observation, we suggest the possibility of a Put to Spm canalization in response to *Pst* DC3000.

Such canalization has been shown in response to drought stress (Alcázar et al., 2011). The lack of Spd accumulation in response to *Pst* DC3000 might be due to terminal oxidation of Spd (**Figure 29**).

In addition to biotic stresses, the involvement of Spm in abiotic responses has been studied. *Arabidopsis* plants with enhanced levels of Spm, exhibit higher tolerance to salt and drought stresses (Marco et al., 2011, 2019). The Spm-deficient mutants show hypersensitivity in response to salt treatment. However, this phenotype is rescued by exogenous application of Spm (Yamaguchi et al., 2006). Earlier, Huh et al., 2002 revealed that salt stress triggers PCD in *Arabidopsis* roots resulted by ion disequilibrium (Huh et al., 2002), and suppression of PCD, as an plant defensive response, diminishes salt tolerance in *Arabidopsis* (Bahieldin et al., 2016). This work is in agreement with a protective role of Spm during the salt stress response, which demonstrated by cell death triggered in *spms* mutant in response to salinity (**Figure 34**). According to our observation, (i) the absence of Spm enhances cell death during salinity (**Figure 34**), whilst (ii) high concentration of Spm triggers cell death (**Figure 27**). We suggest

a dual role for Spm, ROS scavenger at low dosages (physiological concentrations) and ROS producer at higher dosages.

New genes modulating polyamine metabolism

The study of natural variation has allowed to determine the genetics of plant developmental processes (Alonso-Blanco et al., 2009). Natural variation has been used to study secondary metabolism (Kliebenstein, 2009), plant development (Alonso-Blanco et al., 2005), pathogen resistance (Holub, 2007) and other quantitative traits. Here we used GWAS mapping in 204 *Arabidopsis* natural accessions to identify new genes involved in polyamine homeostasis. GWAS mapping identified a cluster of SAM- dependent methyltransferases (**Table 12**), which indicates a possible role for SAM metabolism in modulation of polyamine content. SAM as an essential molecule and universal methyl donor, also participates in metabolism pathways in plants, like the biosynthesis of polyamines, ethylene and nicotianamine (Sauter et al., 2013). The competition for SAM as a common substrate for ethylene and polyamine biosynthesis is a matter of controversy in the last decades. SAM is synthesized from methionine and ATP by the enzymic activity of SAMS (Binet et al., 2011). The involvement of *SAMS* gene family in several metabolic pathways such as ethylene, polyamines and plant cell signaling is demonstrated (Chen et al., 2016; Roje, 2006; Wang et al., 2016). However, the expression pattern of *SAMS* genes family varies in response to biotic and abiotic stresses. Enhanced expression level of tomato *SAMS* genes was evidenced in response to salinity (Sánchez-Aguayo et al., 2004). Soybean *SAMS* genes were downregulated in response to drought and flooding stresses (Wang et al., 2016). Moreover, a positive correlation among SAMDC and SAMS activity in response to abiotic stress was suggested. For instance, in response to cold stress, alfalfa (*Medicago sativa*) exhibited an association between *SAM1* gene expression and polyamine levels (Guo et al., 2014). Overexpression of *SAMS* gene in transgenic tobacco plants, enhanced *SAMDC* expression in response to salinity (Y. C. Qi et al., 2010). Despite of the *SAMS* contribution to polyamine metabolism in response to several stresses, we show that under non-stress conditions, compromised SAM synthetase does not modify polyamine metabolism (**Figure 38**).

Earlier studies showed that genes involved in SAM pathways (**Figure 37**) also play a critical role in stress responses. Among them, SAMDC is a key enzyme for higher polyamines synthesis. Downregulation of *SAMDC* in tobacco negatively affected salt stress tolerance

(Moschou et al., 2008). In contrary, *SAMDC* overexpression enhanced resistance to salinity (Ji et al., 2019). We provide evidence that under non-stress conditions, single *SAMDC* loss-of-function mutations do not affect polyamine metabolism, which suggests the occurrence of redundancy (**Figure 39**).

A positive role for *SAMDC* in biotic responses is also documented. Marco et al., 2014 demonstrated that *SAMDC1* overexpression enhanced *Arabidopsis* resistance to *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis* (Marco et al., 2014). The effect of *SAMDC* overexpression in resistance of *Arabidopsis* plants to powdery mildew caused by *Podosphaera xanthii* (*P. xanthii*) was also reported (Liu et al., 2014). Interestingly, our data indicates that *SAMDC1* expression is not required for polyamine metabolism in response to hemi-biotrophic *Pst* DC3000 (**Figure 40**). *samdc* mutants in response to *Pst* DC3000 also accumulate Put whereas no differences in Spd or Spm contents are observed (**Figure 40**). These results emphasize the tight regulation of Spd and Spm levels in *Arabidopsis*.

S*AMDC* loss-of-function mutations do not compromise resistance to *Pst* DC3000 (**Figure 42**). However, *SAMDC* silencing in tomato resulted in reduced resistance to fungal pathogen *Cladosporium fulvum* (Zhao et al., 2018).

In this work we also studied the interaction between polyamines and ethylene metabolism. by using ethylene overproducer (*eto1* and *eto3*) and *constitutive triple response (ctr1)* mutants. Kieber et al., 1993 reported that ethylene production was elevated up to 100-fold in *eto3* and 10-fold in *eto1* due to increased ACS activity (Kieber et al., 1993; Woeste et al., 1999). We show that higher ethylene biosynthesis (*eto3*) lead to enhanced Put content under non-stress conditions (**Figure 43**), which might be associated with a stress response to high ethylene. In any case, the high ethylene production was not accompanied by a reduction in polyamine contents.

The involvement of ethylene in defense has been shown (Argueso et al., 2007; Broekaert et al., 2006). For instance, in ETI triggered by *Pst avrRpt2*, enhanced ethylene biosynthesis, as a part of defense response was evidenced (Guan et al., 2015). We investigated the effect of ethylene competition in polyamine metabolism during the compatible interaction between *Arabidopsis* and *Pst* DC3000. We show that Put accumulates in *eto1* and *eto3* mutants in response to Pst DC3000, although with a lower response compared to the wild type and *ctr1* (**Figure 44**). Moreover, no differences in DAP levels were detected between the ethylene mutants and the wild type (**Figure 45**). Overall, our data argues against a competition for SAM between polyamine and ethylene biosynthesis pathways.

Conclusions

BibliographyC

1- Polyamine metabolism is affected by *Pseudomonas syringae* infection in *Arabidopsis thaliana*, thus being part of the metabolic response against bacterial infection.

2- The defense response triggered by the virulent *Pst* DC3000 strain induces Put accumulation and Spd depletion independently of SA, *EDS1, PAD4* and *NPR1*. Spd depletion might be due to its oxidation to aldehyde forms or back-conversion into Put.

3- During ETI triggered by *Pst AvrRpm1* and *Pst AvrRps4,* putrescine accumulates independently of SA, *EDS1, PAD4* and *NPR1*. Therefore, Put accumulation is a SA-pathway independent response. We also conclude that the alteration of polyamine metabolism is not different between CNL or TNL - triggered signaling.

4- Temperature-conditioned ETI activation in autoimmune hybrids results in Put accumulation. We also observe a competition between polyamine biosynthesis and autoimmunity for nitrogen resources.

5- The contribution of coronatine to putrescine accumulation is revealed by the lower levels of Put in plants inoculated with the coronatine deficient Pst DC3000 Cor^- strain. The response to coronatine may also underlie the common metabolic response observed between *Pst* DC3000 strains used in this study. However, coronatine does not explain the total increases in Put content.

6- Exogenously supplied Put does not trigger cell death, but it contributes to a faster cell death in response to flg22. We suggest that Put might amplify already activated PTI defense responses.

7- In contrast to Put, exogenously supplied Spm leads to cell death as revealed by TB staining and ion leakage assays. This response is independent of *EDS1* and SA. However, Spm does not lead to enhanced *Pst* DC3000 resistance in systemic leaves (SAR establishment) and the *spms* mutant is not compromised in disease resistance in response to *Pst* DC3000 infiltration. It remains to be determined whether Spm triggered cell death is related to defense activation.

8- GWAS mapping identified SAM-dependent methyl transferases in the natural variation of polyamine levels in *Arabidopsis*. However, individual mutation of SAM synthases and *SAMDC* genes family do not affect polyamine metabolism or disease resistance to *Pst* DC3000. The high redundancy in *SAMS* and *SAMDC* genes might mask the effect of SAM and dcSAM metabolism in polyamine contents and defense responses.

9- We find no obvious evidence for a competition for SAM between polyamine and ethylene biosynthesis. Rather, high levels of ethylene trigger Put accumulation under non-stress conditions.

Bibliography

Bibliography

Abreu, M. E., & Munné-Bosch, S. (2009). Salicylic acid deficiency in NahG transgenic lines and *sid2* mutants increases seed yield in the annual plant *Arabidopsis thaliana*. *Journal of Experimental Botany*, ***60***(4), 1261–1271.

Agrios, G. (2005). *Plant pathology*. Elsevire Academic Press.

Agrios, G. (2009). Plant pathogens and disease: general introduction. *Elsevier*.

Agurla, S., Gayatri, G., & Raghavendra, A. S. (2018). Polyamines increase nitric oxide and reactive oxygen species in guard cells of *Arabidopsis thaliana* during stomatal closure. *Protoplasma*, ***255***, 153–162.

Ahou, A., Martignago, D., Alabdallah, O., Tavazza, R., Stano, P., Macone, A., Pivato, M., Masi, A., Rambla, J. L., Vera-sirera, F., Angelini, R., Federico, R., & Tavladoraki, P. (2014). A plant spermine oxidase / dehydrogenase regulated by the proteasome and polyamines. *Journal of Experimental Botany*, ***65***(6), 1585–1603.

Akatsuka, H., Kawai, E., Omori, K., & Shibatani, T. (1995). The three genes lipB , lipC , and lipD involved in the extracellular secretion of the *Serratia marcescens* lipase which lacks an N-Terminal signal peptide. *Journal of Bacteriology*, ***177***(22), 6381–6389.

Alcázar, R., Altabella, T., Marco, F., Bortolotti, C., Reymond, M., Koncz, C., Carrasco, P., & Tiburcio, A. F. (2010). Polyamines : molecules with regulatory functions in plant abiotic stress tolerance. *Planta*, ***231*** (6), 1237–1249.

Alcázar, R., Bitrián, M., Bartels, D., Koncz, C., Altabella, T., & Tiburcio, A. F. (2011). Polyamine metabolic canalization in response to drought stress in *Arabidopsis* and the resurrection plant Craterostigma plantagineum. *Plant Signaling and Behavior*, ***6***(2), 243–250.

Alcázar, R., Bueno, M., & Tiburcio, A. F. (2020). Polyamines: small amines with large effects on plant abiotic stress tolerance. *Cells*, ***9***, 2373.

Alcázar, R., García, A. V., Kronholm, I., De Meaux, J., Koornneef, M., Parker, J. E., & Reymond, M. (2010). Natural variation at strubbelig receptor kinase 3 drives immune-triggered incompatibilities between *Arabidopsis thaliana* accessions. *Nature Genetics*, ***42***(12), 1135–1139.

Alcázar, R., García, A. V., Parker, J. E., & Reymond, M. (2009). Incremental steps toward incompatibility revealed by *Arabidopsis* epistatic interactions modulating salicylic acid pathway activation. *Proceedings of the National Academy of Sciences of the United States of America*, ***106***(1), 334–339.

Alcázar, R., Marco, F., Cuevas, J. C., Patron, M., Ferrando, A., Carrasco, P., Tiburcio, A. F., & Altabella, T. (2006). Involvement of polyamines in plant response to abiotic stress. *Biotechnol Lett*, ***28***, 1867–1876.

Alcázar, R., & Parker, J. E. (2011). The impact of temperature on balancing immune responsiveness and growth in *Arabidopsis*. *Trends in Plant Science*, ***16***(12), 666–675.

Alet, A. I., Sánchez, D. H., Ferrando, A., Tiburcio, A. F., Alcazar, R., Cuevas, J. C., Altabella,

T., Pico, F. M., Carrasco, P., Menéndez, A. B., & Ruiz, O. A. (2011). Homeostatic control of polyamine levels under long-term salt stress in *Arabidopsis*: Changes in putrescine content do not alleviate ionic toxicity. *Plant Signaling and Behavior*, ***6***(2), 237–242.

Alfano, J. R., & Collmer, A. (1997). The type III (Hrp) secretion pathway of plant pathogenic bacteria: Trafficking harpins, Avr proteins, and death. *Journal of Bacteriology*, ***179***(18), 5655–5662.

Alfano, J. R., & Collmer, A. (2004). Type III secretion system effector proteins: Double agents in bacterial disease and plant defense. *Annual Review of Phytopathology*, ***42***(1), 385–414.

Alonso-Blanco, C., Aarts, M. G. M., Bentsink, L., Keurentjes, J. J. B., Reymond, M., Vreugdenhil, D., & Koornneef, M. (2009). What has natural variation taught us about plant development, physiology, and adaptation? *Plant Cell*, ***21***, 1877–1896.

Alonso-Blanco, C., Mendez-Vigo, B., & Koornneef, M. (2005). From phenotypic to molecular polymorphisms involved in naturally occurring variation of plant development. *International Journal of Developmental Biology*, ***49***(5–6), 717–732.

Alvarez-Martinez, C. E., & Christie, P. J. (2009). Biological diversity of prokaryotic Type IV secretion systems. *Microbiology and Molecular Biology Reviews*, ***73***(4), 775–808.

Ames, B. N., Dubin, D. T., & Rosenthal, S. M. (1958). Presence of polyamines in certain bacterial viruses. *Science*, ***127***(3302), 814-5.

An, C., Wang, C., & Mou, Z. (2017). The *Arabidopsis* elongator complex is required for nonhost resistance against the bacterial pathogens *Xanthomonas citri* subsp. *citri* and *Pseudomonas syringae* pv. phaseolicola NPS3121. *New Phytologist*, ***214***(3), 1245–1259.

Andolfo, G., Sanseverino, W., Rombauts, S., Van de Peer, Y., Bradeen, J. M., Carputo, D., Frusciante, L., & Ercolano, M. R. (2013). Overview of tomato (*Solanum lycopersicum*) candidate pathogen recognition genes reveals important Solanum R locus dynamics. *New Phytologist*, ***197***(1), 223–237.

Angelini, R., Cona, A., Federico, R., Fincato, P., Tavladoraki, P., & Tisi, A. (2010). Plant physiology and biochemistry plant amine oxidases " on the move ": An update. *Plant Physiology et Biochemistry*, ***48***(7), 560–564.

Argueso, C. T., Hansen, M., & Kieber, J. J. (2007). Regulation of ethylene biosynthesis. *Journal of Plant Growth Regulation*, ***26***, 92–105.

Arnold, R., Jehl, A., & Rattei, T. (2010). Targeting effectors: the molecular recognition of Type III secreted proteins. *Microbes and Infection*, ***12***, 346–358.

Asthir, B., Spoor, W., & Duffus, C. M. (2004). Involvement of polyamines, diamine oxidase and polyamine oxidase in resistance of barley to *Blumeria graminis* f. sp. *hordei*. *Euphytica*, ***136***, 307–312.

AtanasovEvgeniev, K. (2019). Genetics of natural variation and environmental modulation of

immune-related hybrid incompatibilities in *Arabidopsis*. University of Barcelona.

Averyanov, A. (2009). Oxidative burst and plant disease resistance. *Front.Biosi*, ***1***, 142–152.

Axtell, M., & Staskawicz, B. J. (2003). Initiation of RPS2-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4. *Cell*, ***112***, 369–377.

Bachrach, U., Abzug, S., & Bekierkunst, A. (1967). Cytotoxic effect of oxidized Spermine on Ehrlich Ascites cells. *Biochimica et Biophysica*, ***134***, 174–181.

Bachrach, Uriel. (2010). The early history of polyamine research. *Plant Physiology et Biochemistry*, ***48***(7), 490–495.

Back, M. A., Haydock, P. P. J., & Jenkinson, P. (2002). Disease complexes involving plant parasitic nematodes and soilborne pathogens. *Plant Pathology*, ***51***(6), 683–697.

Backer, R., Naidoo, S., & Van den Berg, N. (2019). The Nonexpressor of pathogenesis-related gene 1 (NPR1) and related family: Mechanistic insights in plant disease resistance. *Frontiers in Plant Science*, ***10***, 1–21.

Bagni, N., & Fracassini, D. (1974). The role of polyamines as growth factors in higher plants and their mechanism of action. *In Plant Growth Substances; Proceedings of the International Conference*.

Bagni, N., & Tassoni, A. (2001). Biosynthesis , oxidation and conjugation of aliphatic polyamines in higher plants. *Amino Acids*, ***20***, 301–317.

Bahieldin, A., Alqarnia, D., Atef, A., Gadalla, N., Al-matarya, M., Edris, S., Kordy, M., Makki, R., A.Al-Doss, A., S.M.Sabir, J., H.Z.Mutwakil, M., & M.El-Domyatia, F. (2016). Suppression of PCD-related genes affects salt tolerance in *Arabidopsis*. *Comptes Rendus Biologies*, ***3339***(3–4), 105–114.

Baker, B., Zambryski, P., & Staskawicz, B. (1997). Signaling in plant-microbe interactions. *Science,* ***276***, 726-733.

Balint-Kurti, P. (2019). The plant hypersensitive response : concepts , control and consequences. *Molecular Plant Pathology*, ***20***(8), 1163–1178.

Baltrus, D. A., Nishimura, M. T., Romanchuk, A., Chang, J. H., Mukhtar, M. S., Cherkis, K., Roach, J., Grant, S. R., Jones, C. D., & Dangl, J. L. (2011). Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates. *Plos Pathog*, ***7***(7).

Bender, C. L., Alarcón-Chaidez, F., & Gross, D. C. (1999). *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Mirobiology and Molecular Biology Reviews*, ***63***(2), 266–292.

Bhandari, D. D., Lapin, D., Kracher, B., Von Born, P., Bautor, J., Niefind, K., & Parker, J. E. (2018). An EDS1 EP-domain surface mediating timely transcriptional reprogramming of immunity genes. *BioRxiv*, 362921.

Bhandari, D. D., Lapin, D., Kracher, B., Von Born, P., Bautor, J., Niefind, K., & Parker, J. E. (2019). An EDS1 heterodimer signalling surface enforces timely reprogramming of immunity genes in *Arabidopsis*. *Nature Communications*, ***10***, 772.

Bhattacharjee, S., Halane, M. K., Kim, S. H., & Gassmann, W. (2011). Pathogen effectors target *Arabidopsi*s EDS1 and alter its interactions with immune regulators. *Science*, ***334.***

Bhatty, M., Gomez, J. A. L., & Christie, P. J. (2013). The expanding bacterial Type IV secretion lexicon. *Res Microbiol*, ***164***(6).

Binet, R., Fernandez, R. E., Fisher, D. J., & Maurelli, A. T. (2011). Identification and characterization of the *Chlamydia trachomatis* L2 S-adenosylmethionine transporter. *MBio*, ***2***(3), 1–9.

Bingle, L. E., Bailey, C. M., & Pallen, M. J. (2008). Type VI secretion: a beginner's guide. *Current Opinion in Microbiology*, ***11***, 3–8.

Birkenbihl, R. P., Liu, S., & Somssich, I. E. (2017). Transcriptional events defining plant immune responses. *Current Opinion in Plant Biology*, ***38***, 1–9.

Birren, B., Fink, G., & Lander, E. (2002). Fungal Genome Initiative: White Paper developed by the Fungal Research Community. *Cambridge, MA:Whitehead Institue Center for Genome Research*.

Bisgrove, S. R., Simonich, M. T., Smith, N. M., Sattler, A., & Innes, R. W. (1994). A disease resistance gene in *Arabidopsis* with specificity for two different pathogen avirulence genes. *Plant Cell*, ***6***(7), 927–933.

Bitrián, M., Zarza, X., Altabella, T., Tiburcio, A. F., & Alcázar, R. (2012). Polyamines under abiotic stress: metabolic crossroads and hormonal crosstalks in plants. *Metabolites*, ***2***, 516–528.

Bleves, S., Viarre, V., Salacha, R., Michel, G. P. F., Filloux, A., & Voulhoux, R. (2010). Protein secretion systems in *Pseudomonas aeruginosa*: A wealth of pathogenic weapons. *International Journal of Medical Microbiology*, ***300***, 534–543.

Block, A., & Alfano, J. R. (2011). Plant targets for *Pseudomonas syringae* type III effectors : virulence targets or guarded decoys ? *Current Opinion in Microbiology*, ***14***(1), 39–46.

Bogdanove, A. J., Beer, S. V, Bonas, U., Boucher, C. A., Collmer, A., Coplin, D. L., Cornelis, G. R., Huang, H. C., Hutcheson, S. W., Panopoulos, N. J., & Gijsegem, F. Van. (1996). Unified nomenclature for broadly conserved *hrp* genes of phytopathogenic bacteria. *Molecular Microbiology*, ***20***, 681–683.

Boisson, B., Giglione, C., & Meinnel, T. (2003). Unexpected protein families including cell defense components feature in the N-myristoylome of a higher eukaryote. *Journal of Biological Chemistry*, ***278***(44), 43418–43429.

Boller, T. (1991). Ethylene in pathogenesis and disease resistance. In M. AK & S. JC (Eds.),

The plant hormone ethylene (293–314). CRC Press.

Boller, T., & Felix, G. (2009). A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual Review of Plant Biology*, ***60***, 379–407.

Bommarco, R., Kleijn, D., & Potts, S. G. (2013). Ecological intensification: Harnessing ecosystem services for food security. *Trends in Ecology and Evolution*, ***28***(4), 230–238.

Bonneau, L., Carré, M., & Martin-Tanguy, J. (1994). Polyamines and related enzymes in rice seeds differing in germination potential. *Plant Growth Regulation*, ***15***, 75–82.

Bordenave, C. D., Mendoza, C. G., Jiménez Bremont, J. F., Gárriz, A., & Rodríguez, A. A. (2019). Defining novel plant polyamine oxidase subfamilies through molecular modeling and sequence analysis. *BMC Evolutionary Biology*, ***19***.

Bouchereau, A., Aziz, A., & Larher, F. (1999). Polyamines and environmental challenges : recent development. *Plant Science*, ***140***, 103–125.

Boyer, F., Fichant, G., Berthod, J., Vandenbrouck, Y., & Attree, I. (2009). Dissecting the bacterial type VI secretion system by a genome wide in silico analysis: What can be learned from available microbial genomic resources? *BMC Genomics*, ***10***, 104.

Bozkurt, T. O., Schornack, S., Banfield, M. J., & Kamoun, S. (2012). Oomycetes , effectors , and all that jazz. *Current Opinion in Plant Biology*, ***15***, 483–492.

Brodersen, P., Malinovsky, F. G., Hématy, K., Newman, M. A., & Mundy, J. (2005). The role of salicylic acid in the induction of cell death in *Arabidopsis acd11*. *Plant Physiology*, ***138***,

Brodersen, P., Petersen, M., Nielsen, H. B., Zhu, S., Newman, M. A., Shokat, K. M., Rietz, S., Parker, J., & Mundy, J. (2006). *Arabidopsis* MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. *Plant Journal*, ***47***, 532–546.

Broekaert, W. F., Delauré, S. L., De Bolle, M. F. C., & Cammue, B. P. A. (2006). The role of ethylene in host-pathogen interactions. *Annual Review of Phytopathology*, ***44***, 393–416.

Broetto, F., Marchese, J., Leonardo, M., & Regina, M. (2005). Fungal elicitor-mediated changes in polyamine content, phenylalanine ammonia-lyase and peroxidase activities in bean cell culture. *Gen Appl Plant Physiol*, ***31***(3–4), 235–246.

Brooks, D. M., Hernández-Guzmán, G., Kloek, A. P., Alarcón-Chaidez, F., Sreedharan, A., Rangaswamy, V., Peñaloza-Vázquez, A., Bender, C. L., & Kunkel, B. N. (2004). Identification and characterization of a well-defined series of coronatine biosynthetic mutants of *Pseudomonas syringae* pv. tomato DC3000. *Molecular Plant-Microbe Interactions*, ***17***(2), 162–174.

Brown, J. K. M., & Tellier, A. (2011). Plant-parasite coevolution: bridging the gap between genetics and ecology. *Annual Review of Phytopathology*, ***49***(1), 345–367.

Buell, C. R., Joardar, V., Lindeberg, M., Selengut, J., Paulsen, I. T., Gwinn, M. L., Dodson, R. J., Deboy, R. T., Durkin, A. S., Kolonay, J. F., Madupu, R., Daugherty, S., Brinkac, L., Beanan, M. J., Haft, D. H., Nelson, W. C., Davidsen, T., Zafar, N., Zhou, L., Collmer, A. (2003). The complete genome sequence of the *Arabidopsis* and tomato pathogen *Pseudomonas syringae* pv. tomato DC3000. *Proc. Natl. Acad. Sci. USA*, ***100***(18), 10181–10186.

Buonaurio, R. (2008). Infection and plant defense responses during plant- bacterial interaction. *Plant-Microbe Interaction*, ***661***(2), 169–197.

Burkholder, W. H. (1948). Bacteria as plant pathogens. *Annu. Rev. Microbiol*, *2*(5), 389–412.

Büttner, D., & He, S. Y. (2009). Type III protein secretion in plant pathogenic bacteria. *Plant Physiology*, ***150***(4), 1656–1664.

Cai, Q., Zhang, J., Guo, C., & Al, E. (2006). Reviews of the physiological roles and molecular biology of polyamines in higher plants. *Fujian Educ. Coll*, ***7***, 118–124.

Cao, H., Bowling, S. A., Gordon, A. S., & Dong Xinnian. (1994). Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell*, ***6***(11), 1583–1592.

Cao, H., Glazebrook, J., Clarke, J. D., Volko, S., & Dong, X. (1997). The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell*, ***88***, 57–63.

Cascales, E. (2008). The type VI secretion toolkit. *EMBO Reports*, ***9***(8), 735–741.

Cervelli, M., Bianchi, M., Cona, A., Crosatti, C., Stanca, M., Angelini, R., Federico, R., & Mariottini, P. (2006). Barley polyamine oxidase isoforms 1 and 2 , a peculiar case of gene duplication. *The FEBS Journal*, ***273***, 3990–4002.

Cesari, S., Bernoux, M., Moncuquet, P., Kroj, T., & Dodds, P. N. (2014). A novel conserved mechanism for plant NLR protein pairs: The “integrated decoy” hypothesis. *Frontiers in Plant Science*, ***5***, 1–10.

Chandra-Shekara, A. C., Navarre, D. R., Kachroo, A., Kang, H. G., Klessig, D., & Kachroo, P. (2004). Signaling requirements and role of salicylic acid in HRT- and rrt-mediated resistance to *Turnip crinkle virus* in *Arabidopsis*. *Plant Journal*, ***40***(5), 647–659.

Chang, J. H., Desveaux, D., & Creason, A. L. (2014). The ABCs and 123s of bacterial secretion systems in plant pathogenesis. *Annual Review of Phytopathology*, ***52***, 317–345.

Chang, J. H., Urbach, J. M., Law, T. F., Arnold, L. W., Hu, A., Gombar, S., Grant, S. R., Ausubel, F. M., & Dangl, J. L. (2005). A high-throughput, near-saturating screen for type III effector genes from *Pseudomonas syringae*. *Proceedings of the National Academy of Sciences of the United States of America*, ***102***(7), 2549–2554.

Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. (2019). Polyamine Function in Plants : Metabolism , Regulation on Development , and Roles in Abiotic Stress Responses. *Frontiers in Plant Science*, ***9***, 1–13.

Chen, M., Chen, J., Fang, J., Guo, Z., & Lu, S. (2014). Down-regulation of S - adenosylmethionine decarboxylase genes results in reduced plant length , pollen viability , and abiotic stress tolerance. *Plant Cell Tiss Organ Cult*, ***116***, 311–322.

Chen, T., Li, W., Hu, X., Guo, J., Liu, A., & Zhang, B. (2015). A cotton MYB transcription factor , GbMYB5 , is positively involved in plant adaptive response to drought stress. *Plant and Cell Physiology*, ***56***(5), 917–929.

Chen, Y., Zou, T., & McCormick, S. (2016). S-adenosylmethionine synthetase 3 is important for pollen tube growth. *Plant Physiology*, ***172***, 244–253.

Chen, Y., Holmes, E. C., Rajniak, J., Kim, J., Tang, S., Fischer, C. R., Beth, M., & Sattely, E. S. (2018). N -hydroxy-pipecolic acid is a mobile metabolite that induces systemic disease resistance in *Arabidopsis*. *PNAS*, ***115***(21).

Chiang, Y. H., & Coaker, G. (2015). Effector Triggered Immunity: NLR immune perception and downstream defense responses. *Amercian Society of Plant Biologists*, ***13***.

Chisholm, S. T., Coaker, G., Day, B., & Staskawicz, B. J. (2006). Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell*, ***124***(4), 803–814.

Chitarra, G. S., Abee, T., Rombouts, F. M., Posthumus, M. A., & Dijksterhuis, J. (2004). Germination of *Penicillium paneum* conidia is regulated by 1-octen-3-ol, a volatile self-inhibitor. *Applied and Environmental Microbiology*, ***70***(5), 2823–2829.

Chung, E., Cunha, L., Wu, A., Gao, Z., Cherkis, K., Afzal, A. J., Mackey, D., & Dangl, J. L. (2011). Specific threonine phosphorylation of a host target by two unrelated type III effectors activates ahost innate immune receptor in plants. *Cell Host and Microbe*, ***9***, 125–136.

Clément, M. V., Ponton, A., & Pervaiz, S. (1998). Apoptosis induced by hydrogen peroxide is mediated by decreased superoxide anion concentration and reduction of intracellular milieu. *FEBS Letters*, ***440***, 13–18.

Cohen, S. S. (1998). A guide to the Polyamines. Oxford University.

Coleman, C. S., Hu, G., & Pegg, A. E. (2004). Putrescine biosynthesis in mammalian tissues. *Biochem Journal*, ***379***, 849–855.

Coll, N. S., Epple, P., & Dangl, J. L. (2011). Programmed cell death in the plant immune system. *Cell Death and Differentiation*, ***18***(8), 1247–1256.

Collins, N., Drake, J., Ayliffe, M., Sun, Q., Ellis, J., Hulbert, S., & Pryor, T. (1999). Molecular characterization of the maize Rp1-D rust resistance haplotype and its mutants. *Plant Cell*, ***11***, 1365–1376.

Cona, A., Rea, G., Angelini, R., Federico, R., & Tavladoraki, P. (2006). Functions of amine oxidases in plant development and defence. *Trends in Plant Science*, ***11***(2).

Cowley, T., & Walters, D. R. (2002). Polyamine metabolism in barley reacting

hypersensitively to the powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. *Plant Cell and Environment*, ***25***, 461–468.

Crute, I., Beynon, J., Dangl, J., Huolub, E., Mauch-Mani, B., Slusarenko, A., Staskawicz, B. ., & Ausubel, F. (1994). Microbial pathogenesis of *Arabidopsis*. In E. M. Meyerowitz & C. R. Somerville (Eds.), *Arabidopsis* (705–747). Cold Spring Harbor Laboratory Press.

Cui, H., Gobbato, E., Kracher, B., Qiu, J., Bautor, J., & Parker, J. E. (2017). A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in *Arabidopsis* immunity. *New Phytologist*, ***213***(4), 1802–1817.

Cui, H., Tsuda, K., & Parker, J. E. (2015). Effector-triggered immunity: From pathogen perception to robust defense. *Annual Review of Plant Biology*, ***66***, 487–511.

Culver, J. N., & Padmanabhan, M. S. (2007). Virus-induced disease: altering host physiology one interaction at a time. *Annual Review of Phytopathology*, ***45***(1), 221–243.

Cunnac, S., Lindeberg, M., & Collmer, A. (2009). *Pseudomonas syringae* type III secretion system effectors : repertoires in search of functions. *Current Opinion in Microbiology*, ***12***, 53–60.

Dangl, J. L., Horvath, D. M., & Staskawich, B. J. (2013). Pivoting the plant immune system. *Science*, ***341***, 745–751.

Dangl, J. L., & Jones, J. D. G. (2001). Plant pathogens and integrated defence responses to infection. *Nature*, ***411***.

Dangl, J. L., Ritter, C., Gibbon, M. J., Mur, L. A. J., Wood, J. R., Goss, S., Mansfield, J., Taylor, J. D., & Vivian, A. (1992). Functional homologs of the *Arabidopsis* RPM1 disease resistance gene in bean and pea. *Plant Cell*, ***4***(11), 1359–1369.

Day, B., Dahlbeck, D., & Staskawicz, B. J. (2006). NDR1 interaction with RIN4 mediates the differential activation of multiple disease resistance pathways in *Arabidopsis*. *Plant Cell*, ***18***(10), 2782–2791.

Delledonne, M., Zeier, J., Marocco, A., & Lamb, C. (2001). Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proceedings of the National Academy of Sciences of the United States of America*, ***98***(23), 13454–13459.

Deslandes, L., Olivier, J., Peeters, N., Feng, D. X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S., & 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. *Proc Natl Acad Sci*, ***100***(13), 8024–8029.

Després, C., DeLong, C., Glaze, S., Liu, E., & Fobert, P. R. (2000). The *Arabidopsis* NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. *Plant Cell*, ***12***(2), 279–290.

Desvaux, M., Parham, N. J., Scott-Tucker, A., & Henderson, I. R. (2004). The general secretory pathway: A general misnomer? *Trends in Microbiology*, ***12***(7), 306–309.

Dewdney, J., Reuber, T. L., Wildermuth, M. C., Devoto, A., Cui, J., Stutius, L. M., Drummond, E. P., & Ausubel, F. M. (2000). Three unique mutants of *Arabidopsis* identify eds loci required for limiting growth of a biotrophic fungal pathogen. *The Plant Journal*, ***24***(2), 205–218.

Dick, M. (2001). Straminipilous Fungi. Springer Netherlands.

Dirix, G., Monsieurs, P., Dombrecht, B., Daniels, R., Marchal, K., Vanderleyden, J., & Michiels, J. (2004). Peptide signal molecules and bacteriocins in Gram-negative bacteria: A genome-wide in silico screening for peptides containing a double-glycine leader sequence and their cognate transporters. *Peptides*, ***25***, 1425–1440.

Dixon, M. S., Jones, D. A., Keddie, J. S., Thomas, C. M., Harrison, K., & Jones, J. D. G. (1996). The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. *Cell*, ***84***, 451–459.

Dodds, P. N., & Rathjen, J. P. (2010). Plant immunity: Towards an integrated view of plant pathogen interactions. *Nature Reviews Genetics*, ***11***, 539–548.

Dong, X., Mindrinos, M., Davis, K. R., & Ausubel, F. M. (1991). Induction of *Arabidopsis* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. *Plant Cell*, ***3***(1), 61–72.

Doyle, S. M., Diamond, M., & McCabe, P. F. (2010). Chloroplast and reactive oxygen species involvement in apoptotic-like programmed cell death in *Arabidopsis* suspension cultures. *Journal of Experimental Botany*, ***61***(2), 473–482.

Dropkin, V. H. (1955). The relations between nematodes and plants. *Experimental Parasitology*, ***4***(3), 282–322.

Duong, F., Lazdunski, A., Carni, B., & Murgier, M. (1992). Sequence of a cluster of genes controlling synthesis and secretion of alkaline protease in *Pseudomonas aeruginosa*: relationships to other secretory pathways. *Gene*, ***121***(1), 47–54.

Dutt, M., Barthe, G., Irey, M., & Grosser, J. (2015). Transgenic citrus expressing an *Arabidopsis NPR1* gene exhibit enhanced resistance against Huanglongbing (HLB; Citrus greening). *PLoS ONE*, ***10***(9), 1–17.

Eitas, T. K., Nimchuk, Z. L., & Dangl, J. L. (2008). *Arabidopsis* TAO1 is a TIR-NB-LRR protein that contributes to disease resistance induced by the *Pseudomonas syringae* effector AvrB. *Proceedings of the National Academy of Sciences of the United States of America*, ***105***(17), 6475–6480.

El Kasmi, F., Chung, E. H., Anderson, R. G., Li, J., Wan, L., Eitas, T. K., Gao, Z., & Dangl, J. L. (2017). Signaling from the plasma-membrane localized plant immune receptor RPM1 requires self-association of the full-length protein. *Proceedings of the National Academy of*

Sciences of the United States of America, ***114***(35), E7385–E7394.

El Oirdi, M., El Rahman, T. A., Rigano, L., El Hadrami, A., Rodriguez, M. C., Daayf, F., Vojnov, A., & Bouarab, K. (2011). *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in Tomato. *Plant Cell*, ***23***(6), 2405–2421.

Elejalde-palmett, C., Bernonville, T. D. De, Glevarec, G., Pichon, O., Papon, N., Courdavault, V., St-pierre, B., Giglioli-guivarc, N., Lanoue, A., & Besseau, S. (2015). Characterization of a spermidine hydroxycinnamoyltransferase in *Malus domestica* highlights the evolutionary conservation of trihydroxycinnamoyl spermidines in pollen coat of core Eudicotyledons. *Journal of Experimental Botany*, ***66***(22), 7271–7285.

Falk, A., Feys, B. J., Frost, L. N., Jones, J. D. G., Daniels, M. J., & Parker, J. (1999). EDS1 , an essential component of R gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Plant Biology*, ***96*,** 3292–3297.

Fawke, S., Doumane, M., & Schornack, S. (2015). Oomycete Interactions with Plants: Infection Strategies and Resistance Principles. *Microbiology and Molecular Biology Reviews*, ***79***, 263–280.

Felix, G., Duran, J. D., Volko, S., & Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant Journal*, ***18***(3), 265–276.

Fellenberg, C., Böttcher, C., & Vogt, T. (2009). Phytochemistry Phenylpropanoid polyamine conjugate biosynthesis in *Arabidopsis thaliana* flower buds. *Phytochemistry*, ***70***(11–12), 1392–1400.

Feng, F., & Zhou, J. M. (2012). Plant-bacterial pathogen interactions mediated by type III effectors. *Current Opinion in Plant Biology*, ***15***(4), 469–476.

Fernández-Crespo, E., Scalschi, L., Llorens, E., García-Agustín, P., & Camañes, G. (2015). $NH4^+$ protects tomato plants against *Pseudomonas syringae* by activation of systemic acquired acclimation. *Journal of Experimental Botany*, ***66***(21), 6777–6790.

Feys, B. J., Moisan, L. J., Newman, M. A., & Parker, J. E. (2001). Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4. *EMBO Journal*, ***20***(19), 5400–5411.

Feys, B. J., Wiermer, M., Bhat, R. A., Moisan, L. J., Medina-Escobar, N., Neu, C., Cabral, A., & Parker, J. E. (2005). *Arabidopsis* Senescence-Associated Gene101 stabilizes and signals within an Enhanced Disease Susceptibility 1 complex in plant innate immunity. *Plant Cell*, ***17***(9), 2601–2613.

Fincato, P., Moschou, P. N., Spedaletti, V., Tavazza, R., Angelini, R., Federico, R., Roubelakis-angelakis, K. A., & Tavladoraki, P. (2011). Functional diversity inside the *Arabidopsis* polyamine oxidase gene family. *Journal of Experimental Botany*, ***62***(3), 1155–1168.

Foster, S. A., & Walters, D. R. (1992). Polyamine concentrations and activities of ornithine

and arginine decarboxylase in wheat iInfected with the stem rust fungus. *Journal of Plant Physiology*, ***140***, 134–136.

Franceschetti, M., Fornalè, S., Tassoni, A., Zuccherelli, K., Mayer, M. J., & Bagni, N. (2004). Effects of spermidine synthase overexpression on polyamine biosynthetic pathway in tobacco plants. *Journal of Plant Physiology*, ***161***, 989–1001.

Franceschetti, M., Hanfrey, C., Scaramagli, S., Torrigiani, P., Bagni, N., Burtin, D., & Michael, A. J. (2001). Characterization of monocot and dicot plant S-adenosyl-L-methionine decarboxylase gene families including identification in the mRNA of a highly conserved pair of upstream overlapping open reading frames. *Biochem Journal*, ***353***, 403–409.

Freitasa, V. S., Mirandab, R. de S., Costac, J. H., Oliveirac, D. F. de, Paulac, S. de O., Migueld, E. de C., Freired, R. S., Priscoc, J. T., & Gomes-Filho, E. (2018). Ethylene triggers salt tolerance in maize genotypes by modulating polyamine catabolism enzymes associated with H_2O_2 production. *Environmental and Experimental Botany*, ***145***, 75–86.

Fu, X., Chen, C., Wang, Y., Liu, J., & Moriguchi, T. (2011). Ectopic expression of MdSPDS1 in sweet orange (*Citrus sinensis* Osbeck) reduces canker susceptibility : involvement of H_2O_2 production and transcriptional alteration. *BMC Plant Biology*, ***11***, 55.

Fu, Z. Q., & Dong, X. (2013). Systemic acquired resistance: Turning local infection into global defense. *Annual Review of Plant Biology*, ***64***, 839–863.

Gallois, J. L., Moury, B., & German-Retana, S. (2018). Role of the genetic background in resistance to plant viruses. *International Journal of Molecular Sciences*, ***19***(10), 1–20.

Galston, A. W., & Sawhney, R. K. (1990). Polyamines in plant physiology. *Plant Physiol.*, ***94*****,** 406–410.

Gantner, J., Ordon, J., Kretschmer, C., Guerois, R., & Stuttmann, J. (2019). An EDS1-SAG101 complex is essential for tnl-mediated immunity in *Nicotiana benthamiana*. *Plant Cell*, ***31***(10), 2456–2474.

Gao, Q. M., Zhu, S., Kachroo, P., & Kachroo, A. (2015). Signal regulators of systemic acquired resistance. *Frontiers in Plant Science*, ***6***, 228.

Gao, Z., Chung, E. H., Eitas, T. K., & Dangl, J. L. (2011). Plant intracellular innate immune receptor resistance to *Pseudomonas syringae* pv. *maculicola 1* (RPM1) is activated at, and functions on, the plasma membrane. *Proceedings of the National Academy of Sciences of the United States of America*, ***108***(18), 7619–7624.

García-Heredia, J. M., Hervás, M., Rosa, M. A. D. la, & Navarro, J. A. (2008). Acetylsalicylic acid induces programmed cell death in *Arabidopsis* cell cultures. *Planta*, ***228***(1), 89–97.

García, A. V., Blanvillain-Baufumé, S., Huibers, R. P., Wiermer, M., Li, G., Gobbato, E., Rietz, S., & Parker, J. E. (2010). Balanced nuclear and cytoplasmic activities of EDS1 are required for a complete plant innate immune response. *PLoS Pathogens*, ***6***(7), 1–15.

Garcion, C., Lohmann, A., Lamodière, E., Catinot, J., Buchala, A., Doermann, P., & Métraux,

J. P. (2008). Characterization and biological function of the Isochorismate Synthase2 gene of *Arabidopsis*. *Plant Physiology*, ***147***, 1279–1287.

Gardiner, D. M., Kazan, K., Praud, S., Torney, F. J., Rusu, A., & Manners, J. M. (2010). Early activation of wheat polyamine biosynthesis during Fusarium head blight implicates putrescine as an inducer of trichothecene mycotoxin production. *BMC Plant Biology*, ***10***, 289.

Garzón, L. N., Oliveros, O. A., Rosen, B., Ligarreto, G. A., Cook, D. R., & Blair, M. W. (2013). Isolation and characterization of nucleotide-binding site resistance gene homologues in common bean (*Phaseolus vulgaris*). *Phytopathology*, ***103***(2), 156–168.

Ge, C., Cui, X., Wang, Y., Hu, Y., Fu, Z., Zhang, D., Cheng, Z., & Li, J. (2006). BUD2 , encoding an S-adenosylmethionine decarboxylase , is required for *Arabidopsis* growth and development. *Nature*, ***16***, 446–456.

Gehrig, H., Schüssler, A., & Kluge, M. (1996). *Geosiphon pyriforme*, a fungus forming endocytobiosis with Nostoc (Cyanobacteria), is an ancestral member of the Glomales: Evidence by SSU rRNA analysis. *J. Mol. Evol*, ***43***, 71–81.

Geng, X., Cheng, J., Gangadharan, A., & Mackey, D. (2012). The coronatine toxin of *Pseudomonas syringae* is a multifunctional suppressor of *Arabidopsis* defense. *The Plant Cell*, ***24***, 4763–4774.

Ghoshal, B., & Sanfaçon, H. (2015). Symptom recovery in virus-infected plants: Revisiting the role of RNA silencing mechanisms. *Virology*, ***479–480***, 167–179.

Ghuge, S. A., Tisi, A., Carucci, A., Rodrigues-pousada, R. A., Franchi, S., Tavladoraki, P., Angelini, R., & Cona, A. (2015). Cell Wall Amine Oxidases: new players in root xylem differentiation under stress conditions. *Plants*, ***4***, 489–504.

Gimenez, E., Salinas, M., & Manzano-Agugliaro, F. (2018). Worldwide research on plant defense against biotic stresses as improvement for sustainable agriculture. *Sustainability (Switzerland)*, ***10***(2), 1–19.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, ***43***(1), 205–227.

Glazebrook, J., Chen, W., Estes, B., Chang, H., Nawrath, C., & Me, J. (2003). Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. *The Plant Journal*, ***34*****,** 217–228.

Glazebrook, J., Rogers, E. E., & Ausubel, F. M. (1996). Isolation of new *Arabidopsis* mutants with enhanced disease susceptibility to *Pseudomonas syringae* by direct screening. *Genetics*, ***149***(2), 537–548.

Glazebrook, J., Zook, M., Mert, I. F., Kagan, I., Rogers, E. E., Crute, I. R., Holub, E. B., Hammerschmidt, R., & Ausubelt, F. M. (1997). Phytoalexin-deficient mutants of *Arabidopsis* reveal that PAD4 encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. *Genetics*, ***146***, 381–392.

Gomez-Jimenez, M. C., Paredes, M. A., Gallardo, M., Fernandez-Garcia, N., Olmos, E., & Sanchez-Calle, I. M. (2010). Tissue-specific expression of olive S-adenosyl methionine decarboxylase and spermidine synthase genes and polyamine metabolism during flower opening and early fruit development. *Planta*, ***232***, 629–647.

Gong, B., Li, X., Vandenlangenberg, K. M., Wen, D., Sun, S., Wei, M., Li, Y., Yang, F., Shi, Q., & Wang, X. (2014). Overexpression of S-adenosyl-l-methionine synthetase increased tomato tolerance to alkali stress through polyamine metabolism. *Plant Biotechnology Journal*, ***12***, 694–708.

González-Fernández, R., Prats, E., & Jorrín-Novo, J. V. (2010). Proteomics of plant pathogenic fungi. *Journal of Biomedicine and Biotechnology*, ***2010***.

Gonzalez, M. E., Marco, F., Minguet, E. G., Carrasco-Sorli, P., Blazquez, M. A., Carbonell, J., Ruiz, O. A., & Pieckenstain, F. L. (2011). Perturbation of spermine synthase gene expression and transcript profiling provide new insights on the role of the tetraamine Spermine in *Arabidopsis* defense against *Pseudomonas viridiflava*. *Plant Physiology*, ***156***, 2266–2277.

González, R., Butković, A., Rivarez, M. P. S., & Elena, S. F. (2020). Natural variation in *Arabidopsis thaliana* rosette area unveils new genes involved in plant development. *Scientific Reports*, ***10***.

Grant, M., Godiard, L., Straube, E., & Ashfield, T. (1995). Structure of the *Arabidopsis* RPM1 gene enabling dual specificity disease resistance. *Scinece*, ***269***(5225), 843–846.

Greenland, A ., & Lewis, D. (1984). Amines in barley leaves infected by brown rust and their possible relevance to formation of "Green Islands." *New Phytol*, ***96***, 283–291.

Groppa, M. D., & Benavides, M. P. (2008). Polyamines and abiotic stress : recent advances. *Amino Acids*, ***34***, 35–45.

Groß, F., Rudolf, E., Thiele, B., Durner, J., & Astier, J. (2017). Copper amine oxidase 8 regulates arginine-dependent nitric oxide production in *Arabidopsis thaliana*. *Journal of Experimental Botany*, ***68***(9), 2149–2162.

Guan, R., Su, J., Meng, X., Li, S., Liu, Y., Xu, J., & Zhang, S. (2015). Multilayered regulation of ethylene induction plays a positive role in *Arabidopsis* resistance against *Pseudomonas syringae*. *Plant Physiology*, ***169***, 299–312.

Guo, M., Tian, F., Wamboldt, Y., & Alfano, J. R. (2009). The majority of the type III effector inventory of *Pseudomonas syringae* pv. tomato DC3000 can suppress plant immunity. *Mol Plant Microbe Interact*, ***22***(9), 1069–1080.

Guo, Z., Tan, J., Zhuo, C., Wang, C., Xiang, B., & Wang, Z. (2014). Abscisic acid, H_2O_2 and nitric oxide interactions mediated cold-induced S-adenosylmethionine synthetase in *Medicago sativa* subsp. Falcata that confers cold tolerance through up-regulating polyamine oxidation. *Plant Biotechnology Journal*, ***12***, 601–612.

Hagel, J. M., & Facchini, P. J. (2005). Elevated tyrosine decarboxylase and tyramine

hydroxycinnamoyltransferase levels increase wound-induced tyramine-derived hydroxycinnamic acid amide accumulation in transgenic tobacco leaves. *Planta*, ***221***, 904–914.

Halane, M. K., Kim, S. H., Spears, B. J., Garner, C. M., Rogan, C. J., Okafor, E. C., Su, J., Bhattacharjee, S., & Gassmann, W. (2018). The bacterial type III-secreted protein AvrRps4 is a bipartite effector. *PLoS Pathogens*, ***14***, 1–19.

Hamasaki-katagiri, N., Katagiri, Y., White, C., & Tabor, H. (1998). Spermine is not essential for growth of *Saccharomyces cerevisiae*: identification of the SPE4 gene (spermine synthase) and characterization of a *spe4* deletion mutant. *Gene*, ***210***, 195–201.

Handa, A. K., Fatima, T., & Mattoo, A. K. (2018). Polyamines : bio-molecules with diverse functions in plant and human health and disease. *Frontiers in Plant Science*, ***6***, 1–18.

Hanfrey, C., Sommer, S., Mayer, M. J., Burtin, D., & Michael, A. J. (2001). *Arabidopsis* polyamine biosynthesis : absence of ornithine decarboxylase and the mechanism of arginine decarboxylase activity. *The Plant Journal*, ***27***(6), 551–560.

Hanzawa, Y., Imai, A., Michael, A. J., Komeda, Y., & Takahashi, T. (2002). Characterization of the spermidine synthase-related gene family in *Arabidopsis thaliana*. *FEBS Letters*, ***527***, 176–180.

Hanzawa, Y., Takahashi, T., & Komeda, Y. (1997). ACL5: An *Arabidopsis* gene required for internodal elongation after flowering. *Plant Journal*, ***12***(4), 863–874.

Hao, Y., Zhang, Z., Kitashiba, H., Honda, C., Ubi, B., Kita, M., & Moriguchi, T. (2005). Molecular cloning and functional characterization of two apple S -adenosylmethionine decarboxylase genes and their different expression in fruit development , cell growth and stress responses. *Gene*, ***350***, 41–50.

Harpaz-Saad, S., Yoon, G. M., Mattoo, A. K., & Kieber, J. J. (2012). The formation of ACC and competition between polyamines and ethylene for SAM. *Annual Plant Reviews*, ***44***, 53–81.

Hassannejad, S., Bernard, F., Mirzajani, F., & Gholami, M. (2012). SA improvement of hyperhydricity reversion in *Thymus daenensis* shoots culture may be associated with polyamines changes. *Plant Physiology and Biochemistry*, ***51***, 40–46.

Hatsugai, N., Igarashi, D., Mase, K., Lu, Y., Tsuda, Y., Chakravarthy, S., Wei, H., Foley, J. W., Collmer, A., Glazebrook, J., & Katagiri, F. (2017). A plant effector-triggered immunity signaling sector is inhibited by pattern-triggered immunity. *The EMBO Journal*, ***36***(18), 2758–2769.

Hatsugai, N., Iwasaki, S., Tamura, K., Kondo, M., Fuji, K., Ogasawara, K., Nishimura, M., & Hara-Nishimura, I. (2009). A novel membrane fusion-mediated plant immunity against bacterial pathogens. *Genes and Development*, ***23***, 2496–2506.

Havelange, A., Lejeune, P., Bemier, G., Kaur-Sawhney, R., & Galston, A. . (1996). Putrescine export from leaves in relation to floral transition in *Sinapis alba*. *Physiol.Plant*, ***96***, 59–65.

Hayes, C. S., Aoki, S. K., & Low, D. A. (2010). Bacterial contact-dependent delivery systems. *Annual Review of Genetics*, ***44***, 71–90.

Hazarika, P., & Rajam, M. V. (2011). Biotic and abiotic stress tolerance in transgenic tomatoes by constitutive expression of S-adenosylmethionine decarboxylase gene. *Physiology and Molecular Biology of Plants*, ***17***(2), 115–128.

He, S. Y. (1998). Type III protein secretion systems in plant and animal pathogenic bacteria. *Annual Review of Phytopathology*, ***36***(1), 363–392.

Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L., & Hedges, S. B. (2001). Molecular evidence for the early colonization of land by fungi and plants. *Science*, ***293***(5532), 1129–1133.

Heidari, P., Mazloomi, F., Nussbaumer, T., & Barcaccia, G. (2020). Insights into the SAM synthetase gene family and its roles in tomato seedlings under abiotic stresses and hormone treatments. *Plants*, ***9***, 586.

Heidrich, K., Wirthmueller, L., Tasset, C., Pouzet, C., Deslandes, L., & Parker, J. E. (2011). *Arabidopsis* EDS1 connects pathogen effector recognition to cell compartment-specific immune responses. *Science*, ***334***(6061), 1401–1404.

Herlihy, J., Ludwig, N. R., Van den Ackerveken, G., & McDowell, J. M. (2019). Oomycetes used in *Arabidopsis* research. *The Arabidopsis Book* (Vol. 17, pp. 1–26). Amercian Society of Plant Biologists.

Hernan Garcia-Ruiz. (2019). **乳鼠心肌提取** HHS Public Access. *Hhs Public Access*, ***123***, 40–43.

Hernández, J. A., Diaz-Vivancos, P., Barba-Espín, G., & Clemente-Moreno, M. J. (2017). On the role of salicylic acid in plant responses to environmental stresses. In R. Nazar, N. Iqbal, & N. A. Khan (Eds.), *Salicylic Acid: A Multifaceted Hormone* (pp. 17–34). Springer Nature.

Hinsch, M., & Staskawicz, B. (1996). Identification of a new *Arabidopsis* disease resistance locus, RPS4, and cloning of the corresponding avirulence Gene, *avrRPS4*, from *Pseudomonas syringae* pv. *pisi*. *MPMI*, ***9***(1), 55–61.

Hofberger, J. A., Zhou, B., Tang, H., Jones, J. D. G., & Schranz, M. E. (2014). A novel approach for multi-domain and multi-gene family identification provides insights into evolutionary dynamics of disease resistance genes in core eudicot plants. *BMC Genomics*, ***15***, 966.

Holub, E. B. (2007). Natural variation in innate immunity of a pioneer species. *Current Opinion in Plant Biology*, ***10***, 415–424.

Hooper, D. U., Chapin, F. S., EwelL, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J. H., Horbach, R., Navarro-Quesada, A. R., Knogge, W., & Deising, H. B. (2011). When and how to kill a plant cell: Infection strategies of plant pathogenic fungi. *Journal of Plant Physiology*, ***168***(1), 51–62.

Horvath, H., Rostoks, N., Brueggeman, R., Steffenson, B., Von Wettstein, D., & Kleinhofs, A. (2003). Genetically engineered stem rust resistance in barley using the *Rpg1* gene. *Proceedings of the National Academy of Sciences of the United States of America*, ***100***(1), 364–369.

Huang, Y. S., Horton, M., Vilhjálmsson, B. J., Seren, Ü., Meng, D., Meyer, C., Amer, M. A., Borevitz, J. O., Bergelson, J., & Nordborg, M. (2011). Analysis and visualization of *Arabidopsis thaliana* GWAS using web 2.0 technologies. *Database*, ***2011***.

Huh, G. H., Damsz, B., Matsumoto, T. K., Reddy, M. P., Rus, A. M., Ibeas, J. I., Narasimhan, M. L., Bressan, R. A., & Hasegawa, P. M. (2002). Salt causes ion disequilibrium-induced programmed cell death in yeast and plants. *Plant Journal*, ***29***(5), 649–659.

Huh, S. U., Cevik, V., Ding, P., Duxbury, Z., Ma, Y., Tomlinson, L., Sarris, P. F., & Jones, J. D. G. (2017). Protein-protein interactions in the RPS4/RRS1 immune receptor complex. *PLoS Pathogens*, ***13***(5), 1–22.

Hulbert, S. H. (1997). Structure and evolution of the *rp1* complex conferring rust resistance in maize. *Annual Review of Phytopathology*, ***35***, 293–310.

Hulbert, S. H., Webb, C. A., Smith, S. M., & Sun, Q. (2001). Resistance gene complexes: evolution and utilization. *Annual Review of Phytopathology*, ***39***, 285–312.

Hurni, S., Scheuermann, D., Krattinger, S. G., Kessel, B., Wicker, T., Herren, G., Fitze, M. N., Breen, J., Presterl, T., Ouzunova, M., & Keller, B. (2015). The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proceedings of the National Academy of Sciences of the United States of America*, ***112***(28), 8780–8785.

Hyodo, H., & Tanaka, K. (1986). Inhibition of 1-aminocyclopropane-l-carboxylic acid synthase activity by pPolyamines, Their related compounds and metabolites of S-adenosylmethionine. *Plant & Cell Physiology*, ***23***(3), 391–398.

Icekson, I., Goldlust, A., & Apelbaum, A. (1985). Influence of ethylene on S-adenosylmethionine decarboxylase activity in etiolated pea seedlings. *Journal of Plant Physiology*, ***119***(4), 335–345.

Illingworth, C., Mayer, M. J., Elliott, K., Hanfrey, C., Walton, N. J., & Michael, A. J. (2003). The diverse bacterial origins of the *Arabidopsis* polyamine biosynthetic pathway. *FEBS Letters*, ***549***, 26–30.

Imai, A., Matsuyama, T., Hanzawa, Y., Akiyama, T., Tamaoki, M., Saji, H., Shirano, Y., Kato, T., Hayashi, H., Shibata, D., Tabata, S., Komeda, Y., & Takahashi, T. (2004). Spermidine synthase genes are essential for survival of *Arabidopsis*. *Plant Physiology*, ***135***, 1565–1573.

Imran, Q. M., & Yun, B. W. (2020). Pathogen-induced defense strategies in plants. *Journal of Crop Science and Biotechnology*, ***23***(2), 97–105.

Ivanov, I. P., Atkins, J. F., & Michael, A. J. (2010). A profusion of upstream open reading frame mechanisms in polyamine-responsive translational regulation. *Nucleic Acids Research*, ***38***(2), 353–359.

Jacob, F., Vernaldi, S., & Maekawa, T. (2013). Evolution and conservation of plant NLR functions. *Frontiers in Plant Scienc*, ***4***, 1–16.

Jain, A., Sarsaiya, S., Wu, Q., Lu, Y., & Shi, J. (2019). A review of plant leaf fungal diseases and its environment speciation. *Bioengineered*, ***10***(1), 409–424.

Jang, S. J., Wi, S. J., Choi, Y. J., An, G., & Park, K. Y. (2012). Increased polyamine biosynthesis enhances stress tolerance by preventing the accumulation of reactive oxygen species: T-DNA mutational analysis of *Oryza sativa* lysine decarboxylase-like protein 1. *Molecules and Cells*, ***34***, 251–262.

Jani, A. J., & Cotter, P. A. (2010). Type VI Secretion: Not just for pathogenesis anymore. *Cell Host and Microbe*, ***8***(1), 2–6.

Janowitz, T., Kneifel, H., & Piotrowski, M. (2003). Identification and characterization of plant agmatine iminohydrolase , the last missing link in polyamine biosynthesis of plants. *FEBS Letters*, ***544***, 258–261.

Ji, M., Wang, K., Wang, L., Chen, S., Li, H., Ma, C., & Wang, Y. (2019). Overexpression of a S-Adenosylmethionine decarboxylase from sugar beet M14 increased *Araidopsis* salt tolerance. *International Journal of Molecular Sciences*, ***20***, 1990.

Jiménez-bremont, J. F., Marina, M., Guerrero-González, M. D. L., Rossi, F. R., Sánchez-rangel, D., Rodríguez-kessler, M., Ruiz, O. A., & Gárriz, A. (2014). Physiological and molecular implications of plant polyamine metabolism during biotic interactions. *Frontiers in Plant Science*, ***5***, 95, 1-14.

Jirage, D., Tootle, T. L., Reuber, T. L., Frosts, L. N., Feys, B. J., Parker, J. E., Ausubel, F. M., & Glazebrook, J. (1999). *Arabidopsis thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. *Proceedings of the National Academy of Sciences of the United States of America*, ***96***(23), 13583–13588.

Johal, G. S., & Briggs, S. P. (1992). Reductase activity encoded by the *HM1* disease resistance gene in maize. *Science*, ***258***(5084), 985–987.

Jones, D. A., Thomas, C. M., Hammond-Kosack, K. E., Balint-Kurti, P. J., & Jones, J. D. G. (1994). Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science*, *266*, 789–793.

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, ***444***(7117), 323–329.

Jones, J. T., Haegeman, A., Danchin, E. G. J., Gaur, H. S., Helder, J., Jones, M. G. K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J. E., Wesemael, W. M. L., & Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, ***14***(9), 946–961.

Jumtee, K., Bamba, T., Okazawa, A., Fukusaki, E., & Kobayashi, A. (2008). Integrated metabolite and gene expression profiling revealing phytochrome A regulation of polyamine biosynthesis of *Arabidopsis thaliana*. *Journal of Experimental Botany*, ***59***(6), 1187–1200.

Kakehi, J., Kuwashiro, Y., Niitsu, M., & Takahashi, T. (2008). Thermospermine is required for stem elongation in *Arabidopsis thaliana*. *Plant Cell Physiol*, ***49***(9), 1342–1349.

Kamoun, S., Furzer, O., Jones, J. D. G., Judelson, H. S., Ali, G. S., Dalio, R. J. D., Roy, S. G., Schena, L., Zambounis, A., Panabières, F., Cahill, D., Ruocco, M., Figueiredo, A., Chen, X. R., Hulvey, J., Stam, R., Lamour, K., Gijzen, M., Tyler, B. M., Govers, F. (2015). The Top 10 oomycete pathogens in molecular plant pathology. *Molecular Plant Pathology*, ***16***(4), 413–434.

Kang, Y., Huang, J., Mao, G., He, L.Y., & Schell, M. A. (1994). Dramatically reduced virulence of mutants of *Pseudomonas solanacearum* defective in export of extracellular proteins. *Mol Plant Microbe Interact*, ***7***(3), 370–377.

Kannan, V., Bastas, K., and Devi, R. (2015). Bacteriophages: emerging biocontrol agents for plant pathogenic bacteria. *Sustainable Approaches to Controlling Plant Pathogenic Bacteria*. CRC press.

Kanonenberg, K., Schwarz, C. K. W., & Schmitt, L. (2013). Type I secretion systems - a story of appendices. *Research in Microbiology*, ***164***, 596–604.

Karim, S. (2007). Exploring plant tolerance to biotic and abiotic stresses. Swedish University of Agricultural Sciences Uppsala.

Kaur-sawhney, R., Altman, A., & Galston, A. W. (1978). Dual mechanisms in polyamine-mediated control of ribonuclease activity in oat leaf protoplasts. *Plant Physiol.*, ***62***, 158–160.

Keller, H., Hohlfeld, H., Wray, V., Hahlbrock, K., Scheel, D., & Strack, D. (1996). Changes in the accumulation of soluble and cell wall-bound phenolics in elicitor-treated cell suspension cultures and fungus infected leaves of *Solanum tubersum*. *Phytochemistry*, ***42***(2), 389–396.

Kieber, J. J., Rothenberg, M., Roman, G., Feldmann, K. A., & Ecker, J. R. (1993). CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. *Cell*, ***72***(3), 427–441.

Kim, D. W., Watanabe, K., Murayama, C., Izawa, S., Niitsu, M., Michael, A. J., Berberich, T., & Kusano, T. (2014). Polyamine oxidase5 regulates *Arabidopsis* growth through thermospermine oxidase activity. *Plant Physioly*, ***165***, 1575–1590.

Kim, M. G., Da Cunha, L., McFall, A. J., Belkhadir, Y., DebRoy, S., Dangl, J. L., & Mackey, D. (2005). Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. *Cell*, ***121***, 749–759.

Kim, M. G., Geng, X., Lee, S. Y., & Mackey, D. (2009). The *Pseudomonas syringae* type III effector AvrRpm1 induces significant defenses by activating the *Arabidopsis* nucleotide-binding leucine-rich repeat protein RPS2. *Plant Journal*, ***57***(4), 645–653.

Kim, N. H., Kim, B. S., & Hwang, B. K. (2013). Pepper arginine decarboxylase is required for polyamine and γ-aminobutyric acid signaling in cell death and defense response. *Plant Physiology*, ***162***, 2067–2083.

Kim, Y. J., Leea, S. H., & Park, K. Y. (2004). A leader intron and 115-bp promoter region necessary for expression of the carnation S -adenosylmethionine decarboxylase gene in the pollen of transgenic tobacco. *FEBS Letters*, ***578***, 229–235.

Kitashiba, H., Honda, C., & Moriguchi, T. (2006). Identification of polyamine oxidase genes from apple and expression analysis during fruit development and cell growth. *Plant Biotechnology Journal*, ***23***, 425–429.

Kliebenstein, D. J. (2009). Advancing genetic theory and application by metabolic quantitative trait loci analysis. *Plant Cell*, ***21***, 1637–1646.

Kloek, A. P., Verbsky, M. L., Sharma, S. B., Schoelz, J. E., Vogel, J., Klessig, D. F., & Kunkel, B. N. (2001). Resistance to *Pseudomonas syringae* conferred by an *Arabidopsis thaliana* coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. *Plant Journal*, ***26***(5), 509–522.

Knepper, C., Savory, E. A., & Day, B. (2011). The role of NDR1 in pathogen perception and plant defense signaling. *Plant Signaling and Behavior*, ***6***(8), 1114–1116.

Knogge, W. (1998). Fungal pathogenicity. *Current Opinion in Plant Biology*, ***1***, 324–328.

Knott, J. M., Romer, P., & Sumper, M. (2007). Putative spermine synthases from *Thalassiosira pseudonana* and *Arabidopsis thaliana* synthesize thermospermine rather than spermine. *FEBS Letters*, ***581***, 3081–3086.

Kooke, R., Kruijer, W., Bours, R., Becker, F., Kuhn, A., Van de Geest, H., Buntjer, J., Doeswijk, T., Guerra, J., Bouwmeester, H., Vreugdenhil, D., & Keurentjes, J. J. B. (2016). Genome-wide association mapping and genomic prediction elucidate the genetic architecture of morphological traits in *Arabidopsis*. *Plant Physiology*, ***170***, 2187–2203.

Kranner, I., Minibayeva, F. V, Beckett, R. P., & Seal, C. E. (2010). What is stress ? Concepts , definitions and applications in seed science. *New phytologist,* ***188*** (3), 655–673.

Krings, M., Taylor, T. N., & Dotzler, N. (2011). The fossil record of the peronosporomycetes (Oomycota). *Mycologia*, ***103***(3), 445–457.

Kroon, L. P. N. M., Brouwer, H., De Cock, A. W. A. M., & Govers, F. (2012). The genus Phytophthora anno 2012. *Phytopathology*, ***102***(4), 348–364.

Kumar, A., Altabella, T., Taylor, M. A., & Tiburcio, A. F. (1997). Recent advances in polyamine research. *Trends in Plant Science*, ***2***(4), 124–130.

Kumar, V., Joshi, S. G., Bell, A. A., & Rathore, K. S. (2013). Enhanced resistance against *Thielaviopsis basicola* in transgenic cotton plants expressing *Arabidopsis NPR1* gene. *Transgenic Research*, ***22***, 359–368.

Kunkel, B. N., Bent, A. F., Dahlbeck, D., Innes, R. W., & Staskawicz, B. J. (1993). RPS2, an *Arabidopsis* disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Plant Cell*, ***5***(8), 865–875.

Kusano, T., Berberich, T., Tateda, C., & Takahashi, Y. (2008). Polyamines : essential factors for growth and survival. *Planta*, ***228***, 367–381.

Kusano, Tomonobu, Yamaguchi, K., Berberich, T., & Takahashi, Y. (2007). The polyamine spermine rescues *Arabidopsis* from salinity and drought stresses. *Plant Signaling and Behavior*, ***2***(4), 251–252.

Laluk, K., & Mengiste, T. (2010). Necrotroph attacks on plants: wanton destruction or covert extortion? *The Arabidopsis Book*, *8*, e0136.

Lapin, D., Kovacova, V., Sun, X., Dongus, J. A., Bhandari, D., Von Born, P., Bautor, J., Guarneri, N., Rzemieniewski, J., Stuttmann, J., Beyer, A., & Parker, J. E. (2019). A coevolved EDS1-SAG101-NRG1 module mediates cell death signaling by TIR-domain immune receptors. *Plant Cell*, ***31***(10), 2430–2455.

Lasanajak, Y., Minocha, R., Minocha, S. C., Goyal, R., Fatima, T., Handa, A. K., & Mattoo, A. K. (2014). Enhanced flux of substrates into polyamine biosynthesis but not ethylene in tomato fruit engineered with yeast S -adenosylmethionine decarboxylase gene. *Amino Acids*, ***46***, 729–742.

Le Roux, C., Huet, G., Jauneau, A., Camborde, L., Trémousaygue, D., Kraut, A., Zhou, B., Levaillant, M., Adachi, H., Yoshioka, H., Raffaele, S., Berthomé, R., Couté, Y., Parker, J. E., & Deslandes, L. (2015). A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. *Cell*, ***161***, 1074–1088.

Lecourieux, D., Raneva, R., & Pugin, A. (2006). Calcium in plant defence-signalling pathways. *New Phytologist*, ***171***, 249–269.

Lee, D. G., Park, Y., Kim, M., Jung, H. J., Seu, Y. B., Hahm, K.-S., & Woo, E.-R. (2004). Anti-fungal effects of phenolic amides isolated from the root bark of *Lycium chinense*. *Biotechnology Letters*, ***26***, 1125–1130.

Lee, D., Lal, N. K., Lin, Z. D., Ma, S., Liu, J., Castro, B., Toruño, T., Dinesh-kumar, S. P., & Coaker, G. (2020). Regulation of reactive oxygen species during plant immunity through phosphorylation and ubiquitination of RBOHD. *Nature Communications*, ***11***, 1838.

Lee, Y. P., Babakov, A., Boer, B. De, Zuther, E., & Hincha, D. K. (2012). Comparison of freezing tolerance , compatible solutes and polyamines in geographically diverse collections of Thellungiella sp and *Arabidopsis thaliana* accessions. *BMC Plant Biology*, ***12***(131).

Lefevere, H., Bauters, L., & Gheysen, G. (2020). Salicylic acid biosynthesis in plants. *Frontiers in Plant Science*, ***11***, 338.

Legaz, M. E., De Armas, R., Piñón, D., & Vicente, C. (1998). Relationships between phenolics-conjugated polyamines and sensitivity of sugarcane to smut (*Ustilago scitaminea*). *Journal of Experimental Botany*, ***49***(327), 1723–1728.

Letoffe, S., Ghigo, J. M., & Wandersman, C. (1994). Secretion of the Serratia marcescens HasA protein by an ABC transporter. *Journal of Bacteriology*, ***176***(17), 5372–5377.

Li, G., Froehlich, J. E., Elowsky, C., Msanne, J., Ostosh, A. C., Zhang, C., Awada, T., & Alfano, J. R. (2014). Distinct Pseudomonas type-III effectors use a cleavable transit peptide to target chloroplasts. *Plant Journal*, ***77***(2), 310–321.

Li, N., Parsons, B. L., Liu, D., & Mattoo, A. K. (1992). Accumulation of wound-inducible ACC synthase transcript in tomato fruit is inhibited by salicylic acid and polyamines. *Plant Molecular Biology*, ***18***, 477–487.

Li, X., Clarke, J. D., Zhang, Y., & Dong, X. (2001). Activation of an EDS1-mediated R-gene pathway in the *snc1* mutant leads to constitutive, *NPR1*-independent pathogen resistance. *Molecular Plant-Microbe Interactions*, ***14***(10), 1131–1139.

Li, Z., Huang, J., Wang, Z., Meng, F., Zhang, S., Wu, X., Zhang, Z., & Gao, Z. (2019). Overexpression of *Arabidopsis* nucleotide-binding and leucine-rich repeat genes *RPS2* and *RPM1*(*D505V*) confers broad-spectrum disease resistance in rice. *Frontiers in Plant Science*, ***10***, 417.

Lin, W., Ma, X., Shan, L., & He, P. (2013). Big roles of small kinases: The complex functions of receptor-like cytoplasmic kinases in plant immunity and development. *Journal Integr Plant Biololy*, ***55***(1), 1188–1197.

Lindgren, P. B. (1997). The role of *hrp* genes during plant-bacterial interactions. *Annual Review of Phytopathology*, ***35***(1), 129–152.

Lindgren, P., Peet, R., & Panopoulos, N. (1986). Gene Cluster of *Pseudomonas syringae* pv. "phaseolicola" controls pathogenicity of bean plants and hypersensitivity on nonhost plants. *Journal of Bacteriology*, *168*, 512–522.

Liu, C., Li, X., Yang, R., Mo, Y., Wang, Y., Xian, F., Zhang, X., & Wang, F. (2014). The protective roles of S-adenosylmethionine decarboxylase (*SAMDC*) gene in melon resistance to powdery mildew infection. *Horticulture Environment and Biotechnology*, ***55***(6), 557–567.

Liu, C., Atanasov, K. E., Arafaty, N., Tiburcio, A. F., Zeier, J., & Alcázar, R. (2020). Putrescine elicits ROS-dependent activation of the salicylic acid pathway in *Arabidopsis thaliana*. *Plant Cell and Environment*, ***43***, 2755–2768.

Liu, C., Atanasov, K. E., Tiburcio, A. F., & Alcázar, R. (2019). The polyamine putrescine contributes to H_2O_2 and *RbohD/F*-dependent positive feedback loop in *Arabidopsis* PAMP-triggered immunity. *Frontiers in Plant Science*, ***10***, 894.

Liu, J. H., Wang, W., Wu, H., Gong, X., & Moriguchi, T. (2015). Polyamines function in stress tolerance: From synthesis to regulation. *Frontiers in Plant Science*, ***6***, 827.

Liu, J., Liu, X., Dai, L., & Wang, G. (2007). Recent progress in elucidating the structure, function and evolution of disease resistance genes in pPlants. *Journal of Genetics and Genomics*, *34*(9), 765–776.

Liu, J., Elmore, J. M., Lin, Z. D., & Coaker, G. (2012). A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune

receptor. *Cell Host Microbe*, ***9***(2), 137–146.

Liu, Y., Schiff, M., Czymmek, K., Tallóczy, Z., Levine, B., & Dinesh-Kumar, S. P. (2005). Autophagy regulates programmed cell death during the plant innate immune response. *Cell*, ***121***, 567–577.

Lolle, S., Stevens, D., & Coaker, G. (2020). Plant NLR-triggered immunity: from receptor activation to downstream signaling. *Current Opinion in Immunology*, ***62***, 99–105.

Louis, J., Gobbato, E., Mondal, H. A., Feys, B. J., Parker, J. E., & Shah, J. (2012). Discrimination of *Arabidopsis* PAD4 activities in defense against green peach aphid and pathogens. *Plant Physiology*, ***158***(4), 1860–1872.

Luo, J., Fuell, C., Parr, A., Hill, L., Bailey, P., Elliott, K., Fairhurst, S. A., Martin, C., & Michael, A. J. (2009). A novel polyamine acyltransferase responsible for the accumulation of spermidine conjugates in *Arabidopsis* Seed. *The Plant Cell*, ***21***, 318–333.

Ma, C., Wang, Y., Gu, D., Nan, J., Chen, S., & Li, H. (2017). Overexpression of S-adenosyl-L-methionine synthetase 2 from sugar beet M14 increased *Arabidopsis* tolerance to salt and oxidative stress. *International Journal of Molecular Sciences*, ***18***, 847.

Ma, Y., Guo, H., Hu, L., Martinez, P. P., Moschou, P. N., Cevik, V., Ding, P., Duxbury, Z., Sarris, P. F., & Jones, J. D. G. (2018). Distinct modes of derepression of an *Arabidopsis* immune receptor complex by two different bacterial effectors. *Proceedings of the National Academy of Sciences of the United States of America*, ***115***(41), 10218–10227.

Macho, A. P., & Zipfel, C. (2014). Plant PRRs and the activation of innate immune signaling. *Molecular Cell*, ***54***(2), 263–272.

Macho, A. P., & Zipfel, C. (2015). Targeting of plant pattern recognition receptor-triggered immunity by bacterial type-III secretion system effectors. *Current Opinion in Microbiology*, ***23***, 14–22.

Mackey, D., Belkhadir, Y., Alonso, J. M., Ecker, J. R., & Dangl, J. L. (2003). *Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell*, ***112***(3), 379–389.

Mackey, D., Holt, B. F., Wiig, A., & Dangl, J. L. (2002). RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. *Cell*, ***108***(6), 743–754.

Majumdar, R., Barchi, B., Turlapati, S. A., Gagne, M., Minocha, R., Long, S., & Minocha, S. C. (2016). Glutamate, ornithine, arginine, proline, and polyamine metabolic interactions: The pathway is regulated at the post-transcriptional level. *Frontiers in Plant Science*, ***7***, 78.

Majumdar, R., Shao, L., Turlapati, S. A., & Minocha, S. C. (2017). Polyamines in the life of *Arabidopsis* : profiling the expression of S - adenosylmethionine decarboxylase (*SAMDC*) gene family during its life cycle. *BMC Plant Biology*, ***17***.

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., & Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, ***13***(6), 614–629.

Marathe, R., & Dinesh-Kumar, S. P. (2003). Plant defense: One post, multiple guards?! *Molecular Cell*, ***11***(2), 284–286.

Marcé, M., Brown, D. S., Capell, T., Figueras, X., & Tiburcio, A. F. (1995). Rapid high-performance liquid chromatographic method for the quantitation of polyamines as their dansyl derivatives: application to plant and animal tissues. *Journal of Chromatography B: Biomedical Sciences and Applications*, ***666***, 329–335.

Marco, F., Alcazar, R., Tiburcio, A. F., & Carrasco, P. (2011). Interactions between polyamines and abiotic stress pathway responses unraveled by transcriptome analysis of polyamine overproducers. *OMICS A Journal of Integrative Biology*, ***15***(11).

Marco, F., Busó, E., & Carrasco, P. (2014). Overexpression of *SAMDC1* gene in *Arabidopsis thaliana* increases expression of defense-related genes as well as resistance to *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis*. *Frontiers in Plant Science*, ***5***, 115.

Marco, F., Busó, E., Lafuente, T., & Carrasco, P. (2019). Spermine confers stress resilience by modulating abscisic acid biosynthesis and stress responses in *Arabidopsis* Plants. *Frontiers in Plant Science*, ***10***, 972.

Marco, F., & Carrasco, P. (2002). Expression of the pea S-adenosylmethionine decarboxylase gene is involved in developmental and environmental responses. *Planta*, ***214***, 641–647.

Marina, M., Maiale, S. J., Rossi, F. R., Romero, M. F., Rivas, E. I., Gárriz, A., Ruiz, O. A., & Pieckenstain, F. L. (2008). Apoplastic polyamine oxidation plays different roles in local responses of tobacco to infection by the necrotrophic fungus *Sclerotinia sclerotiorum* and the biotrophic bacterium *Pseudomonas viridiflava*. *Plant Physiology*, ***147***, 2164–2178.

Marini, F., Betti, L., Scaramagli, S., Biondi, S., & Torrigiani, P. (2001). Polyamine metabolism is upregulated in response to *tobacco mosaic virus* in hypersensitive, but not in susceptible, tobacco. *New Phytologist*, ***149***, 301–309.

Martin-tanguy, J. (1997). Conjugated poiyamines and reproductive deveiopment : Biochemicai , moiecuiar and physioiogical approaches. *Physiologia Plantarum*, ***100*****,** 675–688.

Martin-tanguy, J. (2001). Metabolism and function of polyamines in plants : recent development (new approaches). *Plant Growth Regulation*, *34*, 135–148.

Martin, G. B., Brommonschenkel, S. H., Chunwongse, J., Frary, A., Ganal, M. W., Spivey, R., Wu, T., Earle, E. D., & Tanksley, S. D. (1993). Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science*, ***262***(5138), 1432–1436.

Maruri-López, I., Aviles-Baltazar, N. Y., Antony, B., & Serrano, M. (2019). Intra and extracellular journey of the phytohormone Salicylic acid. *Frontiers in Plant Science, **10*** (423), 1–11.

Masson, P. H., Takahashi, T., Angelini, R., & Angelini, R. (2017). Editorial : Molecular mechanisms underlying polyamine functions in plants. *Frontiers in Plant Science*, ***8***, 1–3.

Mattoo, A. K., Minocha, S. C., Minocha, R., & Handa, A. K. (2010). Polyamines and cellular metabolism in plants : transgenic approaches reveal different responses to diamine putrescine versus higher polyamines spermidine and spermine. *Amino Acids*, ***38***, 405–413.

McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology*, ***40***, 349–379.

McLachlan, D. H., Kopischke, M., & Robatzek, S. (2014). Gate control: Guard cell regulation by microbial stress. *New Phytologist*, ***203***, 1049–1063.

Mellidoua, I., Karamanolia, K., Berisb, D., Haralampidisb, K., Constantinidoua, H.-I. A., & Roubelakis-Angelakis, K. A. (2017). Underexpression of apoplastic polyamine oxidase improves thermotolerance in *Nicotiana tabacum*. *Journal of Plant Physiology*, ***218***, 171–174.

Meyers, B. C., Chin, D. B., Shen, K. A., Sivaramakrishnan, S., Lavelle, D. O., Zhang, Z., & Michelmore, R. W. (1998). The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. *Plant Cell*, ***10***, 1817–1832.

Meyers, B. C., Dickerman, A. W., Michelmore, R. W., Sivaramakrishnan, S., Sobral, B. W., & Young, N. D. (1999). Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. *Plant Journal*, ***20***(3), 317–332.

Meyers, B. C., Kozik, A., Griego, A., Kuang, H., & Michelmore, R. W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. *Plant Cell*, ***15***(4), 809–834.

Miedes, E., Vanholme, R., Boerjan, W., & Molina, A. (2014). The role of the secondary cell wall in plant resistance to pathogens. *Frontiers in Plant Science*, ***5***, 1–13.

Miller, G., Schlauch, K., Tam, R., Cortes, D., Torres, M. A., Shulaev, V., Dangl, J. L., & Mittler, R. (2009). The Plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Plant Biology*, ***2***(84).

Mitsuya, Y., Takahashi, Y., Berberich, T., Miyazakia, A., Matsumurab, H., Takahashic, H., Terauchib, R., & Kusano, T. (2009). Spermine signaling plays a significant role in the defense response of *Arabidopsis thaliana* to *Cucumber mosaic virus*. *Journal of Plant Physiology*, ***166***, 626–643.

Mittal, S., & Davis, K. R. (1995). Role of the phytotoxin coronatine in the infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. tomato. *Mol Plant Microbe Interact*, ***8***(1), 165–171.

Mo, H., Wang, X., Zhang, Y., Yang, J., & Ma, Z. (2015). Cotton *ACAULIS5* is involved in stem elongation and the plant defense response to *Verticillium dahliae* through thermospermine alteration. *Plant Cell Reports*, ***34***, 1975–1985.

Mo, H., Wang, X., Zhang, Y., Zhang, G., Zhang, J., & Ma, Z. (2015). Cotton polyamine

oxidase is required for spermine and camalexin signalling in the defence response to *Verticillium dahliae*. *Plant Journal*, ***83***, 962–975.

Molla, K. A., Karmakar, S., Chanda, P. K., Sarkar, S. N., Datta, S. K., & Datta, K. (2016). Tissue-specific expression of *Arabidopsis NPR1* gene in rice for sheath blight resistance without compromising phenotypic cost. *Plant Science*, ***250***, 105–114.

Møller, S. G., & Mcpherson, M. J. (1998). Developmental expression and biochemical analysis of the *Arabidopsis atao1* gene encoding an H_2O_2- generating diamine oxidase. *The Plant Journal*, ***13***(6), 781–791.

Moschou, P. N., Wu, J., Cona, A., Tavladoraki, P., Angelini, R., & Roubelakis-Angelakis, K. A. (2012). The polyamines and their catabolic products are significant players in the turnover of nitrogenous molecules in plants. *Journal of Experimental Botany*, ***63***(14), 5003–5015.

Moschou, P., Paschalidis, K. A., & Roubelakis-Angelakis, K. A. (2008). Plant polyamine catabolism the state of the art. *Plant Signaling & Behavior*, *December*, 1061–1066.

Moschou, P. N., Paschalidis, K. A., Delis, I. D., Andriopoulou, A. H., Lagiotis, G. D., Yakoumakis, D. I., & Roubelakis-angelakis, K. A. (2008). Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H_2O_2 signatures that direct tolerance responses in tobacco. *The Plant Cell*, ***20***, 1708–1724.

Moss, B. (2008). Water pollution by agriculture. *Phil. Trans. R. Soc. B*, ***363***, 659–666.

Mou, Z., Fan, W., & Dong, X. (2003). Inducers of plant systemic acquired resistance regulate *NPR1* function through redox changes. *Cell*, ***113***(7), 935–944.

Moustafa-Farag, M., Almoneafy, A., Mahmoud, A., Elkelish, A., Arnao, M. B., Li, L., & Ai, S. (2020). Melatonin and its protective role against biotic stress impacts on plants. *Biomolecules*, ***10***(1), 1–12.

Mur, L. A. J., Kenton, P., Lloyd, A. J., Ougham, H., & Prats, E. (2008). The hypersensitive response; The centenary is upon us but how much do we know? *Journal of Experimental Botany*, ***59***(3), 501–520.

Muroi, A., Ishihara, A., Tanaka, C., Ishizuka, A., Takabayashi, J., Miyoshi, H., & Nishioka, T. (2009). Accumulation of hydroxycinnamic acid amides induced by pathogen infection and identification of agmatine coumaroyltransferase in *Arabidopsis thaliana*. *Planta*, ***230***, 517–527.

Naconsie, M., Kato, K., Shoji, T., & Hashimoto, T. (2014). Molecular evolution of N-methylputrescine oxidase in tobacco. *Plant and Cell Physiology*, ***55***(2), 436–444.

Nagai, A., Torres, P. B., Duarte, L. M. L., Chaves, A. L. R., Macedo, A. F., Floh, E. I. S., de Oliveira, L. F., Zuccarelli, R., & Dos Santos, D. Y. A. C. (2020). Signaling pathway played by salicylic acid, gentisic acid, nitric oxide, polyamines and non-enzymatic antioxidants in compatible and incompatible Solanum-tomato *Mottle mosaic virus* interactions. *Plant Science*, ***290***.

Nahar, K., Hasanuzzaman, M., Rahman, A., Alam, M., Mahmud, J.-A., Suzuki, T., & Fujita, M. (2016). Polyamines confer salt tolerance in mung bean (*Vigna radiata* L .) by reducing sodium uptake , improving nutrient homeostasis , antioxidant defense , and methylglyoxal detoxification systems. *Frontiers in Plant Science*, ***7***, 1–14.

Narusaka, M., Shirasu, K., Noutoshi, Y., Kubo, Y., Shiraishi, T., Iwabuchi, M., & Narusaka, Y. (2009). RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens. *Plant Journal*, ***60***(2), 218–226.

Nawrath, C., Heck, S., Parinthawong, N., & Métraux, J. (2002). EDS5 , an essential component of salicylic acid – dependent signaling for disease resistance in *Arabidopsis* , is a member of the MATE transporter family. *The Plant Cell*, ***14***, 275–286.

Nawrath, C., & Métraux, J. P. (1999). Salicylic acid induction-deficient mutants of *Arabidopsis* express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. *Plant Cell*, *11*(8), 1393–1404.

Ndamukong, I., Abdallat, A. Al, Thurow, C., Fode, B., Zander, M., Weigel, R., & Gatz, C. (2007). SA-inducible *Arabidopsis* glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription. *Plant Journal*, ***50***(1), 128–139.

Negrel, J., & Martin, C. (1984). The biosynthesis of feruloyltyramine in *Nicotiana tabacum*. *Phytochemistry*, ***23***(12), 2797–2801.

Negrel, J., Vallee, J.-C., & Martin, C. (1984). Ornithine decarboxylase activity and the hypersensitive reaction to *Tobacco musaic virus* in *Nicotiana tabacum*. *Phytochemistry*, ***23***(12), 2747–2751.

Nelson, R., Wiesner-Hanks, T., Wisser, R., & Balint-Kurti, P. (2018). Navigating complexity to breed disease-resistant crops. *Nature Reviews Genetics*, ***19***, 21–33.

Németh, M., Janda, T., Horváth, E., Páldi, E., & Szalai, G. (2002). Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. *Plant Science*, ***162***, 569–574.

Neubauer, M., Serrano, I., Rodibaugh, N., Bhandari, D. D., Bautor, J., Parker, J. E., & Innes, R. W. (2020). *Arabidopsis* EDR1 protein kinase regulates the association of EDS1 and PAD4 to inhibit cell death. *Molecular Plant-Microbe Interactions*, ***33***(4), 693–703.

Newman, M., Roepenack-lahaye, E. Von, Parr, A., Daniels, M. J., & Dow, J. M. (2001). Induction of hydroxycinnamoyl-tyramine conjugates in pPepper by *Xanthomonas campestris* , a plant defense response activated by *hrp* gene-dependent and *hrp* gene-independent mechanisms. *MPMI*, ***14***(6), 785–792.

Ngou, B. P. M., Ahn, H.-K., Ding, P., & Jones, J. D. (2020). Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *BioRxiv*.

Nicaise, V. (2014). Crop immunity against viruses: Outcomes and future challenges. *Frontiers in Plant Science*, ***5***, 1–18.

Nicol, J. M., Turner, S. J., Coyne, D. L., Nijs, L. Den., Hockland, S. & Maafi, Z. T. (2011). Current nematode threats to world agriculture. In *Genomics and molecular genetics of plant-nematode interactions* (pp. 21-43). Springer, Dordrecht.

Nimchuk, Z., Eulgem, T., Holt III, B. F., & Dangl, J. L. (2003). Recognition and response in the plant immune system. *Annual Review of Genetics*, ***37***(1), 579–609.

Nimchuk, Z., Marois, E., Kjemtrup, S., Leister, R. T., Katagiri, F., & Dangl, J. L. (2000). Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. *Cell*, ***101***, 353–363.

Noman, A., Aqeel, M., & Lou, Y. (2019). PRRs and NB-LRRs: From signal perception to activation of plant innate immunity. *International Journal of Molecular Sciences*, ***20***, 1882.

Nowicka, E. S. (2017). Polyamine catabolism adds fuel to leaf senescence. *Amino Acids*, ***49***(1), 49–56.

Nürnberger, T., Brunner, F., Kemmerling, B., & Piater, L. (2004). Innate immunity in plants and animals: Striking similarities and obvious differences. *Immunological Reviews*, ***198***, 249–266.

Ono, Y., Kim, D. W., Watanabe, K., Sasaki, A., Niitsu, M., Berberich, T., Kusano, T., & Takahashi, Y. (2012). Constitutively and highly expressed *Oryza sativa* polyamine oxidases localize in peroxisomes and catalyze polyamine back conversion. *Amino Acids*, ***42***, 867–876.

Osherov, N., & May, G. S. (2001). The molecular mechanisms of conidial germination. *FEMS Microbiology Letters*, ***199***(2), 153–160.

Pál, M., Kovács, V., Vida, G., Szalai, G., & Janda, T. (2011). Changes in salicylic acid and polyamine contents following powdery mildew infection of near-isogenic thatcher-based wheat lines carrying different Lr genes. *Acta Biologica Szegediensis*, ***55***(1), 139–141.

Pál, M., Szalai, G., & Janda, T. (2015). Speculation : Polyamines are important in abiotic stress signaling. *Plant Science*, ***237***, 16–23.

Palomares-Rius, J. E., Escobar, C., Cabrera, J., Vovlas, A., & Castillo, P. (2017). Anatomical alterations in plant tissues induced by plant-parasitic nematodes. *Frontiers in Plant Science*, ***8***, 1–16.

Panicot, M., Minguet, E. G., Ferrando, A., Alcazar, R., Blazquez, M. A., & Carbonell, J. (2002). A polyamine metabolon involving aminopropyl transferase complexes in *Arabidopsis*. *Plant Cell*, ***14***, 2539–2551.

Parihar, P., Singh, S., Singh, R., Singh, V. P., & Prasad, S. M. (2015). Effect of salinity stress on plants and its tolerance strategies: a review. *Environmental Science and Pollution Research*, ***22***(6), 4056–4075.

Parkhi, V., Kumar, V., Campbell, L. A. M., Bell, A. A., Shah, J., & Rathore, K. S. (2010). Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants

expressing *Arabidopsis NPR1*. *Transgenic Research*, ***19***(6), 959–975.

Paschalidis, K. A., Moschou, P. N., Toumi, I., & Roubelakis-Angelakis, K. A. (2009). Polyamine anabolic/catabolic regulation along the woody grapevine plant axis. *Journal of Plant Physiology,* ***166***(14), 1508–1519.

Paschalidis, K., Tsaniklidis, G., Wang, B. Q., Delis, C., Trantas, E., Loulakakis, K., Makky, M., Sarris, P. F., Ververidis, F., & Liu, J. H. (2019). The interplay among polyamines and nitrogen in plant stress responses. *Plants*, ***8***, 315.

Passera, A., Compant, S., Casati, P., Maturo, M. G., Battelli, G., Quaglino, F., Antonielli, L., Salerno, D., Brasca, M., Toffolatti, S. L., Mantegazza, F., Delledonne, M., & Mitter, B. (2019). Not just a pathogen? Description of a plant-beneficial *Pseudomonas syringae* strain. *Frontiers in Microbiology*, ***10***.

Pedros, A. R., MacLeod, M. R., Ross, H. A., McRae, D., Tiburcio, A. F., Davies, H. V., & Taylor, M. A. (1999). Manipulation of S-adenosylmethionine decarboxylase activity in potato tubers. *Planta*, ***209***, 153–160.

Pegg, A. E. (2016). Functions of Polyamines in Mammals. *The Journal of Biological Chemistry*, ***291***(29), 14904–14912.

Pegg, A. E., & Michael, A. J. (2011). Spermine synthase Anthony. *Cell Mol Life Sci*, ***67***(1), 1–13.

Peghaire, E., Hamdache, S., Galien, A., Sleiman, M., Halle, A., Alaoui, H. El, Kocer, A., Richard, C., & Goupil, P. (2020). Inducing plant defense reactions in tobacco plants with phenolic-rich extracts from red maple leaves : A characterization of main active ingredients. *Forests*, ***11***(705).

Pieterse, C. M. J., Van Wees, S. C. M., Van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P. J., & Van Loon, L. C. (1998). A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell*, ***10***(9), 1571–1580.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., & Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology*, ***52***(1), 347–375.

Piotrowski, M., Janowitz, T., & Kneifel, H. (2003). Plant C-N hydrolases and the identification of a plant N-carbamoylputrescine amidohydrolase involved in polyamine biosynthesis. *The Journal of Biological Chemistry*, ***278***, 1708–1712.

Planas-portell, J., Gallart, M., Tiburcio, A. F., & Altabella, T. (2013). Copper-containing amine oxidases contribute to terminal polyamine oxidation in peroxisomes and apoplast of *Arabidopsis thaliana*. *BMC Plant Biology*, ***13***.

Podlešáková, K., Ugena, L., Spíchal, L., Doležal, K., & Diego, N. De. (2019). Phytohormones and polyamines regulate plant stress responses by altering GABA pathway. *New Biotechnology*, ***48***, 53–65.

Pokotylo, I., Kravets, V., & Ruelland, E. (2019). Salicylic acid binding proteins (SABPs): The hidden forefront of salicylic acid signalling. *International Journal of Molecular Sciences*, ***20***, 4377.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: Trends, impacts and drivers. *Trends in Ecology and Evolution*, ***25***(6), 345–353.

Preston, G. M. (2000). *Pseudomonas syringae* pv. tomato: the right pathogen, of the right plant, at the right time. *Molecular Plant Pathology*, ***1***(5), 263–275.

Preston, G. M., Studholme, D. J., & Caldelari, I. (2005). Profiling the secretomes of plant pathogenic Proteobacteria. *FEMS Microbiology Reviews*, ***29***, 331–360.

Pugsley, A. (1993). The general secretory pathway in bacteria. *Microbiological Reviews*, ***57***, 50–108.

Qi, G., Chen, J., Chang, M., Chen, H., Hall, K., Korin, J., Liu, F., Wang, D., & Fu, Z. Q. (2018). Pandemonium breaks out: disruption of salicylic acid-mediated defense by plant pathogens. *Molecular Plant*, ***11***, 1427–1439.

Qi, Y. C., Wang, F. F., Zhang, H., & Liu, W. Q. (2010). Overexpression of *Suadea salsa* S-adenosylmethionine synthetase gene promotes salt tolerance in transgenic tobacco. *Acta Physiologiae Plantarum*, ***32*****,** 263–269.

Radhakrishnan, R., & Lee, I. J. (2013). Spermine Promotes Acclimation to osmotic stress by modifying antioxidant, abscisic acid, and jasmonic acid signals in soybean. *Journal of Plant Growth Regulation*, ***32***, 22–30.

Radojičić, A., Li, X., & Zhang, Y. (2018). Salicylic acid: a double-edged sword for programed cell death in plants. *Frontiers in Plant Science*, ***9***, 1133.

Rafiqi, M., Bernoux, M., Ellis, J. G., & Dodds, P. N. (2009). In the trenches of plant pathogen recognition: Role of NB-LRR proteins. *Seminars in cell & developmental biology, **20*** (9), 1017–1024. Academic press.

Rahdari, P., & Hoseini, S. M. (2013). Roll of polyamines (Spermidine and Putrescine) on protein , chlorophyll and phenolic compounds in wheat (*Triticum aestivum* L .) under salinity stress. *J Nov. Appl Sci.*, ***2***(12), 746–751.

Raina, A., Janne, J., Hannonen, P., & Holtta, E. (1970). Synthesis and accumulation of polyamines in generation rat liver. *Annals of the New York Academy of Sciences*, ***171***.

Rampersad, S. N. (2020). Pathogenomics and management of *Fusarium* diseases in plants. *Pathogens*, ***9***(340).

Ray, S. K., Rajeshwari, R., & Sonti, R. V. (2000). Mutants of *Xanthomonas oryzae* pv. *oryzae* deficient in general secretory pathway are virulence deficient and unable to secrete xylanase. *Molecular Plant-Microbe Interactions*, ***13***(4), 394–401.

Rayapuram, C., & Baldwin, I. T. (2007). Increased SA in *NPR1*-silenced plants antagonizes JA and JA-dependent direct and indirect defenses in herbivore-attacked *Nicotiana attenuata* in nature. *Plant Journal*, ***52***(4), 700–715.

Records, A. R. (2011). The type VI secretion system: A multipurpose delivery system with a phage-like machinery. *Molecular Plant-Microbe Interactions*, ***24***(7), 751–757.

Rêgo, A. T., Chandran, V., & Waksman, G. (2010). Two-step and one-step secretion mechanisms in Gram-negative bacteria: Contrasting the type IV secretion system and the chaperone-usher pathway of pilus biogenesis. *Biochemical Journal*, ***425***, 475–488.

Richards, F. J., & Coleman, R. G. (1952). Occurrence of putrescine in potassium-deficient barley. *Nature*, ***170***, 1952.

Rietz, S., Stamm, A., Malonek, S., Wagner, S., Becker, D., Medina-Escobar, N., Corina Vlot, A., Feys, B. J., Niefind, K., & Parker, J. E. (2011). Different roles of Enhanced Disease Susceptibility1 (EDS1) bound to and dissociated from Phytoalexin Deficient4 (PAD4) in *Arabidopsis* immunity. *New Phytologist*, ***191***(1), 107–119.

Roberts, D. R., Walker, M. A., Thompson, J. E., & Dumbroff, E. B. (1984). The effects of inhibitors of polyamine and ethylene biosynthesis on senescence, ethylene production and polyaminel levels in cut carnation flowers. *Plant & Cell Physiology*, ***25***(2), 315–322.

Roberts, S. C., Jiang, Y., Jardim, A., Carter, N. S., Heby, O., & Ullman, B. (2001). Genetic analysis of spermidine synthase from *Leishmania donovani*. *Molecular & Biochemical Parasitology*, ***115*****,** 217–226.

Rodrıguez, A. A., Maiale, S. J., Menendez, A. B., & Ruiz, O. A. (2009). Polyamine oxidase activity contributes to sustain maize leaf elongation under saline stress. *Journal of Experimental Botany*, ***60***(15), 4249–4262.

Roeder, S., Dreschler, K., Wirtz, M., Cristescu, S. M., Harren, F. J. M. van, Hell, R., & Piechulla, B. (2009). SAM levels , gene expression of SAM synthetase , methionine synthase and ACC oxidase , and ethylene emission from *N. suaveolens* flowers. *Plant Mol Biol*, ***70***, 535–546.

Rohila, A. K., Ansul, Maan, D., Kumar, A., & Kumar, K. (2017). Impact of agricultural practices on environment. *Asian Jr. of Microbiol. Biotech. Env. Sc.*, ***19***(J2), 145–148.

Roje, S. (2006). S-Adenosyl-l-methionine: Beyond the universal methyl group donor. *Phytochemistry*, ***67***, 1686–1698.

Romero, F. M., Maiale, S. J., Rossi, F. R., Marina, M., Ruız, O. A., & Garriz, A. (2018). polyamine metabolism responses to biotic and abiotic stress. *Methods Mol. Biol*, ***1694***, 37–49.

Roos, W. H., Ivanovska, I. L., Evilevitch, A., & Wuite, G. J. L. (2007). Viral capsids: mechanical characteristics, genome packaging and delivery mechanisms. *Cellular and Molecular Life Sciences*, ***64***(12), 1484–1497.

Roossinck, M. J. (2010). Lifestyles of plant viruses. *Philosophical Transactions of the Royal*

Society B: Biological Sciences, ***365***(1548), 1899–1905.

Ropenack, E. von., Parr, A., & Schulze-Lefert, P. (1998). Structural analyses and dynamics of soluble and cell wall-bound phenolics in a broad spectrum resistance to the powdery mildew fungus in barley. *The Journal of Biological Chemistry*, ***273***(15), 9013–9022.

Rosenheim, O. (1924). The isolation of Spermine phosphate from semen and testis. *Biochem Journal*, ***18***, 1253-1263.

Rossi, F. R., Gárriz, A., Marina, M., & Pieckenstain, F. L. (2020). Modulation of polyamine metabolism in *Arabidopsis thaliana* by salicylic acid. *BioRxiv*, 1–40.

Rossman, A. Y. (2009). The impact of invasive fungi on agricultural ecosystems in the United States. *Biological Invasions*, ***11***(1), 97–107.

Rouxel, T., & Balesdent, M. H. (2013). From model to crop plant-pathogen interactions: cloning of the first resistance gene to *Leptosphaeria maculans* in *Brassica napus*. *New Phytologist*, ***197***, 356–358.

Roy, M., & Wu, R. (2002). Overexpression of S-adenosylmethionine decarboxylase gene in rice increases polyamine level and enhances sodium chloride-stress tolerance. *Plant Science*, ***163***, 987–992.

Sagor, G. H. M., Cong, R. Z., Berberich, T., Takahashi, H., Takahashi, Y., & Kusano, T. (2009). Spermine signaling in defense reaction against avirulent viral pathogen in *Arabidopsis thaliana*. *Plant Signaling and Behavior*, ***4***(4), 316–318.

Sánchez-Aguayo, I., Rodríguez-Galán, J. M., García, R., Torreblanca, J., & Pardo, J. M. (2004). Salt stress enhances xylem development and expression of S-adenosyl-L-methionine synthase in lignifying tissues of tomato plants. *Planta*, ***220***, 278–285.

Sandkvist, M. (2001). Biology of type II secretion. *Molecular Microbiology*, ***40***(2), 271–283.

Sanseverino, W., Roma, G., De Simone, M., Faino, L., Melito, S., Stupka, E., Frusciante, L., & Ercolano, M. R. (2010). PRGdb: A bioinformatics platform for plant resistance gene analysis. *Nucleic Acids Research*, ***38***.

Sarris, P. F., Duxbury, Z., Huh, S. U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G., Saucet, S. B., Wirthmueller, L., Menke, F. L. H., Sohn, K. H., & Jones, J. D. G. (2015). A plant immune receptor detects pathogen effectors that target WRKY transcription factors. *Cell*, ***161***, 1089–1100.

Saucet, S. B., Ma, Y., Sarris, P. F., Furzer, O. J., Sohn, K. H., & Jones, J. D. G. (2015). Two linked pairs of *Arabidopsis* TNL resistance genes independently confer recognition of bacterial effector AvrRps4. *Nature Communications*, ***6***, 6338.

Sauter, M., Moffatt, B., Saechao, M. C., Hell, R., & Wirtz, M. (2013). Methionine salvage and S-adenosylmethionine: essential links between sulfur, ethylene and polyamine biosynthesis. *Biochemical Journal*, ***451***, 145–154.

Schornack, S., Huitema, E., Cano, L. M., Bozkurt, T. O., Oliva, R., Van Damme, M., Schwizer, S., Raffaele, S., Chaparro-Garcia, A., Farrer, R., Segretin, M. E., Bos, J., Haas, B. J., Zody, M. C., Nusbaum, C., Win, J., Thines, M., & Kamoun, S. (2009). Ten things to know about oomycete effectors. *Molecular Plant Pathology*, ***10***(6), 795–803.

Schulze-Lefert, P., & Panstruga, R. (2011). A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends in Plant Science*, ***16***(3), 117–125.

Schwarz, S., Hood, R. D., & Mougous, J. D. (2010). What is type VI secretion doing in all those bugs? *Trends Microbiol*, ***18***(12), 531–537.

Sebela, M., Radova, A., Angelini, R., Tavladoraki, P., Frebort, I., & Pec, P. (2001). FAD-containing polyamine oxidases : a timely challenge for researchers in biochemistry and physiology of plants. *Plant Science*, ***160***, 197–207.

Sephton. C, V. K. (2018). Spore germination of pathogenic filamentous fungi. *Advances in Applied Microbiology*, ***102***, 117–157.

Serapiglia, M. J., Minocha, R., & Minocha, S. C. (2008). Changes in polyamines, inorganic ions and glutamine synthetase activity in response to nitrogen availability and form in red spruce (*Picea rubens*). *Tree Physiology*, ***28***, 1793–1803.

Seyfferth, C., & Tsuda, K. (2014). Salicylic acid signal transduction: The initiation of biosynthesis, perception and transcriptional reprogramming. *Frontiers in Plant Science*, ***5***, 1–10.

Shan, L., He, P., Li, J., Heese, A., Peck, S. C., Nürnberger, T., Martin, G. B., & Sheen, J. (2008). Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. *Cell Host and Microbe*, ***4***(1), 17–27.

Shang, Y., Li, X., Cui, H., He, P., Thilmony, R., Chintamanani, S., Zwiesler-Vollick, J., Gopalan, S., Tang, X., & Zhou, J. M. (2006). RAR1, a central player in plant immunity, is targeted by *Pseudomonas syringae* effector AvrB. *Proceedings of the National Academy of Sciences of the United States of America*, ***103***(50), 19200–19205.

Shao, Z. Q., Xue, J. Y., Wu, P., Zhang, Y. M., Wu, Y., Hang, Y. Y., Wang, B., & Chen, J. Q. (2016). Large-scale analyses of angiosperm nucleotide-binding site-leucine-rich repeat genes reveal three anciently diverged classes with distinct evolutionary patterns. *Plant Physiology*, ***170***(4), 2095–2109.

Shao, Z. Q., Zhang, Y. M., Hang, Y. Y., Xue, J. Y., Zhou, G. C., Wu, P., Wu, X. Y., Wu, X. Z., Wang, Q., Wang, B., & Chen, J. Q. (2014). Long-term evolution of nucleotide-binding site-leucine-rich repeat genes: Understanding gained from and beyond the legume family. *Plant Physiology*, ***166***(1), 217–234.

Shapiguzov, A., Vainonen, J. P., Wrzaczek, M., & Kangasjärvi, J. (2012). ROS-talk - how the apoplast, the chloroplast, and the nucleus get the message through. *Frontiers in Plant Science*, ***3***, 292.

Shuping, D. S. S., & Eloff, J. N. (2017). The use of plants to protect plants and food against fungal pathogens: a Review. *African Journal of Traditional, Complementary, and Alternative Medicines : AJTCAM*, ***14***(4), 120–127.

Sigee, D. C. (1993). *Bacterial Plant Pathology: Cell and Molecular Aspects*. Cambridge Univ. Press.

Silverman, J. M., Brunet, Y. R., Cascales, E., & Mougous, J. D. (2012). Structure and regulation of the type VI secretion system. *Annu. Rev. Microbiol*, ***66***, 453–472.

Simonich, M. T., & Innes, R. W. (1995). A disease resistance gene in *Arabidopsis* with specificity for the *avrPph3* gene of *Pseudomonas syringae* pv. *phaseolicola*. *Mol Plant Microbe Interact*, ***8***(4), 637–640.

Sinha, R., & Venkat, M. (2013). RNAi silencing of three homologues of S-adenosylmethionine decarboxylase gene in tapetal tissue of tomato results in male sterility. *Plant Molecular Biology*, ***82***, 169–180.

Skopelitis, D. S., Paranychianakis, N. V., Paschalidis, K. A., Pliakonis, E. D., Delis, I. D., Yakoumakis, D. I., Kouvarakis, A., Papadakis, A. K., Stephanou, E. G., & Roubelakis-Angelakis, K. A. (2006). Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. *Plant Cell*, ***18***(10), 2767–2781.

Smith, T. A. (1971). The occurrence, metabolic and functions of Amines in plants. *Bid . Rev*, ***46***, 201–241.

Smith, T. A. (1991). A historical perspective on research in plant polyamine biology. In *Biochemistry and Physiology of Polyamines in Plants*, 1-22.

Sohn, K. H., Zhang, Y., & Jones, J. D. G. (2009). The *Pseudomonas syringae* effector protein, AvrRPS4, requires in planta processing and the KRVY domain to function. *Plant Journal*, ***57***(6), 1079–1091.

Song, W., Forderer, A., Yu, D., & Chai, J. (2020). Structural biology of plant defence. *New Phytologist*, ***229***(2), 692-711.

Spoel, S. H., Johnson, J. S., & Dong, X. (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences of the United States of America*, ***104***(47), 18842–18847.

Spoel, S. H., Koornneef, A., Claessens, S. M. C., Korzelius, J. P., Van Pelt, J. A., Mueller, M. J., Buchala, A. J., Métraux, J. P., Brown, R., Kazan, K., Van Loon, L. C., Dong, X., & Pieterse, C. M. J. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *Plant Cell*, ***15***(3), 760–770.

Stathopoulos, C., Hendrixson, D. R., Thanassi, D. G., Hultgren, S. J., St. Geme, J. W., & Curtiss, R. (2000). Secretion of virulence determinants by the general secretory pathway in

Gram-negative pathogens: An evolving story. *Microbes and Infection*, ***2***(9), 1061–1072.

Strange, R. N., & Scott, P. R. (2005). Plant disease: A threat to global food security. *Annual Review of Phytopathology*, ***43***(1), 83–116.

Straus, M. R., Rietz, S., Ver Loren Van Themaat, E., Bartsch, M., & Parker, J. E. (2010). Salicylic acid antagonism of EDS1-driven cell death is important for immune and oxidative stress responses in *Arabidopsis*. *Plant Journal*, ***62***, 628–640.

Sun, X., Lapin, D., Feehan, J. M., Stolze, S. C., Kramer, K., Dongus, J. A., Rzemieniewski, J., Blanvillain-Baufumé, S., Harzen, A., Bautor, J., Derbyshire, P., Menke, F. L. H., Finkemeier, I., Nakagami, H., Jones, J. D.G. (2020). Pathogen effector recognition-dependent association of NRG1 with EDS1 and 2 SAG101 in TNL receptor immunity. *BioRxiv*.

Swiderski, M. R., Birker, D., & Jones, J. D. G. (2009). The TIR domain of TIR-NB-LRR resistance proteins is a signaling domain involved in cell death induction. *Molecular Plant-Microbe Interactions*, ***22***(2), 157–165.

Szalai, G., Janda, K., Darkó, É., Janda, T., Peeva, V., & Pál, M. (2017). Comparative analysis of polyamine metabolism in wheat and maize plants. *Plant Physiology and Biochemistry*, ***112***, 239–250.

Szczesny, R., Jordan, M., Schramm, C., Schulz, S., Cogez, V., Bonas, U., & Büttner, D. (2010). Functional characterization of the Xcs and Xps type II secretion systems from the plant pathogenic bacterium *Xanthomonas campestris* pv *vesicatoria*. *New Phytologist*, ***187***(4), 983–1002.

Szepesi, Á., Gémes, K., Orosz, G., Petô, A., Takács, Z., Vorák, M., & Tari, I. (2011). Interaction between salicylic acid and polyamines and their possible roles in tomato hardening processes. *Acta Biologica Szegediensis*, ***55***(1), 165–166.

Tabor, C. W., & Tabor, H. (1984). Polyamines. *Ann. Rev. Biochem.*, ***53***, 749–790.

Taiz, L., & Zeiger, E. (2006). *Plant physiology, 4*.

Takahashi, Y. (2016). The role of polyamines in plant disease resistance. *Environ.Control Biol*, ***54***(1), 17–21.

Takahashi, Y., Tahara, M., Yamada, Y., Mitsudomi, Y., & Koga, K. (2018). Characterization of the polyamine biosynthetic pathways and salt stress response in *Brachypodium distachyon*. *Journal of Plant Growth Regulation*, ***37***(2), 625–634.

Takano, A., Kakehi, J., & Takahashi, T. (2012). Thermospermine is not a minor polyamine in the plant kingdom. *Plant Cell Physiology*, ***53***(4), 606–616.

Takken, F. L. W., & Goverse, A. (2012). How to build a pathogen detector: Structural basis of NB-LRR function. *Current Opinion in Plant Biology*, ***15***(4), 375–384.

Tavladoraki, P., Cona, A., & Angelini, R. (2016). Copper-containing amine oxidases and FAD-dependent polyamine oxidases are key players in plant tissue differentiation and organ

development. *Frontiers in Plant Science*, ***7***.

Tavladoraki, P., Rossi, M. N., Saccuti, G., Perez-amador, M. A., Polticelli, F., Angelini, R., & Federico, R. (2006). Heterologous expression and biochemical characterization of a Polyamine oxidase from *Arabidopsis* involved in polyamine back conversion. *Plant Cell Physiology*, ***141***, 1519–1532.

Tavladoraki, P., Schinina, M. E., Cecconi, F., Di Agostinoa, S., Manera, F., Rea, G., Mariottini, P., Federico, R., & Angelini, R. (1998). Maize polyamine oxidase : primary structure from protein and cDNA sequencing. *FEBS Letters*, ***426***, 62–66.

Tena, G., Boudsocqb, M., & Sheena, J. (2011). Protein kinase signaling networks in plant innate immunity. *Curr Opin Plant Biol.*, ***14***(5), 519–529.

Thines, M. (2014). Phylogeny and evolution of plant pathogenic oomycetes-a global overview. *European Journal of Plant Pathology*, ***138***, 431–447.

Thomma, B. P., Penninckx, I. A., Broekaert, W. F., & Cammue, B. P. (2001). The complexity of disease signaling in *Arabidopsis*. *Current Opinion in Immunology*, ***13***(1), 63–68.

Thu-Hang, P., Bassie, L., Safwat, G. M., Trung-Nghia, P., Christou, P., & Capell, T. (2002). Expression of a heterologous S-adenosylmethionine decarboxylase cDNA in plants demonstrates that changes in S-adenosyl-L-methionine decarboxylase activity determine levels of the higher polyamines spermidine and spermine. *Plant Physiology*, ***129***, 1744–1754.

Tiburcio, A. F., & Alcazar, R. (2018). Potential applications of polyamines in agriculture and plant biotechnology. *Methods Mol. Biol*, ***1694***, 489–508.

Tiburcio, A. F., Altabella, T., & Bitrián, M. (2014). The roles of polyamines during the lifespan of plants : from development to stress. *Planta*, ***240*** (1), 1–18.

Tiburcio, A. F., Altabella, T., Borrell, A., & Masgrau, C. (1997). Polyamine metabolism and its regulation. *Physiologia Plantarum,* ***100***, 664–674.

Tiburcio, A., Kaur-Sawhney, R., & Galston, A. (1990). Polyamine metabolism. *The Biochemistry of plants,* ***16,*** 235–283 Academic press.

Tipping, A. J., & McPherson, M. J. (1995). Cloning and molecular analysis of the pea seddling copper amine oxidase. *The Journal of Biological Chemistry*, ***270***(28), 16939–16946.

Torres, M. A., Dangl, J. L., & Jones, J. D. G. (2002). *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proceedings of the National Academy of Sciences*, ***99***(1), 517-522.

Torres, M., Mansfield, J. W., Grabov, N., Brown, I. R., Ammouneh, H., Tsiamis, G., Forsyth, A., Robatzek, S., Grant, M., & Boch, J. (2006). *Pseudomonas syringae* effector AvrPtoB suppresses basal defence in *Arabidopsis*. *Plant Journal*, ***47***(3), 368–382.

Torres, M. A., Jones, J. D. G., & Dangl, J. L. (2005). Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in *Arabidopsis thaliana*. *Nature Genetics*, ***37***, 1130–1134.

Torres, M. A., Jones, J. D. G., & Dangl, J. L. (2006). Reactive Oxygen Species Signaling in Response to Pathogens 1. *Plant Physiology*, ***141***, 373–378.

Toruño, T., Stergiopoulos, I., & Coaker, G. (2016). Plant-pathogen effectors: Cellular probes interfering with plant defenses in spatial and temporal manners. *Annu Rev Phytopathol*, ***54***, 419–441.

Toth, I. K., Bell, K. S., Holeva, M. C., & Birch, P. R. J. (2003). Soft rot erwiniae: From genes to genomes. *Molecular Plant Pathology*, ***4***(1), 17–30.

Toum, L., Torres, P. S., Gallego, S. M., Benavídes, M. P., Vojnov, A. A., & Gudesblat, G. E. (2016). Coronatine inhibits stomatal closure through guard cell-specific inhibition of NADPH oxidase-dependent ROS production. *Frontiers in Plant Science*, ***7*****,** 1851.

Tsuda, K., & Katagiri, F. (2010). Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. *Current Opinion in Plant Biology*, ***13***(4), 459–465.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., & Katagiri, F. (2009). Network properties of robust immunity in plants. *PLoS Genetics*, ***5***(12).

Urano, K, Yoshiba, Y., Nanjo, T., Igarashi, Y., Seki, M., Sekiguchi, F., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2003). Characterization of *Arabidopsis* genes involved in biosynthesis of polyamines in abiotic stress responses and developmental stages. *Plant, Cell and Environment*, ***26***, 1917–1926.

Urano, Kaoru, Hobo, T., & Shinozaki, K. (2005). *Arabidopsis ADC* genes involved in polyamine biosynthesis are essential for seed development. *FEBS Letters* ***579***, *579*, 1557–1564.

Van Der Biezen, E. A., & Jones, J. D. G. (1998). Plant disease-resistance proteins and the gene-for-gene concept. *Trends in Biochemical Sciences*, ***23***(12), 454–456.

Van Leeuwenhoek, A. (1678). Observationes D. Anthonii Leeuwenhoek, de natis e semine genitali animalculis. *Philos Trans R Soc Lond*, ***12***, 1040–1043.

Van Loon, L. C., Geraats, B. P. J., & Linthorst, H. J. M. (2006). Ethylene as a modulator of disease resistance in plants. *Trends in Plant Science*, ***11***(4), 184–191.

Venugopal, S. C., Jeong, R. D., Mandal, M. K., Zhu, S., Chandra-Shekara, A. C., Xia, Y., Hersh, M., Stromberg, A. J., Navarre, D. R., Kachroo, A., & Kachroo, P. (2009). Enhanced disease susceptibility 1 and salicylic acid act redundantly to regulate resistance gene-mediated signaling. *PLoS Genetics*, ***5***(7), 22–24.

Vickers, R. (2004). The Stressconcept in plants: An introduction. *Political Studies*, ***6***(2), 182–194.

Vlot, C., Dempsey, D. A., & Klessig, D. F. (2009). Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, ***47***, 177–206.

Wagner, S., Stuttmann, J., Rietz, S., Guerois, R., Brunstein, E., Bautor, J., Niefind, K., & Parker, J. E. (2013). Structural basis for signaling by exclusive EDS1 heteromeric complexes with SAG101 or PAD4 in plant innate immunity. *Cell Host and Microbe*, ***14***(6), 619–630.

Walden, R., Cordeiro, A., & Tiburcio, A. F. (1997). Polyamines : Small molecules triggering pathways in plant growth and developmentlt. *Plant Ph*, ***113***, 1009–1013.

Wallden, K., Rivera-Calzada, A., & Waksman, G. (2010). Type IV secretion systems: Versatility and diversity in function. *Cellular Microbiology*, ***12***(9), 1203–1212.

Walldén, K., Williams, R., Yan, J., Lian, P. W., Wang, L., Thalassinos, K., Orlova, E. V., & Waksman, G. (2012). Structure of the VirB4 ATPase, alone and bound to the core complex of a type IV secretion system. *Proceedings of the National Academy of Sciences of the United States of America*, ***109***(28), 11348–11353.

Walters, D. R. (2003). Resistance to plant pathogens: Possible roles for free polyamines and polyamine catabolism. *New Phytologist*, ***159***, 109–115.

Walters, D. R., Wilson, P. W. F., & Shuttleton, M. A. (1985). Relative changes in levels of polyamines and activities of their biosynthetic enzymes in barley infected with powdrey mildew fungus, *Erysiphe graminis* DC ex MERAT f.sp. *Hordei marchal*. *New Phytologist*, ***101***(4), 695–705.

Walters, D. R. (2000). Polyamines in plant - microbe interactions. *Physiological and Molecular Plant Pathology*, ***57***, 137–146.

Walters, D. R. (2003). Polyamines and plant disease. *Phytochemistry*, ***64***, 97–107.

Wang, B., Zhou, J., Wang, Y., Zhu, L., & Teixeira da Silva, Jeime, A. (2006). Physical stress and plant growth. *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues, Vol III*, 68–85.

Wang, W., Paschalidis, K., Feng, J. C., Song, J., & Liu, J. H. (2019). Polyamine catabolism in plants : A universal process with diverse functions. *Frontiers in Plant Science*, ***10***.

Wang, W., & Liu, J. (2015). Genome-wide identification and expression analysis of the polyamine oxidase gene family in sweet orange (*Citrus sinensi*s). *Gene*, ***555***, 421–429.

Wang, X., Oh, M. W., & Komatsu, S. (2016). Characterization of S-adenosylmethionine synthetases in soybean under flooding and drought stresses. *Biologia Plantarum*, ***60***(2), 269–278.

Wang, Y., Tyler, B. M., & Wang, Y. (2019). Defense and counterdefense during plant-pathogenic oomycete infection. *Annual Review of Microbiology*, ***73*,** 667–696.

Warmerdam, S., Sterken, M. G., Sukarta, O. C. A., van Schaik, C. C., Oortwijn, M. E. P., Lozano-Torres, J. L., Bakker, J., Smant, G., & Goverse, A. (2020). The TIR-NB-LRR pair

DSC1 and WRKY19 contributes to basal immunity of *Arabidopsis* to the root-knot nematode *Meloidogyne incognita*. *BMC Plant Biology*, ***20***(1), 73.

Warren, R. F., Henk, A., Mowery, P., Holub, E., & Innes, R. W. (1998). A mutation within the leucine-rich repeat domain of the *Arabidopsis* disease resistance gene RPS5 partially suppresses multiple bacterial and downy mildew resistance genes. *Plant Cell*, ***10***(9), 1439–1452.

Warren, R. F., Merritt, P. M., Holub, E., & Innes, R. W. (1999). Identification of three putative signal transduction genes involved in *R* gene-specified disease resistance in *Arabidopsis*. *Genetics*, ***152***(1), 401–412.

Wersch, R. Van, Li, X., & Zhang, Y. (2016). Mighty dwarfs: *Arabidopsis* autoimmune mutants and their usages in genetic dissection of plant immunity. *Frontiers in Plant Science*, ***7***, 1717.

Whalen, M. C., Innes, R. W., Bent, A. F., & Staskawicz, B. J. (1991). ldentification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *The Plant Cell*, ***3***, 49–59.

Wi, S. J., Kim, S. J., Kim, W. T., & Park, K. Y. (2014). Constitutive S-adenosylmethionine decarboxylase gene expression increases drought tolerance through inhibition of reactive oxygen species accumulation in *Arabidopsis*. *Planta*, ***239***, 979–988.

Wi, S. J., Kim, W. T., & Park, K. Y. (2006). Overexpression of carnation S-adenosylmethionine decarboxylase gene generates a broad-spectrum tolerance to abiotic stresses in transgenic tobacco plants. *Plant Cell Rep*, ***25***, 1111–1121.

Wiermer, M., Feys, B. J., & Parker, J. E. (2005). Plant immunity: The EDS1 regulatory node. *Current Opinion in Plant Biology*, ***8***(4), 383–389.

Wildermuth, M. C., Dewdney, J., Wu, G., & Ausubel, F. M. (2002). Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*, ***417***.

Wirthmueller, L., Zhang, Y., Jones, J. D. G., & Parker, J. E. (2007). Nuclear accumulation of the *Arabidopsis* immune receptor RPS4 is necessary for triggering EDS1-dependent defense. *Current Biology*, ***17***, 2023–2029.

Woeste, K. E., Ye, C., & Kieber, J. J. (1999). Two *Arabidopsis* mutants that overproduce ethylene are affected in the posttranscriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. *Plant Physiology*, ***119***(2), 521–529.

Wojtasik, W., Kulma, A., Namysł, K., Preisner, M., & Szopa, J. (2015). Polyamine metabolism in flax in response to treatment with pathogenic and non–pathogenic *Fusarium* strains. *Frontiers in Plant Science*, ***6***, 291.

Wu, D., Roepenack-Lahaye, E. Von, Buntru, M., Lange, O. de, Schandry, N., Rez-Quintero, A. L. P., Weinberg, Z., Lowe-Power, T. M., Szurek, B., Michael, A. J., Allen, C., Schillberg, S., & Lahaye, T. (2019). A plant pathogen type III effector protein subverts translational regulation to boost host polyamine levels. *Cell Host and Microbe*, ***26***, 638–649.

Wu, J., Shang, Z., Wu, J., Jiang, X., Moschou, P. N., Sun, W., Roubelakis-Angelakis, K. A., & Zhang, S. (2010). Spermidine oxidase-derived H_2O_2 regulates pollen plasma membrane hyperpolarization-activated Ca^{2+}-permeable channels and pollen tube growth. *Plant Journal*, ***63***, 1042–1053.

Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindle, I. D., Luca, V. De, & Despre, C. (2012). The *Arabidopsis* NPR1 protein is a receptor for the plant defense hormone salicylic acid. *Cell Reports*, ***1***(6), 639–647.

Xiang, T., Zong, N., Zou, Y., Wu, Y., Zhang, J., Xing, W., Li, Y., Tang, X., Zhu, L., Chai, J., & Zhou, J. M. (2008). *Pseudomonas syringae* effector AvrPto blocks innate immunity by targeting receptor kinases. *Current Biology*, ***18***(1), 74–80.

Xiao, S., Ellwood, S., Calis, O., Patrick, E., Li, T., Coleman, M., & Turner, J. G. (2001). Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by RPW8. *Science*, ***291***(5501), 118–120.

Xin, X.-F., Kvitko, B., & He, S. Y. (2018). *Pseudomonas syringae*: what it takes to be a pathogen. *Nat Rev Microbiol.*, ***16***(5), 316–328.

Yamaguchi, K., Takahashi, Y., Berberich, T., Imai, A., Miyazaki, A., Takahashi, T., Michael, A., & Kusano, T. (2006). The polyamine spermine protects against high salt stress in *Arabidopsis thaliana*. *FEBS Letters*, ***580***, 6783–6788.

Yamaguchi, K., Takahashi, Y., Berberich, T., Imai, A., Takahashi, T., Michael, A. J., & Kusano, T. (2007). A protective role for the polyamine spermine against drought stress in *Arabidopsis*. *Biochemical and Biophysical Research Communications*, ***352***(2), 486–490.

Yamasaki, H., & Cohen, M. F. (2006). NO signal at the crossroads : polyamine-induced nitric oxide synthesis in plants ? *Trends in Plant Science*, ***11***(11), 522–524.

Yang, L., Li, B., Zheng, X. Y., Li, J., Yang, M., Dong, X., He, G., An, C., & Deng, X. W. (2015). Salicylic acid biosynthesis is enhanced and contributes to increased biotrophic pathogen resistance in *Arabidopsis* hybrids. *Nature Communications*, ***6***.

Yang, S., Feng, Z., Zhang, X., Jiang, K., Jin, X., Hang, Y., Chen, J. Q., & Tian, D. (2006). Genome-wide investigation on the genetic variations of rice disease resistance genes. *Plant Molecular Biology*, ***62***, 181–193.

Yang, S., Li, J., Zhang, X., Zhang, Q., Huang, J., Chen, J. Q., Hartl, D. L., & Tian, D. (2013). Rapidly evolving *R* genes in diverse grass species confer resistance to rice blast disease. *Proceedings of the National Academy of Sciences of the United States of America*, ***110***(46), 18572–18577.

Yang, Y., Zhang, H., Li, G., Li, W., Wang, X., & Song, F. (2009). Ectopic expression of MgSM1, a Cerato-platanin family protein from *Magnaporthe grisea*, confers broad-spectrum disease resistance in *Arabidopsis*. *Plant Biotechnology Journal*, ***7***(8), 763–777.

Yang, D. L., Yang, Y., & He, Z. (2013). Roles of plant hormones and their interplay in rice immunity. *Molecular Plant*, ***6***(3), 675–685.

Yin, C., Ramachandran, S. R., Zhai, Y., Bu, C., Pappu, H. R., & Hulbert, S. H. (2019). A novel fungal effector from *Puccinia graminis* suppressing RNA silencing and plant defense responses. *New Phytologist*, ***222***, 1561–1572.

Yoda, H., Fujimura, K., Takahashi, H., Munemura, I., Uchimiya, H., & Sano, H. (2009). Polyamines as a common source of hydrogen peroxide in host- and nonhost hypersensitive response during pathogen infection. *Plant Mol Biol*, ***70***, 103–112.

Yoda, H., Hiroi, Y., & Sano, H. (2006). Polyamine oxidase is one of the key elements for oxidative burst to induce Programmed Cell Death in tobacco cultured cells. *Plant Physiology*, ***142***, 193–206.

Yoda, H., Yamaguchi, Y., & Sano, H. (2003). Induction of hypersensitive cell death by hydrogen peroxide produced through polyamine degradation in tobacco plants. *Plant Physiology*, ***132***, 1973–1981.

Yokoo, S., Inoue, S., Suzuki, N., Amakawa, N., Matsui, H., Nakagami, H., Takahashi, A., Arai, R., & Katou, S. (2018). Comparative analysis of plant isochorismate synthases reveals structural mechanisms underlying their distinct biochemical properties. *Bioscience Reports*, ***38***(2), 1–13.

Yoshimoto, K., Takamura, H., Kadota, I., Motose, H., & Takahashi, T. (2016). Chemical control of xylem differentiation by thermospermine , xylemin , and auxin. *Scientific Reports*, ***6***, 21487.

Yu, G. L., Katagiri, F., & Ausubel, F. M. (1993). *Arabidopsis* mutations at the RPS2 locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Molecular Plant-Microbe Interactions*, ***6***(4), 434–443.

Yuan, Y., Zhong, S., Li, Q., Zhu, Z., Lou, Y., Wang, L., Wang, J., Wang, M., Li, Q., Yang, D., & He, Z. (2007). Functional analysis of rice *NPR1*-like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. *Plant Biotechnology Journal*, ***5***(2), 313–324.

Zacarés, L., López-Gresa, M. P., Fayos, J., Primo, J., Bellés, J. M., & Conejero, V. (2007). Induction of *p* -Coumaroyldopamine and Feruloyldopamine , two novel metabolites , in tomato by the bacterial pathogen *Pseudomonas syringae*. *MPMI*, ***20***(11), 1439–1448.

Zalguizuri, A., Caetano-Anollés, G., & Lepek, V. C. (2018). Phylogenetic profiling, an untapped resource for the prediction of secreted proteins and its complementation with sequence-based classifiers in bacterial type III, IV and VI secretion systems. *Briefings in Bioinformatics*, ***20***(4), 1395–1402.

Zarei, A., Trobacher, C. P., Cooke, A. R., Meyers, A. J., Hall, J. C., & Shelp, B. J. (2015). Apple fruit copper amine oxidase isoforms : Peroxisomal MdAO1 prefers diamines as substrates , whereas extracellular MdAO2 exclusively utilizes monoamines. *Plant and Cell Physiology*, ***56***(1), 137–147.

Zepeda-Jazo, I., Velarde-Buendía, A. M., Enríquez-Figueroa, R., Bose, J., Shabala, S., Muñiz-Murguía, J., & Pottosin, I. I. (2011). Polyamines interact with hydroxyl radicals in activating

$Ca2^{+}$ and k^{+} transport across the root epidermal plasma membranes. *Plant Physiology*, ***157***, 2167–2180.

Zhang, Y. M., Shao, Z. Q., Wang, Q., Hang, Y. Y., Xue, J. Y., Wang, B., & Chen, J. Q. (2016). Uncovering the dynamic evolution of nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes in Brassicaceae. *Journal of Integrative Plant Biology*, ***58***(2), 165–177.

Zhang, Yaxi, Xu, S., Ding, P., Wang, D., Cheng, Y. T., He, J., Gao, M., Xu, F., Lia, Y., Zhua, Z., Lid, X., & Zhang, Y. (2010). Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *PNAS*, ***107***(42), 18220–18225.

Zhang, Y., Goritschnig, S., Dong, X., & Li, X. (2003). A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of *npr1-1*, constitutive 1. *Plant Cell*, ***15***, 2636–2646.

Zhang, Y., & Li, X. (2019). Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Current Opinion in Plant Biology*, ***50***, 29–36.

Zhao, T., Yang, H., Jiang, J., Liu, G., Zhang, H., Xiao, D., Chen, X., Li, J., & Xu, X. (2018). Silencing of the *SAMDC* gene decreases resistance of tomato to *Cladosporium fulvum*. *Physiological and Molecular Plant Pathology*, ***102***, 1–7.

Zhong, S., & Chang, C. (2012). Ethylene signalling: The CTR1 protein kinase. *Annual Plant Reviews*, ***44***, 147–168.

Zhou, J., Wu, S., Chen, X., Liu, C., Sheen, J., Shan, L., & He, P. (2014). *Pseudomonas syringae* effector HopF2 suppresses *Arabidopsis* immunity by targeting BAK1. *Plant Journal*, ***77***(2), 235–245.

Zhou, N., Tootle, T. L., Tsui, F., Klessig, D. F., & Glazebrook, J. (1998). PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. *Plant Cell*, ***10***(6), 1021–1030.

Zhou, T., Wang, Y., Chen, J. Q., Araki, H., Jing, Z., Jiang, K., Shen, J., & Tian, D. (2004). Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. *Molecular Genetics and Genomics*, ***271***(4), 402–415.

Zipfel, C., & Felix, G. (2005). Plants and animals: A different taste for microbes? *Current Opinion in Plant Biology*, ***8***(4), 353–360.