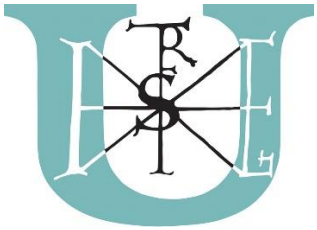
10.14751/SZIE.2017.008



SZENT ISTVÁN UNVERSITY

Powdery mildew-induced gene expression in a susceptible grapevine – the role of a novel grape NAC transcription factor in response to infection

PhD dissertation

Zsófia Makovecz-Tóth

Gödöllő

2016

10.14751/SZIE.2017.008

PhD school

name: Plant Science PhD School

discipline: Plant- and Horticultural Sciences

head: Dr. Lajos Helyes
Professor, DSc.
Szent István University,
Faculty of Agricultural and Environmental Sciences
Institute of Horticulture Technology Research

supervisor: Dr. Erzsébet Kiss
Professor, CSc.
Szent István University,
Faculty of Agricultural and Environmental Sciences
Institute of Genetics and Biotechnology

…… ……

Dr. Erzsébet Kiss
supervisor

Dr. Lajos Helyes
head

10.14751/SZIE.2017.008

TABLE OF CONTENTS

10.14751/SZIE.2017.008

ABBREVIATIONS

ABA	Abscisic Acid
Bgh	*Blumeria graminis* f. sp. *hordei*
BTH	Benzothiadiazole
DAMP	Damage-Associated Molecular Pattern
DTI	DAMP-triggered Immunity
ET	Ethylene
ETI	Effector Triggered Immunity
GA	Gibberellic Acid
GFP	Green Fluorescent Protein
GUS	β-glucuronidase gene
HR	Hypersensitive Response
INA	2,6-Dichloroisonicotinic acid
JA	Jasmonate
MAMP	Microbe-Associated Molecular Pattern
MAS	Marker Assisted Selection
MeSA	Methyl Salicylate
NAC	NAM, (no apical meristem), ATAF (*Arabidopsis* transcription activation factor), CUC (cup-shaped cotyledon)
NB-LRR	Nucleotide-Binding– Leucine-Rich Repeats
PAMP	Pathogen-Associated Molecular Pattern
PCD	Programmed Cell Death
PM	Powdery Mildew
PR	Pathogenesis-Related
PRR	Pattern Recognition Receptors
PTI	PAMP-Triggered Immunity
ROS	Reactive Oxygen Species
qPCR	Quantitative PCR
SA	Salicylic Acid
SAM	Shoot Apical Meristem
SAR	Systemic Acquired Resistance
TF	Transcription Factor

10.14751/SZIE.2017.008

10.14751/SZIE.2017.008

1. INTRODUCTION

Grapevine is an economically important plant due to its diverse consumption and prominent nutritional values of its fruit. Numerous studies demonstrated that a wide variety of berry compounds have therapeutic and health promoting properties, which corroborate the usefulness of grapes in dietetic habits. This plant is one of the oldest cultivated fruit crops. Owing to extensive plant breeding efforts, numerous cultivars are available to supply the market with large variety of products such as fruits, wines, juices, jams and raisins.

Grapes rank 14th among the top agricultural products in the world. According to FAO data the world's total land area dedicated to growing grapes is approximately 75,866 square kilometers. 71 % of the grapes grown is used for wine making, 27% for fresh fruit, and 2% for dried fruit. The greatest grape producer of the world is China with its 12,550,024 tons per year production (2015); the main produce being fresh table grape. The second largest is Italy, the third is the USA based on the FAOSTAT data, but Spain, France and Turkey also belong to the top ten producers (Figure 1). While demands for grapes are increasing each year all over the world, the side-effects related to its production such as diseases are also on the rise.

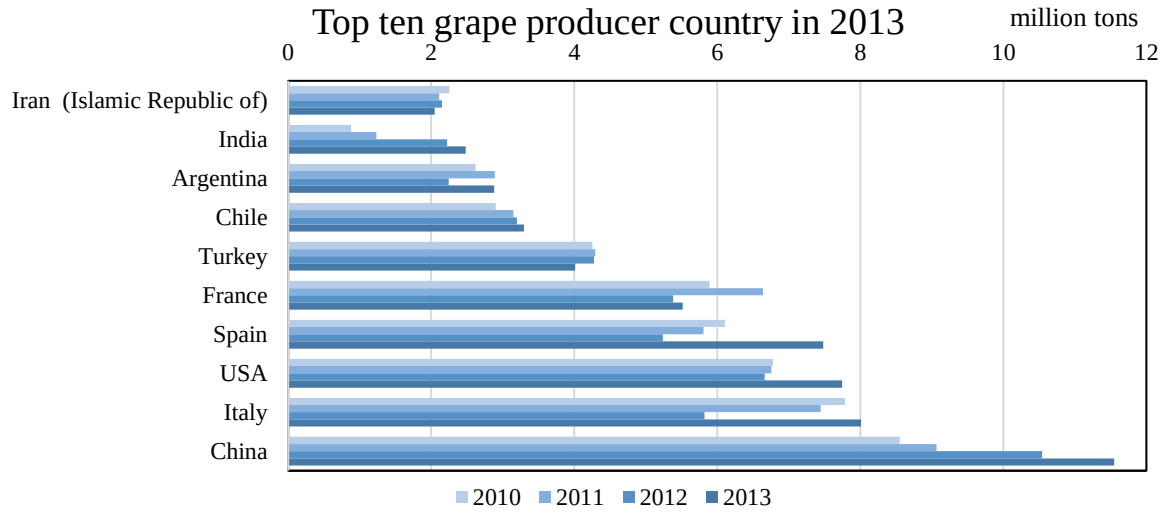


Figure 1. Top ten grape producer countries based on FAOSTAT data. The bars represent the amount of produced grape in million tons per year. The order is estimated based on the amount in 2013. (Data source: Food and Agriculture Organization of the United Nations Statistics Division).

Although numerous studies reported remarkable results with regard to elucidating the genetic and molecular background of diseases the pathogens can still cause extensive damage in the vineyards. The most notorious fungal disease of grapevine is the powdery mildew (PM) caused by *Erysiphe necator*. The growers make extensive efforts to control the spread of the fungus by

the application of chemicals, which are only effective in limiting the disease. Not only are these chemicals expensive and does their application considerably increase the prime cost of production but the applied amounts are rising continuously throughout the world. In addition to their high price, the high risk of their potentially harmful impact on the environment and consumers is also a considerable disadvantage. Due to the toxicity of chemical residues the number of morbidity cases increases all over the world. The human health hazards range from acute dangers to serious chronic diseases such as cancer and endocrine disruption. Therefore, the studies focusing on biologically limiting the disease of grapevine are exceptionally conducive. Researches exploring the molecular background of plant defense and striving to improve the resistance in susceptible varieties support the overall goal of limiting the application of chemicals in vineyards.

Plant-pathogen interactions involve a complex signal exchange and have four main possible consequences: (i) Plants may recognize their aggressors by the signs of the pathogen which then induce an effective immune response. (ii) However, pathogens are able to repress the plant's defense system using their effector molecules, therefore these plants are susceptible. (iii) Some plants have a second line of defense capable of recognizing these effector molecules. These plants have resistance (*R*) genes encoding nucleotide-binding leucine-rich repeat proteins which detect effectors and trigger a massive defense response. (iv) If the R protein is incompatible with the pathogen effector, the R protein does not interact with the effector and the robust response fails. Above all, the evolution of infection is more complex and the level of resistance may also depend on the timing and rate of expression of defense genes. Additionally, phytohormone-mediated signaling also participate effectively in defense regulation.

Earlier studies demonstrated that the levels of salicylic acid (SA) in the leaf tissue of *Vitis vinifera* cv. 'Cabernet Sauvignon' increased in response to *E. necator* infection as a function of time after inoculation. In contrast, the PM-resistant *V. aestivalis* had constitutively high levels of SA which suggests that the SA is responsible for constitutive resistance in plants. The aim of the present study was to test whether exogenous SA application was able to induce the same response as triggered by PM infection in a susceptible grape variety. Our further aim was to investigate the regulation of a PM-dependent gene.

10.14751/SZIE.2017.008

2. **OBJECTIVES**

1. Our goal was to investigate the response of a susceptible grape variety ‘Cabernet Sauvignon’ to PM infection. The reaction was planned to be measured on the basis of gene expression variation.
2. Our further aim was to test whether the SA-treatment was able to change the rate of expression of genes similarly to PM.
3. We planned subsequent investigation of NAC transcription factor identified in the global expression analysis:
    a. We tested the regulation of *NAC* gene. The aim of the investigation was to prove that the SA is not required, or required but insufficient by itself for the induction of *NAC*.
    b. Furthermore, we planned to determine the putative regulatory elements in the *NAC* promoter by using deletional analysis along with *cis*-element databases.
4. We also aimed at comparing the regulation of *NAC* promoter to the regulation of *Arabidopsis* ortholog, *JUB1*.

3. LITERATURE REVIEW

Powdery mildew pathogens are ascomycetous fungi and belong to the *Erysiphales* Order. All PM species are obligate parasites, meaning that the fungi survive only on a living host plant. Therefore, these organisms extract nutrients from plant cells without killing them. PM fungi have a wide range of host plants: they are common on both monocotyledonous and dicotyledonous plants. The 700 known PM species have a collective host range of about 650 monocot and over 9,000 dicot plant species (Schulze-Lefert and Vogel, 2000; Ridout, 2001).

3.1 The major disease of grapevine – powdery mildew

Grapevine PM is caused by the obligate biotrophic Ascomycetes fungus, called *Erysiphe necator* (formerly, *Uncinula necator*) (Schw.) Burr. (*E. necator* is classified in *Erysiphaceae* Family, in *Erysiphales* Order, in *Leotiomycetes* Class, and in *Ascomycota* Division). Races of this pathogen are able to cause infection in the genera within the *Vitaceae* Family, involving *Ampelopsis*, *Cissus*, *Parthenocissus* and *Vitis* species (Pearson and Gadoury, 1992). Among its hosts grapevine is the most economically important besides a wide range of grape cultivars which are extremely susceptible to the powdery mildew disease. This group includes almost all of the *V. vinifera* (n=19) varieties (i.e. Cabernet Sauvignon, Chardonnay). While the fungus is originated from North America many PM-resistant French-American hybrids (‘Chambourcin’, ‘Vidal’, ‘Vignole’) or Native American species (*V. aestivalis* cv. ‘Norton’, *V. labrusca* cv. ‘Catawba’, ‘Cayuga’, ‘Concord’, *V. mustangensis* cv. ‘Mustang’, *V. rotundifolia* cv. ‘Muscadine’) exist, which can be used for vineyard establishment as an alternative approach to controlling PM disease. Regrettably, many of these species fail to produce as high quality wine as *V. vinifera* species. However, these species may still be used as resistance source for improving new varieties, such as the *Muscadinia rotundifolia* (n=20) containing the *RUN1* gene or the *V. vinifera* cv. ‘Kismish vatkana’ (n=19) carrying the *REN1* gene (Pauquet *et al.*, 2001; Donald *et al.*, 2002; Barker *et al.*, 2005; Hoffmann *et al.*, 2008; Coleman *et al.*, 2009; Katula-Debreceni *et al.*, 2010). The new grapevine varieties developed from these gene sources may inherit increased resistance to various pathogens rather than only to PM (Deák *et al.*, 2014; Kozma *et al.*, 2014).

3.1.1 Life cycle of *Erysiphe necator*

E. necator is a heterotallic haploid fungus and has two kinds of mating types. The life cycle is supported by both sexual and asexual overwintering stages. In the asexual form the mycelia and conidia overwinter in dormant buds. The mycelium is a very thin (4-5 μm in diameter) hair-like structure. The specialized branch of mycelium that produces 1-10 conidia is called conidiophore. In spring the mycelium infects the plant from dormant buds (primary infection). The infected buds

produce stunted and distorted shoots, which is a typical phenomenon named ‘flag shoot’. From the conidiophores the conidia are dispersed by wind or insects onto the leaf surface. The conidia germinate at 7-31°C with 30-100% relative humidity to produce the hyphae and the feeding organs called haustoria. In the sexual form the cleistothecia overwinter in bark ruptures or in the soil. Cleistothecia are spherical, dark brown (matured) structures (diameter of 84 to 105 µm), containing 4-6 asci. The asci hold 4-6 oval ascospores, which are released in the spring and spread by rainfall on the leaf surface to develop the secondary infection. The colonies produce cleistothecia in late summer (Pearson and Gadoury, 1992; Gadoury *et al.*, 2012).

The conidia and/or ascospores of the most PM species germinate and develop appressorial germ tube in as little as two hours after the inoculation (hai). Five hai the penetration peg is formed and 24 hai the haustorium is also evolved. The fungus acquires the useful nutrients through this organ (Figure 2) since this is the only direct connection to the host. During its penetration only the cell wall is punctured while the cytoplasm membrane remains intact surrounding the haustorium. At 72 hai the conidiophores are formed and intensive development of peripheral hyphae can be observed. Under ideal conditions five days after inoculation (dai) extensive fungal growth and reproduction may be detected.

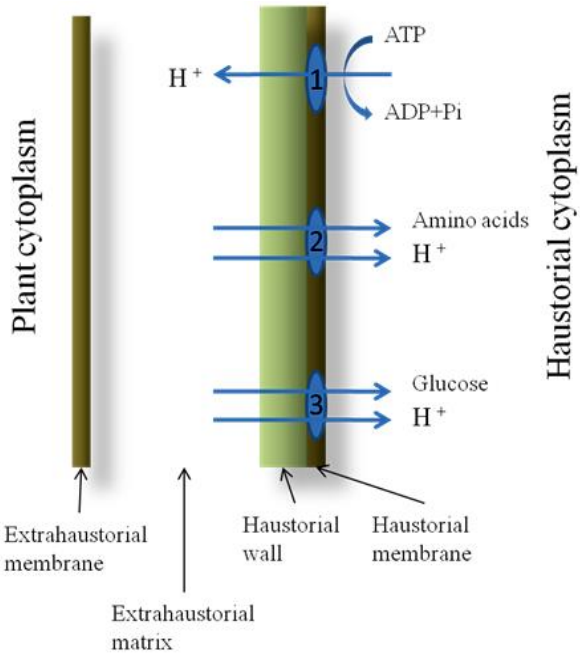


Figure 2. A schematic diagram showing nutrient transport through the haustorial membrane. 1-H^+-ATPase, 2-Amino acid transporter, 3-Glucose/fructose transporter (Szabo and Bushnell, 2001; Voegele *et al.*, 2001).

E. necator is able to infect green, photosynthetically active tissues of the plant and is also able to manipulate plant metabolism to retard senescence of the infected tissue (‘green island’

effect) (Bushnell and Allen, 1962). Macroscopically visible colonies (whitish along with metallic sheen of young colonies; grayish color of senescent colonies) are the signs of the infection on the surface of the plant. Although PMs do not kill the plant, the infection of inflorescence and berries present the gravest economical problems for wine makers. Particularly, the growth of the PM coated young berry skin is blocked, therefore, the berries may split as expanding in the course of time (Gadoury *et al.*, 2012). This provides an opportunity for rotting and the appearance of *Botrytis cinerea*, which adds a musty taste for the wine.

3.1.2 **Plant defense system**

In plant defense system we can distinguish non-host and host resistance (Thordal-Christensen, 2003). In case of non-host protection the infection capability of the pathogen is limited to a certain range of hosts, sometimes to a single genus, as in the case of powdery mildews, rusts and some bacteria. All the other plant species act as non-host plants for these invaders, in which cases the attackers are referred to as non-host pathogens. Non-host resistance provides durable total protection against microbes and this is the most common defense regulatory mechanism in plants. Surprisingly, this compatible host-pathogen interaction is a rare event, since the road to manifestation of disease involves numerous challenges. The infecting microbe must overcome several host defense barriers in order to develop the disease symptoms successfully. Thordal-Christensen (2003) described that pathogen must battle with five main obstacles to cause disease; if it fails to overcome one of them, it is likely a non-host pathogen. The first barrier is (i) the surface pattern (i. e. surface wax component) of the plant, which was found to influence the initiation of fungal cell differentiation in *Blumeria graminis* f. sp. *hordei (Bgh)* (Tsuba *et al.*, 2002). The subsequent barricades are (ii) the pre-formed cell wall, antimicrobial compounds and secondary metabolite production. If the pathogen is able to overcome these components, it will succeed in developing the disease (Papadopoulou *et al.*, 1999).

At certain levels of defense response the host- and non-host resistance may have overlapping components, which are independent of the genotype of the attacker. As the third barrier, (iii) plants may recognize its aggressors by pathogen- or microbe-associated molecular patterns (PAMP, MAMP) (Pieterse *et al.*, 2009). These specific patterns may be the bacterial flagellins, specific surface lipopolysaccharides, nucleic acids (e.g. viral and bacterial DNA/RNA), peptidoglycans, fungal chitins or glucans. These general elicitor molecules may be detected by plant leucine-rich repeat (LRR) receptor kinases, which then induce the PAMP-triggered immunity (PTI). During PTI process mainly those genes are activated which are involved in the biosynthesis of antimicrobial compounds, promote pathogenic cell wall degradation, regulate plant cell wall fortification or induce stomatal closure. A well-studied example for this type of immunity is the papilla formation, which totally blocks the further injection of the fungal peg. This local wall

fortification provides total resistance against host and non-host pathogens as well. The papilla is composed of callose, phenolic compounds and reactive oxygen species (ROS), which are transported to the site of infection by PEN1 and SNARE proteins (Nielsen and Thordal-Christensen, 2013). Furthermore, the papilla formation is negatively regulated by the *Mildew Resistance Locus O* (*MLO*) gene and a mutation in this gene resulted in total resistance against *Bgh* (Jørgensen, 1976). These papilla-related vesicle trafficking components are conserved among the host and non-host plant species (Consonni *et al.*, 2006). Besides PAMPs, the plant cell may detect microbial compounds released by the injured microorganisms. For example, cucumber hypocotyl recognized the α-1,4-linked oligomers of galacturonic acid and oligo-β-glucans released from damaged *Phytophthora megasperma* f. sp. *glycinea* cell wall, which then triggered hydrogen-peroxide production (Svalheim and Robertsen, 1993). This phenomenon is called Damage Associated Molecular Pattern (DAMP), and provokes DAMP-triggered immunity (DTI).

Biotrophs must acquire nutrients from the host plant, therefore, they manipulate plant to develop a specialized cytoplasm membrane around the fungal haustoria. The fungi modulate plant metabolism using its secreted effector proteins and these molecules are translocated through this specialized membrane. The entire process requires compatibility which have been developed in the course of long host-pathogen co-evolution. Therefore, this fourth obstacle (iv) restrict the adaptation of pathogens to a certain host range (Thordal-Christensen, 2003). Once the pathogen overcomes the prevention tricks and enters the plant cell, the plant recognizes the pathogen-secreted effector proteins by its 'receptors'. These 'receptors' are encoded by resistance *(R)* genes while the effector proteins are specified by the pathogenic *avr* genes (the term *avr* is only used in bacterial pathogens). The mutual recognition of effectors by R proteins corresponds to the fifth (v) barrier, which is the host resistance. This is a typical phenomenon in biotrophic infections (Keller *et al.*, 2000). If these two proteins interact, the reaction induces a complex signaling cascade, which may end with systemic acquired resistance (SAR), or with uncontrolled form of cell death referred to as the hypersensitive reaction (HR) (Király *et al.*, 1972; Fodor *et al.*, 1997). Both responses are mediated via SA-signaling and significantly limit the biotrophic pathogen growth with the HR totally inhibiting further spread of the fungus (Thordal-Christensen *et al.*, 1997). This type of immune response is called the effector-triggered immunity (ETI), which is based on the specific recognition of effectors by R proteins.

Typical *R* genes encode the nucleotide-binding site leucine-rich repeat (NB-LRR) domains, which are able to interact with fungal effector proteins. *R* genes are usually dominant alleles and may recognize the PAMPs, may be direct targets in the mutual recognition, may support the target, or may have detoxification function. *R* genes regulate robust resistance against a specific pathogen

or a pathogen race. However, this robustness is only maintained until a new virulent strain appears (Van Schie and Takken, 2014).

3.1.3 **Plant resistance genes**

As the first key element of PTI, the pattern recognition receptors (PRR) detect the PAMPs, or DAMPs of the pathogen. The first PRR discovered was the *Xa21* gene in *Oryza sativa*, which encodes both extracellular LRR and transmembrane protein kinase (Song *et al.*, 1995) (Figure 3). This protein complex was responsible for the recognition of Ax21 (activator of Xa21 triggered immunity) peptide of the bacterium *Xanthomonas oryzae* pv. *oryzae* (Lee *et al.*, 2009). After detection, Xa21 activated intracellular defense response. In plant breeding the integration of a single locus of this gene resulted in increased resistance to several bacterial blight isolates (Wang *et al.*, 1996; Zhai *et al.*, 2002).

As an example for the recognition of fungal PAMP, the chitin of the pathogen cell wall was detected by the CEBiP transmembrane protein and induced immunity in rice (Zipfel and Robatzek, 2010). Similarly, the Elongation Factor Tu (EF-Tu) bacterial peptide was recognized by the plant EF-Tu receptor (EFR). EFR belongs to the same subfamily as the leucine-rich repeat-receptor-like protein kinase (LRR-RLK) flagellin-sensitive 2 (FLS2) (Figure 3), which was also found to regulate defense responses (Zipfel *et al.*, 2006). Insertion of the *EFR* gene into the wheat genome enhanced resistance to bacterial diseases (Schoonbeek *et al.*, 2015). FLS2 protein was found to be ethylene (ET)-dependent, and to be integrated into the plasma membrane. FLS2 bound to the bacterial flagellin 22-amino-acid epitope (Flg22) at an early stage of bacterial infection. This interaction with Flg22 regulated FLS2 to associate with BAK1, another LRR-receptor-like kinase. The FLS2-BAK1 complex then activated *Botrytis-Induced Kinase 1* (*BIK1*) gene, which triggered the mitogen activated protein kinase (MAPK) cascade and governed the defense response (Veronese *et al.*, 2006; Nicaise *et al.*, 2009; Lu *et al.*, 2010). BIK1 was found to positively manage defense against necrotrophs but repressed the response to the virulent biotrophic bacteria *Pseudomonas syringae* pv *tomato* (Veronese *et al.*, 2006). Additionally, the FLS2 physically associated with the resistance proteins RPM1, RPS2 and RPS5, all of which are involved in regulating in ETI (Qi *et al.*, 2011). The results suggest that some of the signaling components of PTI overlap with the ones of ETI (Thomma *et al.*, 2011).

The *R* genes encoding proteins for the mutual recognition of effectors usually belong to the NB-LRR superfamily. These genes code for a central nucleotide binding site (NB) and leucine-rich repeat (LRR) domain at the C-terminal. These proteins are categorized into two groups based on the N-terminal domain: (i) the TIR (Toll/Interleukin-1 receptor-like domain) group genes and (ii) the non-TIR or Coiled-coil (CC) NB-LRRs (Dangl and Jones, 2001; Gururania *et al.*, 2012) (Figure 3).

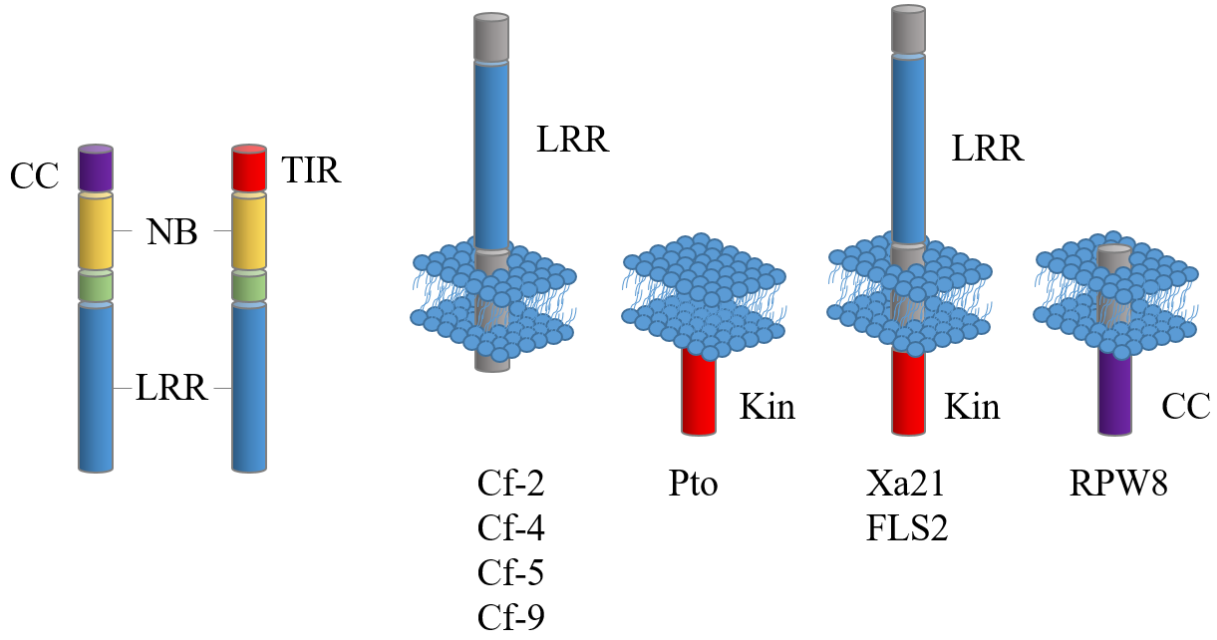


Figure 3. Five main classes of disease resistance proteins. CC-coiled coil domain, TIR- Toll/Interleukin-1 receptor-like domain, NB-nucleotide-binding site, LRR- leucine-rich repeat domain, Kin- serine/threonine kinase domain (Dangl and Jones, 2001).

NB-LRR proteins play a role in the regulation of effector triggered immunity. They may bind directly to the pathogen effector or guard other protein for completion the interaction in order to induce defense response (DeYoung and Innes, 2006). For example, the tomato Bs4 (a TIR-NB-LRR protein) complex was detected to interact directly with the AvrBs4 effector protein of *Xanthomonas campestris* pv. *vesicatoria*. *Bs4* represents high homology to the tobacco *N* and potato *Y-1* resistance genes (Schornack *et al.*, 2004).

The barley *Mla1* and *Mla6* genes were found to be active during PM infection in barley. These genes code CC-NB-LRR proteins; especially the *Mla6* activated *RAR1* and *SGT1* resistance genes for induction of immunity against PM (Shen *et al.*, 2003). Furthermore, the *RAR1* was required for activation of the tobacco *N* gene against TMV too (Liu *et al.*, 2002).

The Pi-ta CC-NB-LRR protein directly interacted with the rice blast fungus AVR-Pita effector. Only a single amino acid change in the protein altered resistance trait to susceptibility (Bryan *et al.*, 2000).

The *RPW8* gene found in *Arabidopsis* codes an N-terminal transmembrane protein and a CC-domain (Figure 3). The *RPW8.1* and *RPW8.2* regulated defense responses through the SA-mediated signaling and associated with *PAD4*, *EDS5*, *NPR1* and *SGT1b* defense genes to activate PM resistance and HR. These *RPW8* genes are independent of COI1 (coronatine-insensitive 1)-, and EIN2 (ethylene-insensitive 2)-mediated signaling pathways. However, the Enhanced Disease

Resistance 1 (EDR1) negatively regulated the activity of *RPW8.1* and *RPW8.2* in response to PM (Xiao *et al.*, 2005).

The tomato *Cf* genes has been used for decades to improve resistance in crop plants. These genes encode extra-cytoplasmic LRRs and C-terminal membrane anchors (Jones *et al.*, 1994) (Figure 3). The Cf-4 protein interacting with Avr4 effector of *Cladosporium fulvum*, triggered ETI and HR in tomato. Interestingly, Cf-4 also recognized homologous cognate effector proteins secreted by other pathogen species (Stergiopoulos *et al.*, 2010).

Most of the *R* genes encoding NB-LRR proteins are putatively localized in the cytoplasm. However, the *A. thaliana RRS1-R* gene coding a TIR-NB-LRR complex harbors a nuclear localization signal and a WRKY-type DNA binding domain at the C-terminal extension. There are several hypotheses concerning how this protein activates defense against the wilt (*Ralstonia solanacearum*): (i) the PopP2 effector contains also a nuclear localization signal and in this way the interaction with RRS1-R is achieved inside the nucleus; or (ii) the RRS1-R is located in the cytoplasm in an inactive form and following the interaction the complex of RRS1-R-PopP2 is transmitted together into the nucleus (Lahaye, 2002; Deslandes *et al.*, 2003).

The tomato Pto intracellular Ser/Thr-protein kinase (Figure 3) activated ETI with guarding the Prf TIR-NB-LRR protein. Pto interacted directly with AvrPto or AvrPtoB elicitors secreted by the bacteria *P. syringae* pv. *tomato* (Oh and Martin, 2011).

Discovery of *R* genes provide further evidence that resistance proteins guide a few host proteins for effector recognition, rather than making a direct contact with pathogen secreted proteins. Based on the guard model a single R protein may be able to interact with multiple effectors and other *R* genes are transcribed in order to guide their interaction and trigger immunity (Zhang *et al.*, 2013b).

Host selective toxins (HSTs) are effective weapons of necrotrophs to kill the plants. These phytotoxins generate necrotic lesions in plant tissues and forward the colonization of the pathogen. Phytoalexins then accumulated in order to detoxify HSTs. For example, camalexin, a phytoalexin specific to a group of cruciferous species accumulated in response to *Alternaria brassicicola* infection in *Arabidopsis* (Saga *et al.*, 2012). Furthermore, camalexin also increased resistance against *Botrytis cinerea* and *Leptosphaeria maculans* (Bohman *et al.*, 2004).

The defensins are small cysteine-rich molecules and have antimicrobial activity. These compounds are able to inhibit the virulence of microorganisms directly by alteration of the fungal membrane permeability, or may enhance plant innate immunity by triggering programmed cell death (Aerts *et al.*, 2008; Hegedüs and Marx, 2013). These genes are widespread among plants, insects and mammals, therefore, they probably have common ancestral origin. The plant defensin proteins inhibit the colonization of a broad range of filamentous ascomycetes such as *Fusarium*

graminearum, *B. cinerea*, or *A. brassicicola* (De Zelicourt *et al.*, 2007; Stotz *et al.*, 2009; Sagaram *et al.*, 2011). Especially the *PDF1.2* plant defensin gene was found to be JA-dependent. The JA signaling-deficient mutant depressed the *PDF1.2* expression and showed high susceptibility to necrotrophic fungus (Veronese *et al.*, 2004). *PDF1.2* probably depends on BIK1-mediated pathway also because induction of the gene was significantly lower in the *bik1* mutant in response to pathogen infection than in wild genotype (Veronese *et al.*, 2006).

Plants are able to express genes which directly deactivate hazardous compounds of the pathogen. For example *Hm1* a specific detoxification gene found in maize and encoding HC toxin reductase neutralized the cyclic tetrapeptide toxin produced by *Colchiobolus carbonum* (Johal and Briggs, 1992).

Effectors triggered conformational changes of R proteins induces a complex avalanche response in plant cell (Figure 4. Schematic representation of major pathogen-related signal transduction pathways in plant cells (Yang *et al.*, 1997).Figure 4). These interacting proteins may phosphorylate MAPK cascade, which then activates transcription factors along with expression of defense genes. Another early response to the compatible interaction is the activation of ion fluxes, which transfuse Ca^{2+} ions inside the cell lumen. Ca^{2+} accumulation activates calmodulin to downstream regulate nitric oxide and ROS abundance (Ma *et al.*, 2008). The early, robust accumulation of ROS results in a "suicide" command for the plant cell and as a result the fungus is prevented from acquiring nutrients from the dead plant tissue.

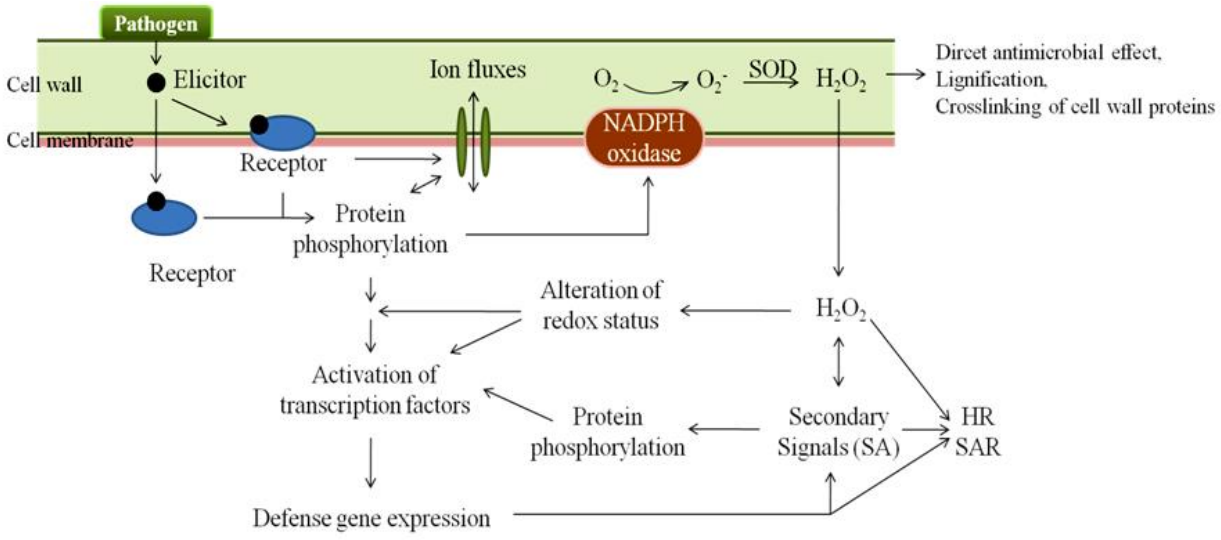


Figure 4. Schematic representation of major pathogen-related signal transduction pathways in plant cells (Yang *et al.*, 1997).

Hydrogen-peroxide is the most stable ROS that may also fulfill signaling function. This molecule regulates the glutathione-related redox status and activates the SA-mediated signal

transduction. The SA is known to be the active signal in defense response against biotrophic pathogens (Glazebrook, 2005). Additionally, this signal induces the expression of pathogenesis-related (*PR*) genes which encode glucanases, chitinases, peroxidases and other antimicrobial compounds. Further results proved that SA is required for HR and to the systemic acquired resistance (SAR) (Vernooij *et al.*, 1994; Ryals *et al.*, 1996; Mauch-Mani and Métraux, 1998; Métraux, 2001). SAR provides innate immunity of the uninfected parts of plant and blocks any further infection of the pathogen.

Mutational analysis is a prominent approach for identifying components of the signaling pathways in immunity. Many recessive mutations result in a constitutive defense response such as the *acd2* (accelerated cell death), *cim3* (constitutive immunity), *cpr1-1* (constitutive expressor of pathogenesis-related (PR) genes), *edr1* and *lsd* (lesion stimulating disease) inactivation. However, other mutations were found to compromise the defense response. The mutants showing SA-signaling deficiency include the *npr1* (non-expressor of pathogenesis-related (*PR*) genes 1)/*nim1* (non-inducible immunity 1), *sai* (SA insensitive); *pad4* (phytoalexin deficient 4), *eds1* (enhanced disease susceptibility 1). The mutants demonstrating ethylene (ET)-signaling deficiency is the *ein2*, while the one with jasmonic acid (JA)-signaling deficiency is the *coi1*. All of these mutant plants displayed increased susceptibility to pathogen infections, meaning that these signaling pathways are also components of defense reaction (Yang *et al.*, 1997; Brodersen *et al.*, 2002; Wang *et al.*, 2002; Glazebrook, 2005; Katsir *et al.*, 2008). Functional screens on these mutants provide better insight into the role of defense genes in signaling pathways.

3.1.4 **Role of salicylic acid in defense response**

The signaling pathways involve a complex array of components between pathogen and plant. In the host plant, the intermediates may include hormonal signals, such as SA, JA or ET, but recent studies demonstrated that growth hormones auxins, cytokinins, abscisic acid (ABA), gibberellic acid (GA) and brassinosteroids (BRs) may also function in defense signaling (Fan *et al.*, 2009; Naseem and Dandekar, 2012; De Bruyne *et al.*, 2014). Biochemical and genetic studies in *A. thaliana* have shown that SA and JA/ET are able to regulate expression of defense genes (Glazebrook, 2005). Increasing endogenous SA content is associated with activation of *PR* gene expression (Shah, 2003) and defense responses in a wide variety of plants. A study showed that in tobacco and in *Arabidopsis*, the same set of *PR* genes responded to exogenous SA application as those activated during the SAR development (Ward *et al.*, 1991; Uknes *et al.*, 1992; 1993). In transgenic *Arabidopsis* and tobacco containing the salicylate hydroxylase gene (*nahG*, originally from *Pseudomonas putida*) SA content was eliminated, and the plant failed to develop SAR (Gaffney *et al.*, 1993; Delaney *et al.*, 1994). Resistance was restored in *nahG* plants with functional analogs of SA (INA, BTH), which are not substrates of SA-hydroxylase (Delaney *et al.*, 1994;

Vernooij *et al.*, 1995; Friedrich *et al.*, 1996). An interesting study showed that *lsd* mutants developed necrotic lesions around pathogen infection but after crossing the mutants with *nahG* plants the F1 progeny lost its resistance along with the SAR-related gene expression being suppressed. Lesion formation was restored after salicylic acid application, which corroborate the fact that SA plays an important role in the defense response against pathogens (Weymann *et al.*, 1995). A similar response was identified in *cpr1*, *cim3* and *cep1* mutants (Bowling *et al.*, 1994; Ryals *et al.*, 1996; Silva *et al.*, 1999) after crossing them with *nahG Arabidopsis* plants.

However, the translocated signal in the activation of SAR is not SA (Vernooij *et al.*, 1994), as it was demonstrated in grafting experiments. Although *nahG*-transgenic tobacco eliminates SA, the rootstock was able to produce a long-distance signal that activates SAR (Vernooij *et al.*, 1994). The SAR inducer mobile signal is the methyl-salicylate (MeSA, SA analog), which is transferred from the infected leaves to the uninoculated part of the plant where it is then transmuted back to SA (Shulaev *et al.*, 1997). In some cases, SAR is independent of SA; *Arabidopsis* roots inoculated with bacteria *P. fluorescens* induced SAR against *F. oxysporum* and *P. syringae* pv. *tomato* without SA accumulation or SA-induced *PRs* expression (Pieterse *et al.*, 1996).

Fung and coworkers (2008) measured the total SA content of PM infected leaf-tissue of *V. aestivalis* (resistant to PM) and *V. vinifera* (susceptible to PM) in comparison with mock-inoculated plants using HPLC. They observed that *V. vinifera* SA levels increased in the infected leaf tissue as a function of time after inoculation. In contrast, *V. aestivalis* had a constitutively high SA level, which suggests that the SA is responsible for constitutive resistance in grapevine. Interestingly, exogenously applied SA did not increase resistance in *V. vinifera*. The SA may play a role in the defense pathway but probably insufficient by itself to increase resistance of a susceptible grape variety.

However, susceptibility of the plant to diseases is not always an obligatory result of host immunity failure. Earlier studies demonstrated that susceptibility of many plant species depends on host compatibility factors, rather than on early responses of PTI or *R* genes. Numerous genes have been identified to play a role in advancing pathogen proliferation, especially of biotrophic fungi, which require cooperation of host compatibility factors for their invasion. The genes impairing pre-penetration requirements – enabling the pathogen to enter the plant cell – or fulfilling post-penetration necessaries are termed as susceptibility *(S)* genes.

3.1.5 **Compatibility factors, evolution of susceptibility**

In contrast to the dominant resistance genes, the susceptibility genes increase resistance if they lose their function; therefore, these genes are beneficial to enhance pathogen tolerance only in recessive form. The susceptibility genes may allow accommodation of the attacker, may

suppress defense response or aid the pathogen to be supplied by nutrients/water (Van Schie and Takken, 2014).

Pathogens enter the plant cell by punching the cell wall, or intrude through wounds and leaf stomata. These entry processes may also be achieved by the assistance of plant. Many genes are identified to be required in the host for compliance of infection. If that particulate gene is not carried by the plant, the pathogen may not be able to infect it. The first *S* gene to be discovered was the earlier mentioned *MLO*, identified in barley (Jørgensen, 1976). The MLO is a seven transmembrane protein, which is integrated into the cell membrane and supports development of haustoria of filamentous biotrophs. This S factor requires Ca^{2+} and calmodulin to suppress defense responses (Ayliffe and Lagudah, 2004) and is independent of JA/ET or SA-mediated signaling in *Arabidopsis* (Consonni *et al.*, 2006). However, a subset of grapevine *MLOs* was found to be SA-inducible (Feechan *et al.*, 2008). The loss of function *mlo* resulted in an interaction between syntaxin, SNARE (Ror2) and SNAP (HvSNAP34) proteins and promoted the fusion of membrane vesicles (Ayliffe and Lagudah, 2004). The vesicle trafficking increased resistance to *Bgh* and other pathogens resembling a 'non-host resistance' trait (Humphry *et al.*, 2006). However, even if *MLOs* are operating, the plant could be resistant if it has the corresponding R proteins that specifically detect effectors.

Similarly to MLO, the BAX inhibitor-1 (BI-1) protein also comprises a transmembrane complex which allows the penetration of *Bgh* and additionally suppresses programmed cell death (Eichmann *et al.*, 2004; 2010). Interestingly, the overexpression of this gene restored the PM penetration function in *mlo* mutants, as well as *MLO* overexpression in *bi-1* mutants (Huckelhoven *et al.*, 2003). BI-1 protein belongs to the Lifeguard protein family, in which the members were found to negatively regulate the cell death, too (Hu *et al.*, 2009).

As the first barrier, the modulation of the cell surface may also limit the invasion of the attacking organism. Leaf surface of *glossy11* maize mutant in which very-long-chain aldehyde levels are decreased inhibited spore germination of PM (Hansjakob *et al.*, 2011). Similarly, the *irg1* and *ram2* mutations altered the *Medicago* leaf cuticle layer, therefore it became resistant to several pathogens (Uppalapati *et al.*, 2012; Wang *et al.*, 2012a).

Expansins are used by plants for cell wall growth and stretch. The expansin EXPA2 provide susceptibility to *B. cinerea* and *A. brassicicola*, probably by allowing pathogen entry. Mutation in EXPA2 resulted in an additional side-effect increasing hypersensitivity to abiotic stresses (Abuqamar *et al.*, 2013). The cellulose synthase-like (*CSLA9*) gene was required for *Agrobacterium* attachment to the plant root surface, suggesting that CSLA9 is an essential cue for host recognition (Zhu *et al.*, 2003). The *AtCLCd* chloride channel encoding gene repressed the Flg22-triggered immunity in *Arabidopsis*. The T-DNA insertional 'knock-out' mutants

represented enhanced response to Flg22, and increased resistance to virulent strain of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (Guo *et al.*, 2014). The small G proteins genes (*RAC*/*ROP*) including the *HvRAC1*, *HvRAC3*, and *HvROP6* encode susceptibility factors in barley and also regulate vesicle trafficking. Overexpression of these genes greatly increased sensitivity to PM infection (Schultheiss *et al.*, 2002; Pathuri *et al.*, 2008). The orthologs identified in rice (*OsRAC4, OsRAC5, OsRACB*) also acted as compatibility factors in response to the adapted fungus, *Magnaporthe oryzae* (Jung *et al.*, 2006; Chen *et al.*, 2010a). However, the *HvRAC1* provided resistance against the non-adapted *M. oryzae* in barley (Pathuri *et al.*, 2008), which indicates the specificity of the gene to the attacker. A thiopurine methyltransferase (ubiquitin-conjugating enzyme), and an ADP ribosylation factor-GTPase-activating protein (ARF-GAP) acted as candidates of *Bgh* effector molecule (Schmidt *et al.*, 2014). Schmidt and co-workers suggest that the *ARF-GAP* vesicle trafficking genes are conserved targets of mildew effectors. Taken together, the genes mediating cytoskeleton rearrangements and vesicle trafficking (*MLO*, *BI-1*, ROP, RAC) are responsible for sensitivity to adapted fungi but facilitate resistance to non-adapted fungi (Van Schie and Takken, 2014).

Pathogens may sustain infection by the inhibition of defense signaling or response. The SA-signaling plays a key role in defense system against biotrophs, therefore enhancing this pathway putatively increases resistance. The SA 3-hydroxylase enzyme degrades SA by converting it into 2,3-DHBA in *Arabidopsis*. The mutation in the gene encoding this enzyme resulted in SA accumulation and increased tolerance against *P. syringae* (Zhang *et al.*, 2013a). SA signaling is escalated in response to biotrophs, but not to necrotrophs. The bHLH3/13/14/17 (basic loop-helix-loop) transcription factors found to suppress JA signaling, while the quadruple knock-out mutant expressively increased innate JA and resistance to *B. cinerea*. However, due to the antagonistic relationship (Robert-Seilaniantz *et al.*, 2011) in these plants the JA signaling was intensified along with the repression of SA pathway, resulting in increased susceptibility to biotrophic pathogens (Song *et al.*, 2013). Mutation in cellulose synthase genes activated the JA and ET mediated defense responses and enhanced resistance against pathogens (Ellis and Turner, 2001; Ellis *et al.*, 2002; Hernandez-Blanco *et al.*, 2007). Interestingly, in the later case the decreased cellulose content triggered the immune response. This is probably in association with accumulation of oligogalacturonides (cellulose precursors), which mimic DAMP and trigger DTI (Van Schie and Takken, 2014). Similarly, although the increased callose content benefit resistance, *pmr4* mutants with inhibited callose synthesis also showed decreased susceptibility to PM species. The accumulated oligosaccharides, which are able to induce the DTI may explain the phenomenon (Nishimura *et al.*, 2003). Down-regulation of *PMR4* generated total resistance to the adapted fungi, but failed to do so to the non-adapted fungi (Jacobs *et al.*, 2003; Huibers *et al.*, 2013).

Sequentially, after recognizing the pathogen, the phosphorylation-mediated MAPK cascade activates the response to biotic stress. Therefore, the molecules inactivating the cascade components can be considered as susceptibility factors, too. The MAPK phosphatases (MKPs) dephosphorylate the cascade components, thereby abolishing its function. The *mkp1* and *mkp2* loss of function mutant provoked decreased susceptibility to virulent *Ralstonia* and *Pseudomonas* bacteria (Bartels *et al.*, 2009; Lumbreras *et al.*, 2010; Anderson *et al.*, 2011). In contrast, some MAPKs repress PTI; the MPK4 of soybean and MAPK5 of rice reduced activity decreased effectiveness of PTI. The mutation in these genes resulted in increased resistance to several pathogens (Xiong and Yang, 2003; Liu *et al.*, 2011). The *EDR1* locus encodes a putative MAPKK kinase, which was found to negatively regulate the SA-mediated responses in *Arabidopsis* (Frye *et al.*, 2000). However, it also depends on the ethylene signal, since the *ein* mutation altered the expression of *EDR1* in response to senescence. The EDR1 probably acts in a cross-talk between ET and SA-mediated pathway operating in cell death and ageing (Tang *et al.*, 2005). Following the activation of MAPKs-mediated cascade, the transcription factors (TFs) actuate the defense reaction. The mostly active TFs during infection are the WRKY transcription factors, which were found to regulate defense mechanism either positively or negatively. Especially the rice *WRKY45-2* gene acted as a susceptibility factor against *X. oryzae* but the homolog gene, which differs only in a few amino acids positively regulated defense against the same pathogen (Tao *et al.*, 2009). The *Arabidopsis AtWRKY18, -40, -60* regulatory genes played a role in tempering the SA-mediated defense pathway. Double or triple mutants increased resistance to biotrophic *P. syringae* and susceptibility to *B. cinerea* compared to wild-type plant (Xu *et al.*, 2006).

The calcium-/calmodulin- and lipid-binding proteins also suppress the defense reaction in host plants. The SR1 calmodulin-binding transcription factor repressed the immunity by directly binding to the *EDS1*, *NDR1* and *EIN3* promoters (Du *et al.*, 2009; Nie *et al.*, 2012). The lipids may act as signaling molecules and are required for ETI and HR (Andersson *et al.*, 2006). The lesion mimic mutant, *acd11* had limited sphingosine (a sphingolipid) transfer protein content, which resulted in an increased level of cellular SA and resistance to biotrophs. Similar function was found with the sphingolipid fatty acid hydroxylase gene, the AtFAH1/2 (Brodersen *et al.*, 2002; König *et al.*, 2012). The SA-mediated defense response was suppressed by fatty acid desaturase (FAD7); the *fad7* mutant the basal SA level did not show alteration, but in response to aphid attack the SA accumulated along with enhanced defense (Avila *et al.*, 2012).

Once the pathogen passes the plant defense barriers the plant is forced to sustain the attacker. Additionally, these organisms are able to manipulate plant metabolism to fulfill their nutritional needs and to facilitate their replication and spread. Maintenance involves the modification of host sugar transport; the cell membrane localized sugar transporters (SWEET11

and SWEET13) were forced by *X. oryzae* to transfer more sugar into the intercellular region. Mutation in these genes abolished *X. oryzae* proliferation (Chen *et al.*, 2010b). SWEET11 associated with copper transporter, COPT1, which was also required for susceptibility to *X. oryzae* (Yuan *et al.*, 2010). The alcohol dehydrogenase gene (ADH) was up-regulated by PM in barley, although the *adh* mutation inhibited PM proliferation (Pathuri *et al.*, 2011). Lipids may also be utilized by the pathogens; the maize *lox3* lipoxygenase mutant plant became full resistant to three different fungus genus. The *lox3* inactivation also blocked the toxin production of *Fusarium* (Gao *et al.*, 2007). The increased resistance to biotrophs may be explained by the repressed JA synthesis in mutant plant. Therefore, inhibition of lipoxygenase activity depresses JA synthesis and SA signaling can be enhanced along with defense against biotrophs (Gao *et al.*, 2009).

Hypertrophy and endoreduplication of plant cells also benefit pathogens maintenance. The increased cell size leads to a rise in the nutrient and water content, while the endoreduplication results in multiplication of chromosomes, which overrides overall metabolism of the host. *Xanthomonas* infection induced cell size enlargement in pepper by triggering bHLH transcription factor Upa20 activity via its AvrBs3 effector (Kay *et al.*, 2007). The *PMR5* probably encodes a putative polysaccharide O-acetyltransferase (Gille and Pauly, 2012), *PMR6* encodes a pectate-lyase-like protein, thereby both of them playing a role in completion the accommodation of PM haustorium. The presence of these genes is required at a later stage of infection and they are independent of SA-mediated signaling (Vogel *et al.*, 2002; 2004). Additionally, recent study demonstrated that these genes were influenced by the pathogen to modulate the ploidy level of mesophyll cells underneath the infected epidermal cells containing haustorium. In this way the metabolic capacity could be enhanced at the site of infection (Chandran *et al.*, 2010; 2013). Although, in *pmr5* and *pmr6* mutants the penetration efficiency was not repressed, the fungus developed fewer hyphae, conidiophores, and conidia (Vogel *et al.*, 2002; 2004). Numerous studies observed that cell cycle regulatory genes were up-regulated in mesophyll cells at the site of infection: some cyclin-dependent kinases (CDKs), CDK inhibitors and a MYB3R4 transcription factor, known to be regulator of G2/M transition. The mutation in *MYB3R4* and in *PUX2* (plant ubiquitin regulatory X domain-containing protein 2) abolished the endoreduplication along with weakened *Golovinomyces orontii* colonization (Chandran *et al.*, 2010).

Among the compatibility factors NAC transcription factors were also found to repress defense response. One of the most intensively studied *Arabidopsis NAC* gene, the *ATAF2* is a repressor gene of PR proteins. Transgenic overexpression of *ATAF2* caused higher susceptibility to *Fusarium oxysporum* in *Arabidopsis*. Furthermore, levels of PR transcripts increased in the *ataf2* mutant lines (Delessert *et al.*, 2005). Another calmodulin-regulated NAC transcription factor was also found to repress defense by down-regulating *PR1* (Kim *et al.*, 2012). The *TaNAC21/22*

was found to negatively regulate defense against stripe rust, *Puccinia striiformis* f. sp. *tritici*. This *NAC* gene was the target of tae-miR164 microRNA, and this miRNA was found to regulate defense responses in earlier studies (Feng *et al.*, 2014). However, most NAC transcription factors were found to manage defense rather than susceptibility in plants.

3.2 NAC transcription factors in plant processes

NAC [NAM, (no apical meristem), ATAF (*Arabidopsis* transcription activation factor), CUC (cup-shaped cotyledon)] transcription factors comprise a large gene family, members of which are plant-specific (Riechmann *et al.*, 2000). NAC transcription factors are well studied proteins and characterized in numerous plant species: almost 30 genes were found in lycophytes and mosses (Zhu *et al.*, 2012a), 45 in *Citrus* (Oliveira *et al.*, 2011), 74 in grapevine (Wang *et al.*, 2013), 101 in soybean (Pinheiro *et al.*, 2009), 116 in maize (Voitsik *et al.*, 2013), 163 in *Populus* (Hu *et al.*, 2010), 167 in banana (Cenci *et al.*, 2014), 117 in *Arabidopsis* and 151 in rice (Nuruzzaman *et al.*, 2010). Phylogenetic analyses revealed that NAC transcription factors evolved over 400 million years ago. The oldest plants, in which *NAC* genes were found are the ancient land plants, the lycophytes (Zhu *et al.*, 2012a). *Selaginella moellendorffii* is the oldest taxon of vascular plants which encodes Vascular-related NAC domains (VNDs) in its genome (Yao *et al.*, 2012). During the course of plant evolution chromosome rearrangements led to the evolution of new *NAC* genes with alternate or multiple functions. The *NACs* may be classified into 21 subfamilies, within the families the function of the genes being relatively conserved. However, these functions may overlap, turning these genes partially or totally redundant within the subfamilies. This redundancy corresponds with the high mutational impact on *NAC* genes (Zhu *et al.*, 2012a). NAC domain containing proteins have a highly conserved N-terminal region and a diversified transcription activating C-terminal domain. The N-terminus consisting of about 160 amino acids is divided into five highly conserved subdomains (A-E; Figure 5) (Kikuchi *et al.*, 2000; Ooka *et al.*, 2003). The C-terminal region is the transcriptional activation domain (transcriptional activation region; TAR) (Figure 5) and it may vary among the proteins (Olsen *et al.*, 2005). The NAC domain was demonstrated to function in transcriptional process (Aida *et al.*, 1999; Xie *et al.*, 1999). A study in 2002 identified AtNAM as a transcription factor and the NAC domain as a putative DNA-binding domain (Duval *et al.*, 2002).

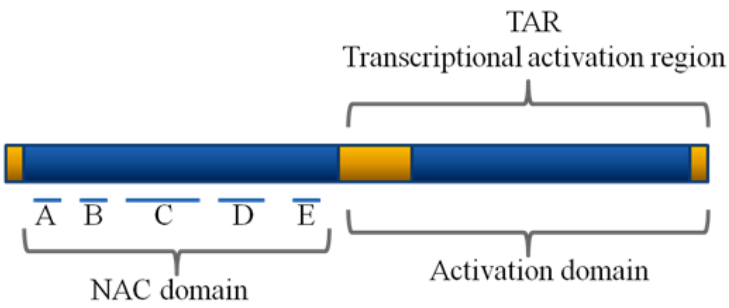


Figure 5. The general structure of NAC transcription factor proteins. A-E are the five highly conserved subdomains, composed of about 160 amino acids. DNA-binding domain is contained within the D and E subdomains. NAC domain is on the N-terminal region, the transcriptional activation domain (TAR) is on the C-terminal region (Ooka *et al.*, 2003)

3.2.1 Role in developmental processes

The NAC domain proteins play a role in various developmental processes such as the shoot apical meristem (SAM), flower, secondary wall formation or the cell longevity. In petunia *no apical meristem* (*nam*, encoding NAC domain protein) mutants the embryos failed to develop SAM, and the *nam* seedlings did not form the leaves and shoots (Souer *et al.*, 1996; Duval *et al.*, 2002). Likewise, the mutated *cuc* (*cup-shaped cotyledon*; *cuc1*, *cuc2*; encoding NAC domain proteins) failed at separation of cotyledons, sepals, stamens just as at the development of SAM (Aida *et al.*, 1997). Overexpressing the *CUC1* gene caused adventitious shoot formation (Hibara *et al.*, 2003). *CUC* genes regulate organ separation also (Aida *et al.*, 1997; Takada *et al.*, 2001; Vroemen *et al.*, 2003). *Cupuliformis* (*cup*) mutants showed a defect in SAM formation too (Weir *et al.*, 2004); *CUP* encodes a NAC domain, which is homologous to the *Petunia* NAM or the *Arabidopsis* CUC proteins.

The NACs are found to be active in floral morphogenesis too (Sablowski and Meyerowitz, 1998). Overexpressing *OsNAC1* increased tiller number and promoted shoot branching in rice. Reduction of this gene expression in *ostil1* mutant rice (tillering rice) recovered the WT phenotype. The results suggest that the *OsNAC1* gene plays a primary role in improving plant structures for higher light-use efficiency and yield (Mao *et al.*, 2007). NACs play an important role in secondary wall thickening (Mitsuda *et al.*, 2005). Expression of chimeric repressors derived from NST1 and NST2 (NAC Secondary wall Thickening promoting factor) resulted in an anther dehiscence defect in *Arabidopsis* (Mitsuda *et al.*, 2005).

Small groups of *NAC* genes have been identified to play a primary role in plant cell wall development. Overexpression of *ANAC104/xylem NAC domain1* negatively regulated lignocellulose synthesis and suppressed the differentiation of tracheary elements (Zhao *et al.*, 2005; Zhao *et al.*, 2008).

NAC genes are found under hormonal control. For example, auxin induced *Arabidopsis NAC1* expression, which then mediated the auxin signaling pathway to promote lateral root morphogenesis (Xie *et al.*, 2000; He *et al.*, 2005). Overexpression of *NAC1* increased, whereas silencing decreased the lateral root development in *Arabidopsis*.

NACs may also control cell cycle (Kim *et al.*, 2006; Willemsen *et al.*, 2008; Kato *et al.*, 2010). In a study, a membrane-bound NAC was found to be associated in cytokinin signaling during cell division in *Arabidopsis* (Kim *et al.*, 2006).

Promoter analysis of numerous *Arabidopsis NAC* genes showed that some *NAC* members are involved in abscisic acid (ABA) hormone signaling pathways. A few genes were regulated by ABA and gibberellins (GAs), while all of the *ATAF* subgroup members responded to ABA and methyl jasmonate (MeJA) (Jensen *et al.*, 2010). The expression of *RD26/ANAC072* (encodes a NAC transcription factor) was induced also by ABA-treatment beside the responsiveness to dehydration and high salinity in *Arabidopsis*. Furthermore, this gene may regulate pathogen responses and ROS detoxification via JA and ABA signals (Fujita *et al.*, 2004). The overexpression of *RD26* caused a hypersensitivity to ABA, and additional ABA- and stress-responsive genes were also up-regulated. The repression of this gene resulted in a reduced response to ABA and the down-stream genes were down-regulated (Fujita *et al.*, 2004).

NACs are associated with the leaf-senescence process and also play a role in grain nutrient remobilization. A T-DNA knockout *atnap* (encode NAC TF) mutant delayed senescence in *Arabidopsis* (Guo and Gan, 2006). The orthologs of this gene were up-regulated in kidney bean and rice during the leaf senescence process. An ancient wild wheat allele (*NAM-B1*) encoding a NAC transcription factor was found to be active during senescence, and substantially regulated remobilization of proteins, zinc and iron content. Since modern cultivated varieties carry a dysfunctional *NAM-B1* allele this offered an opportunity to integrate a wild allele in modern wheat varieties. This would improve their grain nutritional values by enhancing the channeling of these nutrients into the developing wheat endosperm from senescing photosynthetic tissues (Uauy *et al.*, 2006; Waters *et al.*, 2009). The *JUB1/ANAC042* gene was found to negatively regulate senescence, since the overexpression of this genes resulted in delayed bolting of the *Arabidopsis* plants (Wu *et al.*, 2012).

3.2.2 **Role during environmental challenges**

The NAC transcription factors were identified to regulate in both abiotic and biotic stress responses and also found to play a role in phytohormone-mediated signaling cascades. During environmental stresses, a synergistic or an antagonistic crosstalk was detected among the plant hormones in NAC-related pathways (Atkinson and Urwin, 2012).

NACs are found to cooperate in the most typical abiotic stress responses, such as light, cold-heat, wounding, drought, salt and osmotic stresses. Functional studies of *Arabidopsis* lead to the finding that *ANAC078* is evolved in protection of the plant under high light stress. This gene induced flavonoid biosynthesis under high light conditions and its overexpression resulted in the activation of flavonoid biosynthetic genes and intensive anthocyanin synthesis (Morishita *et al.*, 2009). The *Arabidopsis JUB1/ANAC042* gene regulates tolerance to heat stress in cooperation with thermomemory genes (*HSFA2, HSA32, HSP*) and the resistance may be improved by priming the stimulus (Shahnejat-Bushehri *et al.*, 2012). Overexpressed *JUB1* gene induced *DREB2A* expression, which is known to be responsive to dehydration (Wu *et al.*, 2012).

Studies in rice showed that *SNAC1* and *SNAC2* are positively regulating tolerance to drought and salt (Hu *et al.*, 2006; Hu *et al.*, 2008). Similarly, *OsNAC5* also responds to dehydration and was found to interact with the 'CACG' core sequence of the *OsLEA* promoter (Song *et al.*, 2011), which is a key regulator in drought- and salt-stress responses. Interestingly *OsNAC5* expression positively correlates with proline and soluble sugar accumulation (Song *et al.*, 2011). This gene also reacts to cold-stress and found to be ABA- and jasmonic acid (JA)-dependent such as the paralogue *OsNAC6* (Ohnishi *et al.*, 2005; Nakashima *et al.*, 2007; Takasaki *et al.*, 2010). *OsNAC010* regulates gene expression under drought-stress and found to improve grain yield (Jeong *et al.*, 2010).

Functional analysis of *NAC* genes in soybean showed that *GmNAC2*, *-3*, and *-4* are highly expressed in osmotic stress and *GmNAC3* and *-4* are regulated in response to high salinity via ABA and JA signals. The transient expression of *GmNAC1*, *-5* and *-6* caused cell death and enhanced senescence in tobacco (Pinheiro *et al.*, 2009), the *GmNAC5* activated tolerance to wounding, cold and salt stresses independently of ABA (Jin *et al.*, 2013).

Citrullus colocynthis NAC genes (*CcNAC1* and *CcNAC2*) were induced by wounding, drought, cold and salinity stress. Hormone signals (GA, ET, ABA, JA, and SA) also caused alteration in the expression of these genes (Oliveira *et al.*, 2011; Wang *et al.*, 2014).

Using yeast one-hybrid system, the *ANAC019*, *ANAC055* and *ANAC072* genes were found to be activated by ABF3 (ABA-responsive Binding Factor 3) and ABF4 TFs binding to the ABRE element in the promoters of these genes (Zou *et al.*, 2011; Hickman *et al.*, 2013). The CBFs (C-repeat/dehydration responsive element Binding Factor) were found to activate the *ANAC072* gene in drought stress. All three NAC proteins were found to interact with *ERD1* (*Early response to dehydration 1*) promoter during osmotic stress (Tran *et al.*, 2004). The most important TFs regulating in senescence are the CBF1 ABF4 and MYB2 TFs, which affect *ANAC072*, and the bZIP44, HY5 (Long Hypocotyl 5), which affect *ANAC055* expression (Hickman *et al.*, 2013). *ANAC072* and *ANAC055* determine regulation of defense response against *Botrytis cinerea* too.

The most relevant transcription factors were CBF4, ABF4, ABF3, MYB21 and MYB112 inducing *ANAC072*, MYB2, Homeodomain-TF inducing *ANAC019* and MYB2 inducing *ANAC055* during pathogen attack (Hickman *et al.*, 2013). These three proteins compose a prominently complex cascade during both abiotic and biotic stresses.

Previous results showed that a NAC-domain containing protein was highly expressed in response to nematode infection of soybean roots (De Sa *et al.*, 2012). Similar data were found in cyst nematode resistant lines where the *SND1* (*Secondary wall-associated NAC Domain 1*) gene was up-regulated and in susceptible lines the expression was repressed (Mazarei *et al.*, 2011).

Maize *ZmNAC41* and *ZmNAC100* genes reacted to *Colletotrichum graminicola* infection (Voitsik *et al.*, 2013). The wheat *TaNAC4* gene was induced by rust fungal pathogen, found to regulate in response to wounding, high salinity, cold stress and the gene may be governed by JA ABA and/or ET signaling molecules (Xia *et al.*, 2010). The ABA-dependent *TaNAC67* was localized in the nucleus and it was determined to be a positively regulate salt, drought, cold tolerance, simultaneously subserve strengthened cell membrane stability (Mao *et al.*, 2014).

The NAC domain containing proteins also regulate programmed cell death (Zhao *et al.*, 2008; Ohashi-Ito *et al.*, 2010). A recent study showed that *ANAC089* gene reacted to unfolded protein response in endoplasmic reticulum (ER) and this ER stress induced *ANAC089* to switch on programmed cell death (PCD) with downstream regulation of *ANAC094*, a PCD related gene. The cascade resulted in an increase of caspase 3/7-like proteins (regulating in apoptosis) activity and DNA fragmentation in plant cell (Yang *et al.*, 2014).

Cold stress activated the membrane bound NTL6 encoding gene, which enters the nucleus in response to stress inducing the expression of *PR1, PR2, PR5* genes along with conferring resistance against the infection. Reduced activity of this gene resulted in increased sensitivity to pathogens at low temperature. We may suspect that the SA is the responsible signal since SA also manages cold responses besides contributing in defense reactions. However, the study showed that cold induction of these *PR* genes is independent of SA and SA was also able to induce *PRs* in *ntl6* inactivated mutant plants (Seo *et al.*, 2010).

ATAF1 gene is an intensively studied *NAC* gene in *Arabidopsis* and is a positive regulator of penetration resistance in the plant. The ortholog of *ATAF1* in barley, the *HvNAC6* gene has the same function (Jensen *et al.*, 2007). The group reported that *ATAF1* was silenced during powdery mildew, *Bgh* infection, but the overexpression of this gene caused an increase in the resistance of cells against fungal penetration. Interestingly, the ABA biosynthesis gene *(AAO3)* was induced in *ataf1* mutant plants after *Bgh* inoculation and in *ATAF1* overexpressing plants the endogenous ABA level was repressed. Additionally, the *aao3* mutant plant lacked endogenous ABA and showed resistance against *Bgh* penetration (Jensen *et al.*, 2008). The *ATAF1* gene was also

involved in drought stress responses (Lu *et al.*, 2007). A homologue of *ATAF1* in rice (*OsNAC6*) was induced by *Magnaporthe grisea* fungus, which causes blast disease in rice (Nakashima *et al.*, 2007). Interestingly, *OsNAC6* was involved in abiotic-stress (drought, salt, cold, wounding) responses too (Ohnishi *et al.*, 2005; Nakashima *et al.*, 2007), similarly to the *ATAF1* in *Arabidopsis*.

In *Brassica napus* the *BnNAC* gene responded to *Sclerotinia sclerotiorum* infection (Hegedus *et al.*, 2004). The *StNAC* was induced by *Phytophthora infestans* infection in potato and was rapidly expressed in response to wounding, too (Collinge and Boller, 2001).

Previous studies demonstrated that *NAC* genes played a role in various developmental and stress responses. The results also showed that some genes had multiple function in the different handlings of environmental challenges, or the pathway of biotic and abiotic stress responses overlaped at some points.

In grapevine 74 sequences are graded to encode NAC transcription factor in *Vitis vinifera*, but only a few recent studies presented functional analysis results related to *Vitis NAC* genes (Zhu *et al.*, 2012b; Le Henanff *et al.*, 2013; Fang *et al.*, 2016). (Figure 6). Le Henanff *et al.* (2013) demonstrated that the expression of gene *VvNAC1* (*VvNAC60*, Figure 6) is closely associated with leaf, flower and berry development occurring at a later stage suggesting a relationship to the process of senescence. Furthermore, gene *VvNAC1* reacted also to cold, wounding and phytohormone treatments with SA, methyl jasmonate, ET, and ABA. *B. cinerea* infection also induced the expression of *VvNAC1*, while the *Arabidopsis* plants overexpressing *VvNAC1* showed increased tolerance to *B. cinerea* and *Hyaloperonospora arabidopsidis*. Another study isolated *VpNAC1* (*VvNAC41*, Figure 6) gene from *Vitis pseudoreticulata* and they found this gene to positively regulate resistance against PM and *Phytophthora parasitica* var. *nicotianae* Tucker in transgenic tobacco (Zhu *et al.*, 2012b). Additionally, the overexpression of *VpNAC1* enhanced *PR* genes expression. Recent results showed that gene *VaNAC26* (*VvNAC26*, Figure 6) regulated cold, drought and salinity tolerance in *Vitis amurensis* (Fang *et al.*, 2016). Overexpression of this gene in *Arabidopsis* resulted in lower concentrations of H_2O_2 and O_2^- during drought stress and increased drought and salt tolerance. Microarray analysis of overexpressing lines revealed an increased expression rate of genes involved in jasmonic acid (JA) synthesis and signaling. The *NAC042_5* (*VvNAC36*, Figure 6) gene was never analyzed earlier and data were not shown for regulation role in plant processes.

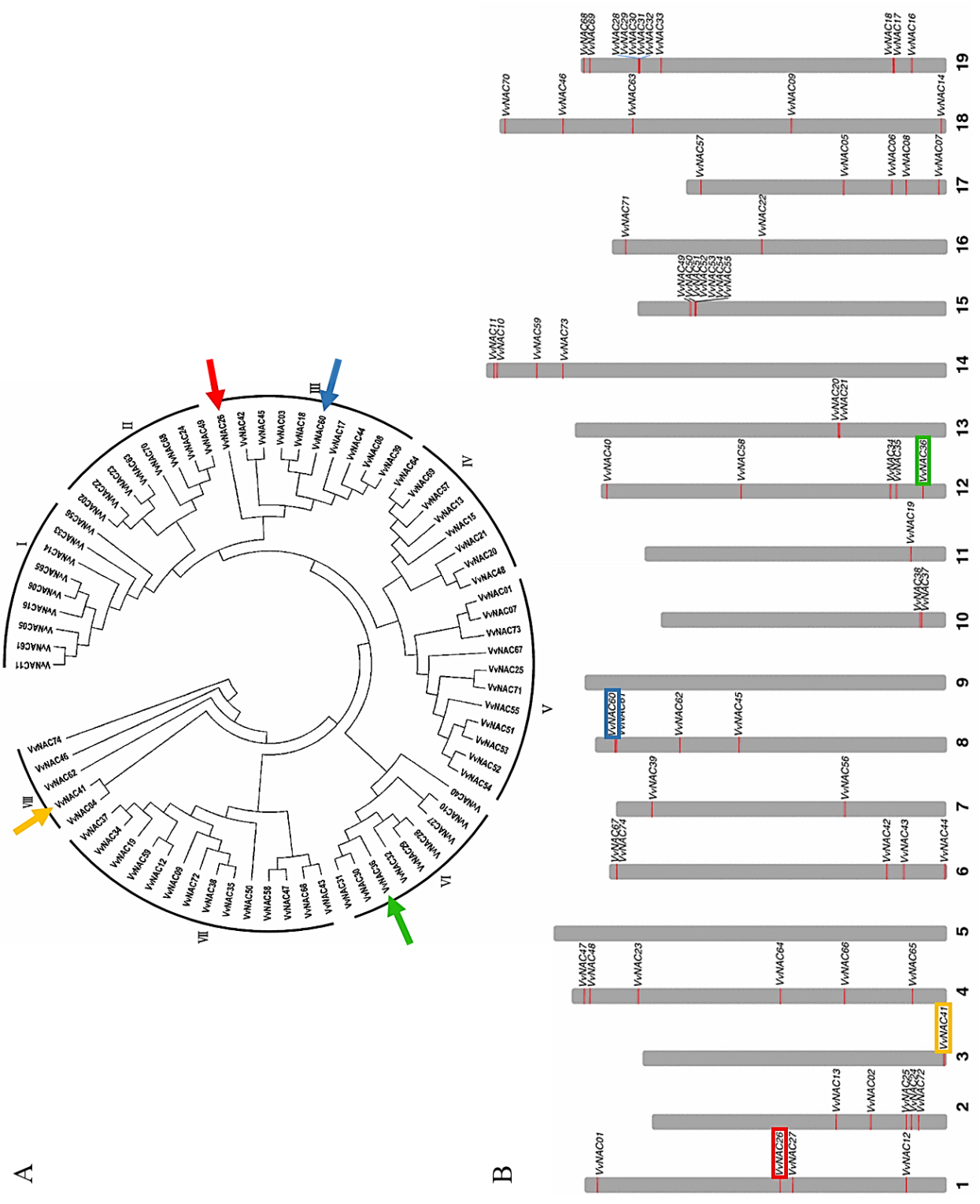


Figure 6. The identified 74 *NAC* genes in the *Vitis* genome. A) Phylogenetic analysis of the 74 *NAC* paralogues (Wang *et al.*, 2013). B) Location of 74 *NAC* paralogues in the *Vitis* genome. The marks point to the functionally analyzed genes. Red marks-*VvNAC26* (Fang *et al.*, 2016), yellow marks-*VvNAC41* (Zhu *et al.*, 2012b), blue marks-*VvNAC60* (Le Henanff *et al.*, 2013), green marks-*VvNAC36* (Toth *et al.*, 2016).

4. MATERIALS AND METHODS

4.1 Global expression analysis of PM-inoculated and MeSA-treated grapevine

4.1.1 Grapevine plant material, growth conditions, and PM-/MeSA-treatments

We grew one-year-old greenhouse-cultivated potted *V. vinifera* L. cv. 'Cabernet Sauvignon' grapevines with a single actively growing herbaceous shoot on each vine. To prepare PM-colonized tissues, two unfolded, but still expanding leaves were mock-inoculated or inoculated with *E. necator* conidia under greenhouse conditions. Inoculation was done by touching the upper surface of the leaf with a detached grapevine leaf covered with *E. necator* colonies actively producing conidia. For SA-induction, leaves at the same developmental stages were mock-inoculated by touching the leaves with detached PM-free healthy grapevine leaves. To prepare healthy reference leaf tissues, plants were treated in the same manner, including mock-inoculation. Three days post-inoculation (dpi), all grapevines were transferred to a PGR15 plant growth chamber (Conviron) in conditions of 85% RH, 14/10 h diurnal cycle, and 26°C temperature. PM-inoculated plants were cultivated in the growth chamber for eight additional days until 11 dpi, at which time the PM-colonized leaves were harvested for RNA extraction. Plants for SA-induction were cultivated in the growth chamber for seven days, at which time they were treated with 15 μM of MeSA, evaporated in the atmosphere of the growth chamber under airflow generated by a computer fan for 24 hours. SA-induced mock-inoculated leaves were harvested at the completion of this 24-hour treatment (11 dpi). Reference plants were also cultivated under identical growth chamber conditions; the mock-inoculated leaves were harvested at 11 dpi. Thus, the PM-colonized and reference samples differed only in the presence/absence of PM treatment, whereas the SA-induced and reference samples differed only in the presence/absence of MeSA treatment. Leaves from all treatments were harvested at 11 dpi and immediately flash-frozen in liquid nitrogen. Each treatment consisted of ten potted vines and two young leaves harvested from each vine of the ten-vine repeat and pooled into a single sample for RNA extraction. Three samples were collected for each treatment. The experimental settings are schematically represented in Figure 7.

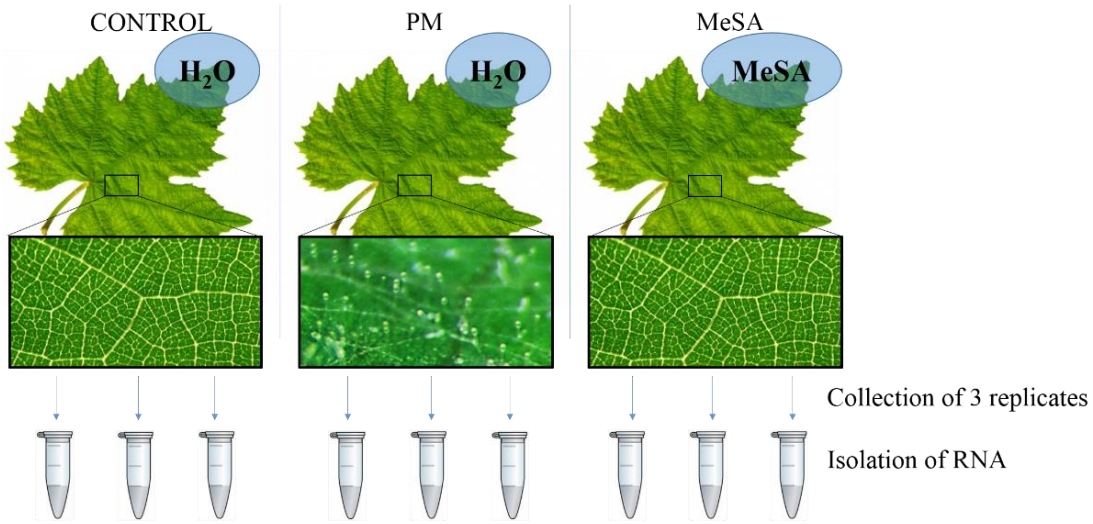


Figure 7. Sample preparation for RT-qPCR and microarray as well (in the microarray, the isolated RNA was hybridized to the Affymetrix GeneChip after the instruction of the company).

4.1.2 RNA isolation

The selected gene *NAC042_5* was independently validated by quantitative real-time PCR (qPCR) to evaluate expression change detected by the microarray experiment. Therefore, leaf tissues of the collected samples were ground in liquid nitrogen and homogenized in extraction buffer (2% hexadecyltrimethyl ammonium bromide (CTAB), 1% SDS, 2.5 M NaCl, 0.5 M Tris, 50 mM EDTA, 5% beta-mercaptoethanol, and 3% polyvinyl poly-pyrolidone). The samples were stored at -80°C until processing. For RNA isolation the frozen samples were thawed at 45ºC and centrifuged (13,000 rpm, 20 min, 4ºC). The supernatant was replenished with 1/2 volume of chloroform, vortexed, and centrifuged (13,000 rpm, 15 min, 4ºC). The supernatant was supplemented with 1/5 volume of 12 M LiCl and incubated for 2 hours at 4ºC. After centrifugation (13,000 rpm, 30 min, 4ºC) the supernatant was discarded and the pellet was washed twice with 80% ethanol and dissolved in RNase-free water. The samples were treated with 1 µl Turbo DNase I (Ambion) in 40 µl reactions, and RNA was purified using an RNeasy MiniElute Cleanup column (Qiagen) following the manufacturer's guidelines.

4.1.3 Microarray

To analyze gene expression changes in response to PM colonization and SA, the Affymetrix GeneChip *V. vinifera* (Grape) Genome Array was employed following the manufacturer's guidelines. Briefly, 4 µg of the purified RNA was used to synthesize double stranded cDNA using the One Cycle cDNA Synthesis kit, then this cDNA was used to produce biotin-labeled cRNA through an *in vitro* transcription (IVT) reaction. The labeled cRNA was fragmented (heated at 94ºC for 35 min to break RNA molecules to 35- to 200-nucleotide fragments) before hybridization to Genechip probes. Hybridization was performed at 45ºC for 16

hours, followed by the washing and staining processes of the array, which were performed on an Affymterix Fluidic Station 450. Fluorescence was amplified with streptavidin-phycoerythrin staining, followed by the addition of a biotinylated antibody (anti-streptavidin) solution and by a final streptavidin-phycoerythrin staining. The prepared chip was then scanned by a GSC3000 laser scanner and the intensity values were processed using the GeneChip Operating Software version 1.2 of Affymetrix. Following Affymetrix guidelines, the background corrections were completed and expression values were calculated. Normalization was performed using the robust multiarray averaging method. Normalized intensity values, as well as raw GeneChip images have been deposited in the Gene Expression Omnibus database in GenBank (accession number: GSE53824). The GeneChip® *Vitis Vinifera* Genome Array assayed the expression of more than 16,000 grape genes, among which 14,000 probes were specific to *Vitis vinifera* and the rest for other *Vitis* species. For one gene 16 oligonucleotides were set on the chip to measure their expression levels. The probe set sequences were from GenBank®, dbEST and RefSeq. Using this technique, it was tested whether there were differences between the response to PM infection and the response to exogenous MeSA application in susceptible *Vitis vinifera* cv. 'Cabernet Sauvignon'. In the experiment three different of treatments were applied (Figure 7). The gene expression levels in the PM-infected and MeSA-treated leaves were individually compared to gene expression levels in the mock inoculated, water treated leaves (reference leaves) (Toth *et al.*, 2016). The microarray experiment and measurement of salicylic acid level of leaves was performed by Dr. Patrick Winterhagen and Dr. Zoltan Szabo at Mountain Grove Experimental Station of Missouri State University.

Raw data produced by Affimetrix *Vitis* GeneChip was statistically analyzed by Dr. Yingcai Su (Missouri State University, Department of Mathematics, 65897-Springfield, USA). Intensity values of the microarray experiment were log2-transformed and submitted to exploratory analysis. ANOVA model with balanced single factor was applied for evaluating data using the statistical package S-plus. The error term is assumed to be normally distributed with mean zero and constant variance. The genes with at least 1.5-fold change compared to the control (p-value < 0.01 and False Discovery Rate 5%) were selected for further analysis.

The annotation of selected probes was performed by Balázs Kalapos (Hungarian Academy of Sciences, Agricultural Institute, Centre of Agricultural Research, 2462-Martonvasar, Hungary) by blasting the Affymetrix GeneIDs (downloaded from http://www.affymetrix.com/estore/) to the EST database of NCBI GeneBank (GeneBank and GeneIndex IDs). The most analogous ESTs were searched for homologs among five species (*V. vinifera, A. thaliana, A. lyrata, S. tuberosum, S. lycopersicum*) using the BLASTx algorithm. The homology between query and database sequences was perceived to be informative only if the *E* value was less than $1e^{-10}$. The identified

transcripts then were analyzed by MapMan, KEGG and Ensembl databases (Kanehisa and Goto, 2000; Hubbard *et al.*, 2002; Thimm *et al.*, 2004; Usadel *et al.*, 2005; Cunningham *et al.*, 2015; Kanehisa *et al.*, 2016) to categorize the putative role of genes in metabolic pathways or other processes.

4.1.4 Reverse transcription and qPCR analysis of *NAC042_5*

The purified RNA was used also for cDNA synthesis using the Taqman Reverse Transcription Reagent kit (Life Technologies) after the manufacturer´s recommendations. Based on sequences available at the Grape Genome Browser/Genoscope (http://www.genoscope.cns.fr) and the DFCI EST databases, gene specific primers were designed for the following grapevine target gene *NAC042_5* (GSVIVT00018864001) and for the reference gene *ACTIN 1* (primer sequences in Appendix Table 3). For qPCR analysis, the SYBR Green Reagent kit (Life Technologies) and the real-time thermal cycler Mx3005P (Stratagene) were used. All samples were run in triplicates under identical reaction settings: the initial activation step of AmpliTaq Gold® was 95°C for 10 min and followed by 40 cycles with denaturation for 15 s at 95°C, primer annealing for 30 s at Tm = 60°C, and after cycling a final segment was applied with denaturation for 1 min at 95°C, 30 s at 60°C and 30 s at 95°C again. Subsequently, a melting curve with temperature steps of 1°C was performed. Primer efficiency was confirmed to be similar (09 +/- 01) for both primer pairs and relative quantitation was calculated using the qPCR analysis software package MxPro-Mx3005P version 3.0 (Stratagene) and the DART-PCR version 1.0 software tool (Peirson *et al.*, 2003) as recommended. R_0 values of target gene was normalized to R_0 values of the reference gene. Statistical significance was determined by Student's T-test to compare the treatment-induced response to the control.

4.2 Analysis of *NAC042_5* regulation in response to PM *in vivo*

4.2.1 Promoter isolation of *NAC042_5* gene and construction of binary vectors

Based on the Grape Genome Browser/Genoscope (http://www.genoscope.cns.fr) database, the *NAC042_5* promoter region was isolated from *V. vinifera* L. cv. 'Cabernet Sauvignon' DNA using the gene specific primers (primer sequences in Appendix Table 3). The primers were designed based on the upstream sequence of GSVIVT00018864001 gene of *V. vinifera* cv. 'Pinot Noir' (12X) data. The Phusion High fidelity Taq polymerase was used for the amplification of the DNA. The produced DNA fragment was electrophoretically separated, dissected and isolated back from the gel using the Wizard® SV Gel and PCR Clean-Up System of Promega. The purified DNA fragment was cloned into the pENTR cloning plasmid (Life technologies™) and then subcloned into pGWB633 binary vector (Nakamura *et al.*, 2010) (vector map in Appendix Figure 18) using the Gateway® technique of Invitrogen. The cloning process was performed according

to the manufacturer's guidelines, and each time *Escherichia coli* was transformed with the recombinant plasmid. Competent *E. coli* cells were prepared manually with repeated ice cold $CaCl_2$ wash. After each transformation, the surviving colonies were tested by colony PCR (primer sequences in Appendix Table 3) and the plasmids were isolated using the PureYield™ Plasmid Miniprep kit of Promega. The multi-cloning site of the final product binary vector was sequenced by the BIOMI Company (Gödöllő, Hungary). The sequence of the *V. vinifera* L. cv. 'Cabernet Sauvignon' *NAC042_5* gene was submitted into 'The National Center for Biotechnology Information' database (GenBank accession number of the promoter sequence: KU297673). In the pGWB633 binary construct, the *NAC042_5* promoter controls the *gusA* reporter gene. The T-DNA of the pGWB633 also contains the *bar* gene – encoding glufosinate-ammonium resistance – which supports the selection of positive transformants. The pGWB633 plasmid with the *NAC042_5* promoter construct was transferred into *Agrobacterium tumefaciens* GV3101 (pMP90) strain after the descriptions of Xu and Li (2008).

The analysis of the promoter was implemented by deletions of the isolated *NAC042_5* promoter. The primer sets were designed to amplify shorter and shorter fragments (four different sizes) of the promoter. These fragments amplified from the same *V. vinifera* L. cv. 'Cabernet Sauvignon' DNA were cloned into pGWB633 and transferred into *Agrobacterium* using the previously described process. For null-promoter control a 23 bp (origin is not 'Cabernet Sauvignon' DNA) fragment was cloned into the Multi Cloning Site of pGWB633, which fragment does not relate to the *NAC042_5* promoter.

The *Agrobacterium* strains containing the various binary vectors were used to transform *A. thaliana*.

4.2.2 Transformation of *Arabidopsis thaliana* plants

The analysis of the NAC promoter regulation was performed in three different *A. thaliana* lines: a wild type, a salicylic acid signaling deficient (*nim1-1*), and a salicylic acid hydrolase gene containing transgenic (*nahG*) line. All three had the origin of Wassilewskija ecotype. The plant transformation was performed following the procedure described by Clough and Bent (1998), referred to as the flower dip method. During this process the flowers of the *Arabidopsis* were dipped into the binary vector containing *Agrobacterium* suspension. The seeds developed from the dipped flowers were germinated in Petri dishes containing high quality soil (Compo Sana). The growing conditions were as follows: cool white light illumination, 16/8 h diurnal cycle, 80% relative humidity at 22°C degree. Ten days after sowing the seedlings were sprayed with 60 mg/l glufosinate-ammonium (Finale) and 0.01% Silvet L-77 solution (Nakamura *et al.*, 2010). The spray was repeated three times, the surviving plants were selected and transplanted into 4X4 pots for further cultivation. Insertion of the corresponding transgene in plants was confirmed using PCR

(primer sequences in Appendix Table 3). The genetically proven transformants were selected to produce the T_2 generation and the transgenic plants were grown till T_3 generation. The lines were selected which had single copy insertion of the corresponding transgene. At least two lines were chosen for each genetic background (WT, *nim1-1*, *nahG*).

4.2.3 Testing the transgenic plants

The chosen lines for each genetic background were tested for basal expression and also for induction by PM. The basal expression was investigated during the life cycle of the transgenic plants; the samples were collected from plants grown under normal conditions (cool white light illumination, 16/8 h diurnal cycle at 24°C degree) without any environmental challenges. For PM-induced expression three-week-old plants were mock-inoculated or inoculated with *Oidium neolycopersici* conidia under growth chamber conditions. The inoculation was performed by touching the upper surface of the leaf with a detached tomato leaf covered with *O. neolycopersici* colonies actively producing conidia. This inoculation method was performed for histochemical assay.

For spectrophotometric measurements, four week-old plants were inoculated by spraying a conidial suspension (Huibers *et al.,* 2013). Control treatment of plants was accomplished by a mock-inoculum spray using healthy tomato leaves. The mock-inoculated and inoculated plants were cultivated under the following growth conditions: cool white light illumination, 16/8 h diurnal cycle at 24°C degree.

4.2.3.1 Spectrophotometric assay

Fourteen days after the inoculation six individuals were collected from each line of the PM-inoculated and mock-treated plants. The infected leaf tissues were excised and were ground in extraction buffer (50 mM NaPO4 pH 7.0, 10 mM β mercaptoethanol, 0.1% Triton X-100). The extract was incubated after addition of 1 mM 4-nitrophenyl β-D-glucuronide (pNPG) at 37°C for 2 hours (Aich *et al.,* 2001; Gilmartin and Bowler, 2002). The conversion of *p*NPG to *p*NP by β-glucuronidase was measured in a spectrophotometric assay at 405 nm absorbance and in 30-min intervals (as repeated measurements) using the Nanodrop 1000 spectrophotometer (Aich *et al.,* 2001). To determine if β-glucuronidase activity was different between the PM-inoculated and mock-treated tissues, we transformed the absorbance values to natural logarithm values (to obtain a reasonably normal distribution), and analyzed them using a mixed linear model implemented by the software package SAS. The mixed linear model was as follows: Log (observation) = effect of gene + effect of treatment + effect of time + interaction effect of gene and treatment + interaction effect of gene and time + interaction effect of treatment and time + interaction effect of gene, treatment and time + error.

4.2.3.2 **Histochemical GUS assay**

Eleven days after the inoculation the plants were investigated by histochemical GUS assay (Jefferson *et al.*, 1987; McCabe *et al.*, 1988). The leaves were incubated overnight at 37°C in the assay solution (100 mM $NaPO_4$ buffer; pH 7.0, 10 mM EDTA, 1% Triton X-100, 0.3% H_2O_2, 0,5 mg/ml X-Gluc/5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid cyclohexylammonium salt). After the staining procedure, the chlorophyll was removed by repeatedly washing the samples with 70% ethanol. To stain fungal tissue, leaves were dipped into cotton blue solution (Thermo Scientific; 30X dilution in 70% ethanol) for 30 s, rinsed with distilled water, and subsequently investigated using a stereo- and light-microscope.

4.2.4 **Promoter analysis of *NAC042_5* sequence**

The promoter of the *NAC042_5* gene was analyzed using the PLACE and PlantPAN 2.0 databases (Higo *et al.*, 1999; Chow *et al.*, 2016) to identify the putative cis-regulatory elements within the sequence.

4.3 ***In vivo* analysis of *ANAC042/JUB1* regulation in response to PM**

To test the regulation of the closest ortholog of *Vitis NAC042_5* in *Arabidopsis thaliana* the promoter of *ANAC042/JUB1* was also isolated using gene specific primers (primer sequences in Appendix Table 3). This fragment was also cloned into the pGWB633 binary vector, and transferred into *Agrobacterium*. This *Agrobacterium* strain was then used to transform the previously mentioned *A. thaliana* types (WT, *nim1-1*, *nahG*). The transformed *Arabidopsis* lines were also inoculated with *O. neolycopersici* and the induced *gusA* expression was investigated using histochemical assay.

5. RESULTS AND DISCUSSION

5.1 Genes identified based on microarray measurement

Numerous studies demonstrated that transcriptome remodeling induced to a great extent by obligate plant pathogens is mediated by SA signaling (Pieterse *et al.*, 2012). However, PM pathogens were shown to induce changes in the transcriptome well beyond SA-induced gene expression levels (Chandran *et al.*, 2009). To distinguish host transcriptome changes triggered exclusively by SA from those triggered more broadly by *E. necator* colonization, we conducted two separate global leaf transcriptome analyses using the Vitis Affymetrix GeneChip platform (Toth et al. 2016).

In the first experiment we compared the leaves with fully established PM colonies to healthy reference leaves, and found that transcript abundance was at least 1.5-fold higher or lower for 373 genes in PM-infected leaves relative to healthy reference leaves. Although the SA was below the threshold of detection in the control leaves (measured by Z. Szabó and P. Winterhagen), SA accumulated in subsamples of PM-infected leaves to 0.92 ± 0.68 μg/g fresh weight (Toth *et al.*, 2016). These SA levels were similar to those measured in PM-infected grapevine leaves at 2 dpi (Fung *et al.*, 2008), indicating that SA levels remained high even when PM colonies became well established on grapevine leaves. This suggests that defense signaling was active in leaves carrying mature, well established PM colonies.

In the second experiment, we assayed MeSA-treated grapevine leaves in comparison with control leaves. The total SA concentration (measured by Z. Szabó and P. Winterhagen) was significantly higher in the MeSA-treated plants (26.33 ± 12.48 μg/g fresh weight) than in control leaves where SA was undetectable (Toth *et al.*, 2016). We found that 481 genes responded to the MeSA treatment with at least 1.5 fold-change in their expression levels, and 179 of them were a subset of the PM-regulated gene list. This suggests that a subset of PM-responsive genes may be regulated via SA signaling.

The Vitis Affymetrix GeneChip included nine probe sets of fungal origin with a nearest homology to genes of ascomycetous fungi. Each of the nine genes was identified in our microarray results as exclusively PM-dependent and were among the genes with the highest expression rates (8- to 284-fold) (Figure 8, yellow mark). The hybridization of these probe sets by transcripts in exclusively PM-treated samples confirmed that *E. necator* inoculum was absent in MeSA-treated and control samples proving that experimental treatments were carried out appropriately.

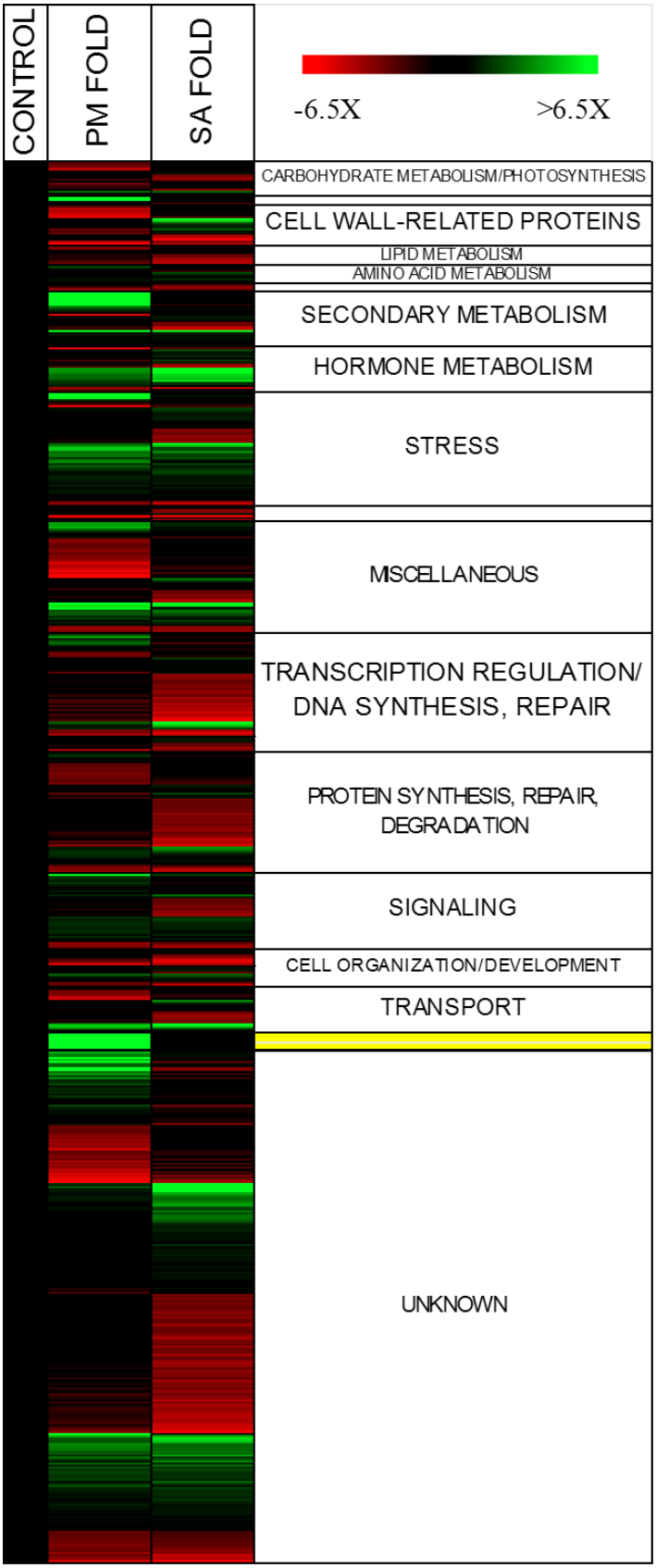


Figure 8. Changes in expression rate measured by microarray analysis. 705 significantly altered probe sets that were up- or down-regulated by at least 1.5-fold relative to control. The yellow color represents the reference fungal genes. Black: 1x expression; red: 6.5-fold down-regulated; green: 6.5-fold or above up-regulated.

The relative transcriptional change of those genes that were found to be modulated by PM infection only, MeSA treatment only, or both PM infection and MeSA treatment are displayed in Figure 9.

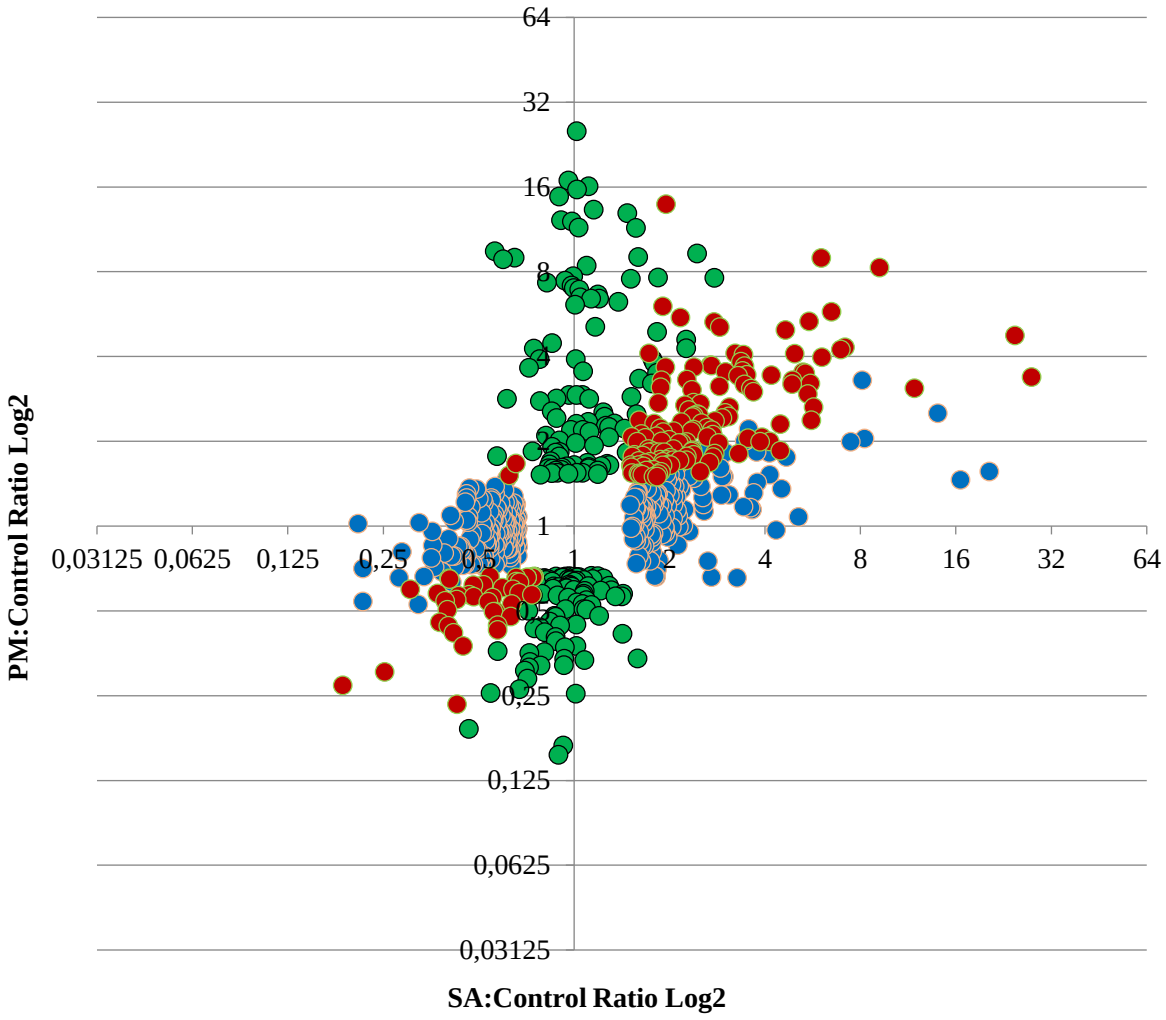


Figure 9. Bivariate plot of expression fold-change for genes that respond to PM colonization and/or MeSA treatment in grapevine leaves. Fold-change of expression induced by PM (vertical axis) plotted against fold-change of expression induced by MeSA (horizontal axis) for genes that respond at the 99% significance level with at least 1.5-fold up- or down-regulation allowing for 5% false discovery rate. Graph does not include data point for the fungal gene derived probe set (1615715_at). Green: PM-responsive only; blue: MeSA-responsive only; red: both treatment-responsive.

5.1.1 Genes induced by both MeSA treatment and PM colonization

Among the 179 transcripts that responded in a similar way to PM and to MeSA, we found genes that function in biotic stress signaling as well as in primary and secondary metabolism (Figure 8). We refer to these genes as the PM- and SA-regulated gene sets (the Ensembl IDs of the identified most interesting genes along with encoded proteins are listed in Table 1). The key

signaling molecule for systemic acquired resistance is MeSA, a mobile form of SA (Park *et al.*, 2007). Gene *SAMTBSCMT* was found to be up-regulated by PM as well as by MeSA treatment. *SAMTBSCMT* encodes a salicylate O-methyltransferase which catalyzes the formation of MeSA from SA and regulates MeSA formation at the site of infection; MeSA is then delivered to the systemic uninfected region of the plant where it can be converted back to SA by SABP2 (SA binding protein 2) to fulfill its function (Vlot *et al.*, 2009). We found that most of the typical defense-associated genes responded to MeSA treatment. During pathogen attack the receptor-like protein kinases (RLKs) are the first key regulator proteins of pathogen-associated molecular patterns-triggered immunity (PTI). Among the identified kinases, there are many belonging to leucine-rich repeat domain-containing RLKs, which regulate a wide variety of defense responses (Matsushima and Miyashita, 2012). From the identified 25 PM-responsive RLKs, 15 were stimulated by MeSA. Three of these were homologous to the *Avr9/Cf-9 Rapidly Elicited 256* gene of tobacco, which is one of the key regulators of the HR during biotic stress (Rowland *et al.*, 2005). Another key defense signaling gene that was found both MeSA- and PM-inducible is *Enhanced Disease Susceptibility1* (*EDS1*). Although EDS1 is an upstream regulator of SA, previous studies demonstrated that abundant SA may feedback-regulate the EDS1/PAD4 complex in *Arabidopsis* (Vlot *et al.*, 2009). It has recently been shown that *V. vinifera EDS1* is induced in response to SA and that its ortholog from a PM-resistant *V. aestivalis* grape variety has a distinct expression pattern (Gao *et al.*, 2010).

Defense signaling downstream from SA is largely continued by activation of NPR1/NIM1, where NPR1 is interacting with NIMIN1, 2, 3 (NIM-interacting1, 2, 3) and several TGA factors to induce defense gene expression (Hermann *et al.*, 2013). Although NIMIN-1 acts as a negative regulator of SA/NPR1 signaling (Weigel *et al.*, 2005), we found a gene, probably encoding the grapevine ortholog of NIMIN-1, which was up-regulated in response to both treatments. NPR1, TGA2, 3, 5, and/or 6 control *WRKY* transcription factor genes, which may positively or negatively regulate the defense response (Wang *et al.*, 2006). The grape ortholog of *WRKY18_2*, an *Arabidopsis* gene known to positively and negatively regulate SA/EDS1-mediated resistance against *Pseudomonas syringae* and *G. orontii*, respectively (Schön *et al.*, 2013), was stimulated by both treatments. In addition, we also found two Myb-type transcription factor (TF) genes, namely, *MYB108* and *MYB14_3*, to be MeSA-inducible. The MYB108 TF belongs to the R2-R3-type MYB family, members of which are known to be involved in the SA-signaling pathway (Eulgem, 2005). *MYB108* is closely related to the ABA-dependent *BOTRYTIS SUSCEPTIBLE1* gene, which is a negative regulator of cell death triggered by wounding or pathogen attack (Cui *et al.*, 2013).

The following pathogenesis-related (*PR*) genes were regulated via both SA, and PM: *PRP1* genes, *BG3* genes, *PR-3*, *CHIV* genes, *CHIB1*, *OSM34* genes, *PRXR11* and *NtPRp27 secretory protein*. *PR* genes were expressed during the course of the infection process with a steady increase starting at early infection stages (Fung *et al.*, 2008). Due to their expression pattern they were all allocated to the same cluster (Fung *et al.*, 2008). It is likely that the regulation of *PR* genes during PM infection is indicative of the coordination of the defense response via SA signaling, as it was found for other plant-pathogen interactions (Wildermuth *et al.*, 2001). Fungal infection-triggered PR protein secretion may be assisted by chaperone proteins (Gupta and Tuteja, 2011). The expression of chaperone genes *calnexin 1* (*CNX1*) and *endoplasmin* (*SHD*) was found to be up-regulated in PM- and SA-dependent manner, as it was earlier shown for their orthologs in *Arabidopsis* (Chandran *et al.*, 2009).

PM infection along with SA signaling may also induce cross-linking of molecules in the plant cell wall and/or deposition of lignin as part of PTI (Bhuiyan *et al.*, 2009), which is indicated by the enhanced expression of *OMT1* (caffeic acid O-methyltransferase), a gene known to be involved in lignin synthesis (Louie *et al.*, 2010).

Genes encoding heat shock proteins (HSPs) (*HSP70-1*, *HSP17.6II*, and *BIP1*), a heat shock-related TF (HSF4), a DNAJ homolog (ERDJ3B) and an Aha1 domain-containing protein functioning as activator of HSPs (Kotak *et al.*, 2004) all responded to both PM and SA. HSPs are involved in abiotic stress signaling and their role in plant responses to pathogen attack is yet to be fully understood. However, these proteins were also found to be active under oxidative stress as ROS and photorespiratory H_2O_2 induces their expression (Shahnejat-Bushehri *et al.*, 2012; Wu *et al.*, 2012). The earliest response after PM infection in *V. vinifera* is an oxidative burst, and rapid up-regulation of genes involved in protection from ROS (Fung *et al.*, 2008). The pathogen-triggered ROS could explain that these heat shock protein encoding genes are up-regulated by both treatments. Furthermore, low levels of H_2O_2 act as a signal for defense gene expression (Neill *et al.*, 2002), which is supported by the PM and MeSA-dependent up-regulation of a reticuline oxidase precursor transcript. Reticuline oxidase (BBE) catalyzes H_2O_2 production by using hexose sugars and it mediates basal resistance against pathogens (Bechtold *et al.*, 2010). However, the *MSS1* (sugar transport protein 13) was also up-regulated by both treatments.

Defense responses along the SA-mediated pathway include redox signaling which is based on the glutathione (GSH) and disulphite (GSSG) ratio. Glutathione S-transferases (GSTs) have both conjugase and peroxidase activity, therefore, GSTs use GSH and reduce H_2O_2 amount, thereby increasing GSSG levels (Rahantaniaina *et al.*, 2013). Indeed, glutaredoxins (GRXs), namely, GRX480 and a cytosol localized GSTU8, which lower H_2O_2 and elevate GSSG levels were found to be up-regulated by both PM colonization and MeSA treatment. It has been

demonstrated that AtGRX480 mediates redox regulation by TGA factors during stress, and linked to SA-dependent pathway (Herrera-Vasquez *et al.*, 2015). We found in grapevine, however, that another glutaredoxin was markedly repressed by both treatments.

Among the PM-responsive ATP binding cassette (ABC) transporters, we identified three genes which were up-regulated by MeSA. One identified transporter probably belongs to the C, the two others to the G family (ABCG7). Notably, the expression of the G family members were induced to very high levels by MeSA (6- and 25-fold). Members of the G family are known to mediate the export of cuticular lipids, with *PEN3* being a key player in the defense response in *Arabidopsis* (Bird *et al.*, 2007; Underwood and Somerville, 2013).

We found several PM-stimulated secondary metabolism-related genes which play a role in the biosynthesis of antimicrobial compounds. The genes encoding HMG-CoA-synthase (*MVA1*) and HMG-CoA-reductase (*HMGR1*) were activated by both PM and MeSA treatments. The MVA1 and HMGR1 proteins are components of the isoprenoid biosynthesis pathway and involved in the synthesis of mevalonate (Miziorko, 2011). Mevalonate is the precursor of phytosterols which play a key role in innate immunity and restrict the nutrient efflux into the apoplastic space where nutrients may be taken up by the pathogen (Wang *et al.*, 2012b). Moreover, it has been demonstrated previously that the over-expression of *Brassica juncea HMG-CoA-Synthase1* in *Arabidopsis* resulted in the constitutive expression of *PRP1*, *PR2* and *PR5* along with suppression of H_2O_2-induced cell death (Wang *et al.*, 2011), which is in agreement with our findings in grapevine presented here.

Genes involved in aromatic amino acid and phenylpropanoid biosynthetic pathways, such as *prephenate dehydratase* (*PD1*), *anthocyanidin O-glucosyltransferase* (*RHGT1* and *GT*), *UGT89B1* and *DMR6* genes were found to be inducible by both MeSA and PM. This is in agreement with the notion that flavonoids and their anthocyanin derivatives have anti-fungal activity in grape varieties (Schaefer *et al.*, 2008). However, the *Arabidopsis AtDMR6* gene was found to provide susceptibility to downy mildew (Zeilmaker *et al.*, 2015). Transcription of the flavonoid biosynthetic gene, *CYP706A4* (encoding flavonoid 3'-hydroxylase) as well as the cytokinin glucosyltransferase gene, *UGT85A2* were down-regulated by both treatments.

SA antagonizes JA signaling in various biotic stresses and it was found that increased SA levels along with repression of JA-signaling resulted in resistance against biotrophic pathogens but provided susceptibility to necrotrophs (Robert-Seilaniantz *et al.*, 2011). This cross-talk may be partially dependent on the cellular redox status, while overexpression of *GRX480* induced *PR-1*, but repressed *PDF1.2* (Ndamukong *et al.*, 2007). Confirming this relationship, MeSA as well as PM induced the expression of *JAZ1_2* in our study. JAZ proteins were shown to repress transcription of JA-responsive genes (Pauwels and Goossens, 2011). However, synergism was also

observed between these two signaling pathways as SA signaling does not always repress JA biosynthesis (Salzman *et al.*, 2005). We found three genes including *LOX2* (lipoxygenase), *CYP74A* (allene oxide synthase), and *OPR2* (12-oxophytodienoate reductase 2) which participated in JA synthesis and which were up-regulated in response to both treatments. This finding is consistent with a recent study which demonstrated that *LOX* expression in cucumber was stimulated not only by PM and SA, but also by JA and ABA (Sang-Keun *et al.*, 2014).

The basal defense of susceptible plants also implicates processes that lead to cell wall fortification in response to pathogen attack. We identified two cell wall-related genes, *EXPA8* and a pectate lyase, which were down-regulated by both treatments. Expansins unlock the network of cell wall polysaccharides and pectate lyases degrade the pectin component of cell wall (Cosgrove, 2000; Marin-Rodriguez *et al.*, 2002), which account for the fact that their repression maintains cell wall integrity. PM-induced repression of these grapevine genes via SA-signaling suggests a regulatory mechanism by the plant to boost structural resistance against the invading pathogen. In *Arabidopsis*, down-regulation of the pectate lyase-like gene *PMR6* was shown to enhance resistance to PM (Vogel *et al.*, 2002). Thus, PM-induced repression of these grapevine genes suggests that their down-regulation may also contribute to enhanced resistance.

Overall, expression of most genes modulated by both MeSA and PM were part of the SA-mediated defense response The majority of the MeSA- and PM-responsive transcripts are downstream of SA in the signaling cascade (as the NIMIN1-1, WRKY or PR proteins), but some upstream regulators (EDS1) are also known to participate in a feedback-regulatory loop with SA.

Table 1. Ensembl IDs of the identified genes along with encoded proteins, of which reacted similarly to both treatments.

Gene	Protein	Ensembl ID
SAMTBSCMT	salicylate O-methyltransferase	VIT_04s0023g02240
EDS1	lipase-like protein	VIT_17s0000g07420
NIMIN-1	NIM-interacting1	VIT_07s0005g02070
WRKY18_2	WRKY transcription factor	VIT_04s0008g05760
MYB108	MYB transcription factor	VIT_05s0077g00500
MYB14_3		VIT_05s0049g01020
PRP1	Pathogenesis-related protein	VIT_03s0088g00710/ VIT_03s0088g00810/ VIT_03s0088g00700/ VIT_00s0207g00130
BG3		VIT_06s0061g00120/ VIT_08s0007g06040
PR-3		VIT_03s0038g03400
CHIV		VIT_05s0094g00360/ VIT_05s0094g00350/ VIT_05s0094g00220

CHIB1		VIT_16s0050g02220
OSM34		VIT_02s0025g04250/ VIT_02s0025g04330/ VIT_02s0025g04340/ VIT_02s0025g04310
PRXR11		VIT_07s0129g00360
NtPRp27	NtPRp27 secretory protein	VIT_03s0091g00160
CNX1	calnexin 1	VIT_00s0283g00030
SHD	endoplasmin	VIT_18s0001g14500
OMT1	caffeic acid O-methyltransferase	VIT_16s0098g00850
HSP70-1	heat shock protein	VIT_08s0007g00130
HSP17.6II		VIT_04s0008g01490
BIP1		VIT_16s0098g01580
HSF4	heat shock-related transcription factor	VIT_07s0031g00670
ERDJ3B	DNAJ homolog	VIT_07s0005g01220
-	Aha1 domain-containing protein	VIT_08s0007g06710
-	reticuline oxidase precursor	VIT_10s0003g05450
MSS1	sugar transport protein 13	VIT_11s0016g03400
GRX480	glutaredoxin	VIT_10s0003g00390
-		VIT_07s0104g01390
GSTU8	glutathione-S-transferase	VIT_08s0007g01400
ABCG7	ATP binding cassette transporter	VIT_00s0625g00020/ VIT_03s0017g01280
MVA1	HMG-CoA-synthase	VIT_02s0025g04580
HMGR1	HMG-CoA-reductase	VIT_03s0038g04100
PD1	prephenate dehydratase	VIT_06s0061g01300
RHGT1	anthocyanidin O-glucosyltransferase	VIT_16s0050g01680
GT		VIT_03s0017g02110/ VIT_12s0034g00130
UGT89B1	UDP-glycosyltransferase	VIT_17s0000g04750
DMR6	2OG-Fe(II) oxygenase	VIT_16s0098g00860/ VIT_13s0047g00210
CYP706A4	flavonoid 3'-hydroxylase	VIT_00s1682g00020
UGT85A2	cytokinin glucosyltransferase	VIT_00s0324g00070
JAZ1_2	jasmonate-zim-domain protein	VIT_09s0002g00890
LOX2	lipoxygenase	VIT_06s0004g01510
CYP74A	allene oxide synthase	VIT_18s0001g11630
OPR2	12-oxophytodienoate reductase 2	VIT_18s0041g02020
EXPA8	expansin	VIT_13s0067g02930
-	pectate lyase	VIT_17s0000g09810

5.1.2 **Genes induced by PM colonization but not by SA treatment**

Among the PM-regulated genes in grapevine, 185 candidates were identified which were not triggered solely by MeSA, indicating that elevated SA levels alone cannot substitute for regulation by PM. These 185 genes are referred to as the “PM-dependent” gene set (the Ensembl IDs of the identified most interesting genes along with encoded proteins are listed in Table 2). These include numerous genes that are involved in primary metabolism, including the pathways

of carbohydrate, protein, and fatty acid metabolism (Figure 8). Since PMs are obligate biotrophic pathogens, they must rely on their host as carbon and nitrogen source and, therefore, modulate plant metabolic processes to fulfill their needs. However, previous results demonstrated that carbohydrates also may have signaling function in defense responses as the increased content of soluble sugar induced the expression of *PR* genes in *Arabidopsis* (Thibaud *et al.*, 2004). Besides the activation of defense-genes, sugar accumulation is also expected to decrease photosynthesis (Araya *et al.*, 2006). In agreement with these expectations, we found that all photosynthesis-related PM-dependent genes, including photosystem II 22 kDa protein, photosystem II light harvesting complex 2.1, NADH dehydrogenase I subunit N, plastocyanin-domain containing protein, LHCII-type I CAB-1, and light-harvesting chlorophyll-binding protein 3, were down-regulated in response to PM infection. Potentially, the down-regulation of these genes could be linked to plant defense responses. For example, PM infection induced the expression of *MES17* pheophorbidase gene which may participate in chlorophyll breakdown (Christ *et al.*, 2012), a consequence of programmed cell death.

An early response to pathogen infection is the apoplastic accumulation of ROS, which may be mediated by aquaporins. However, PM infection repressed *AQUAPORIN TIP1_3* encoding a protein known to translocate H_2O_2 across the plasma membrane (Bienert and Chaumont, 2014). Interestingly, RNAi silenced *tip1-1 Arabidopsis* plants revealed an increased apoplastic carbohydrate content (Ma *et al.*, 2004), suggesting that *AQUAPORIN TIP1_3* suppression in infected grapevine may support the sugar availability for the pathogen. In addition, the transcription of a germin-like protein-encoding gene was also induced by PM. Such proteins were found to catalyze H_2O_2 production (Hu *et al.*, 2003).

Among the PM-dependent gene set, several transcription factors were identified, among them a *NAC*-type transcription factor (*NAC042_5*). Based on the expression pattern reported earlier (Fung *et al.*, 2008) this gene belongs to the same cluster as the genes for pinoresinol forming dirigent protein (*DIRPR*), dicyanin blue copper protein (*BCB*) and isoflavone methyltransferase (Fung *et al.*, 2008). These latter genes were also PM-dependent, albeit their cluster also contains *PR* genes (Fung *et al.*, 2008) which were PM and MeSA-inducible in our current dataset. Two other transcription factors that belong to the WRKY family (*WRKY71_2* and *WRKY21_2*) were in the PM-dependent gene set. Previous studies demonstrated that WRKY71 is involved in the defense response and that it is an upstream regulator of NPR1 in rice (Liu *et al.*, 2006). The WRKY IId subfamily members, including WRKY21, were found to interact with Ca^{2+}/calmodulin binding transcription factors (Park *et al.*, 2005) and mediate the defense response. However, the transcription of a calmodulin-binding protein was found to be up-regulated by both MeSA and PM.

Two typical defense associated genes, namely *PR10* and *Bet v I allergen*, were strongly expressed (8- and 13-fold up-regulation) only in response to PM. Although most PR transcripts were found to be MeSA-inducible, these genes responded only to PM. They were grouped in a cluster along with genes encoding stilbene synthases and the cytochrome P450 84A1 (FAH1) (Fung *et al.*, 2008). Several studies proved that PR-10 proteins which have RNase, DNase and anti-fungal activity play a role in defense responses and cell death and that they are regulated by WRKY TFs (Choi *et al.*, 2012; Agarwal and Agarwal, 2013). It was shown that the expression of the *V. vinifera PR10.1* was transcriptionally regulated by the WRKY33 TF due to *Plasmopara viticola* infection (Merz *et al.*, 2014). Furthermore, it was demonstrated that the *Asparagus PR10* was responding to pathogen infection and H_2O_2 independently from SA (Mur *et al.*, 2004), which suggests that these proteins mediate defense responses upstream or independent of SA signaling in grapevine, too.

Some genes involved in the biosynthesis of stilbenoids, flavonoids and phenylpropanoids were found to be regulated in a PM-dependent manner. The genes encoding DAHP- and EPSP-synthases were found to be up-regulated. The corresponding proteins catalyze the synthesis of aromatic amino acids, which are precursors of flavonoids and stilbenoids in the shikimate pathway (Herrmann, 1995). The expression of stilbene synthase genes (*STS2*, *STS4*, *TT4* genes), as well as the expression of an R2R3-type MYB factor gene (*MYB14_2*) which likely regulates stilbene biosynthesis, was 3- to 7-fold up-regulated by PM in grapevine. Previously, a *MYB14* was found to be co-expressed with *STSs* and to specifically interact with the promoters of *STS41* and *STS29* in grapevine (Holl *et al.*, 2013). Stilbenes in *Vitis* species were proposed to be part of the plant's arsenal against *E. necator* (Dai *et al.*, 2012). The gene encoding chalcone-flavonone isomerase (*TT5*) involved in flavonoid biosynthesis was also regulated in a PM-dependent manner. A similar response was found for a putative *DFRA* and a *UGT75C1* gene, which are involved in secondary metabolism. UGTs along with cytochrome P450 monooxygenases play a key role in creating the structural diversity of triterpenoid saponins (Seki *et al.*, 2015), which are antifungal compounds (Favel *et al.*, 1994). Among the six PM-dependent cytochrome P450 genes identified, four were up-regulated (*FAH1* genes, *CYP87A2*, *CYP716A1*) and two were down-regulated (*CYP714A1*, *CYP87A2*) in response to the pathogen's presence. Corroborating our findings, the *Arabidopsis FAH1* was also found to be up-regulated by PM independently of SA signaling (Chandran *et al.*, 2009). The cytochrome P450 gene *CYP716A1*, whose protein product is involved in antimicrobial saponin biosynthesis, was up-regulated 9-fold in response to PM, supporting the premise that it mediates plant defense (Fukushima *et al.*, 2011). Geraniol 10-hydroxylase is involved in terpenoid indole alkaloid biosynthesis (Collu *et al.*, 2001) and its gene is homologous to *AtCYP76C1*, which was also down-regulated independently from SA in response to PM in *Arabidopsis* (Chandran *et*

al., 2009). Since these genes were not inducible by SA the inducer is likely to be another signal. *CYP87A3* (61% identity to *CYP87A2*) was previously reported to be responsive to auxin (Chaban *et al.*, 2003) which may also act as a defense signal during pathogen attack and it may have an antagonistic regulatory role to SA (Kazan and Manners, 2009).

Four of the six PM-responsive dirigent-like protein genes, which play a role in lignin synthesis (Davin and Lewis, 2000) were strongly up-regulated (3- to 13-fold) in a PM-dependent manner, which is in agreement with previous reports (Borges *et al.*, 2013). Another lignin biosynthetic gene encoding a cinnamoyl-CoA reductase, was found to be regulated by PM only. The protein product of this gene promotes the H-, S-, and G-lignin formation in the monolignol pathway.

The acyl-CoA-binding domain 3 proteins (ACBP3) are proposed to be involved in lipid metabolism. However, the *Arabidopsis* ACBP3 also regulates the NPR1-dependent defense in response to the biotrophic bacterium *P. syringae*, and overexpression of *ACBP3* resulted in enhanced *PR* expression, cell death and H_2O_2 production (Xiao and Chye, 2011). We found that the *ACBP3* grapevine gene is not MeSA-inducible, but it is triggered by the pathogen. In contrast, other genes encoding enzymes involved in the lipid metabolism (3-oxoacyl-[ACP] reductase, a probable sulfotransferase) and in lipid transfer/binding were at least 6-fold down-regulated by PM, in accordance with previously reported results (Fung *et al.*, 2008). Since lipids have a signaling function during pathogen attack, the PM-mediated down-regulation of the expression of such genes may halt activation of defense responses.

Table 2. Ensembl IDs of the identified genes along with encoded proteins, of which reacted solely to PM infection

Gene	Protein	Ensembl ID
NAC042_5	NAC transcription factor	VIT_12s0028g00860
-	photosystem II 22 kDa protein	VIT_18s0001g02740
-	photosystem II light harvesting complex 2.1	VIT_12s0057g00630
-	NADH dehydrogenase I subunit N	VIT_06s0004g08360
-	plastocyanin-domain containing protein	VIT_02s0025g02410
-	LHCII-type I CAB-1	VIT_19s0014g00160
-	light-harvesting chlorophyll-binding protein 3	VIT_00s0181g00200
MES17	pheophorbidase	VIT_13s0067g03260
TIP1_3	aquaporin	VIT_06s0061g00730
-	germin-like protein	VIT_17s0000g05360
DIRPR	pinoresinol forming dirigent protein	VIT_02s0025g00750
BCB	dicyanin blue copper protein	VIT_09s0002g06890
-	isoflavone methyltransferase	VIT_12s0028g01940
WRKY71_2	WRKY transcription factor	VIT_12s0028g00270
WRKY21_2		VIT_00s2547g00010
-	calmodulin-binding protein	VIT_01s0026g01790

PR10	pathogenesis-related protein	VIT_05s0077g01530
Bet v I allergen		VIT_05s0077g01540
-	DAHP-synthase	VIT_00s0391g00070
-	EPSP-synthase	VIT_15s0048g00350
STS2	stilbene synthase	VIT_16s0100g00990
STS4		VIT_16s0100g01000
TT4		VIT_16s0100g01190/ VIT_16s0100g01140/ VIT_16s0100g00840
MYB14_2	MYB transcription factor	VIT_07s0005g03340
TT5	chalcone-flavonone isomerase	VIT_13s0067g03820
DFRA	dihydrofolate reductase	VIT_08s0040g00440
UGT75C1	UDP-glycosyltransferase	VIT_05s0062g00740
FAH1	ferulate-5-hydroxylase	VIT_07s0031g01380/ VIT_04s0023g02900
CYP87A2	cytochrome P450	VIT_02s0025g04080
CYP716A1		VIT_11s0065g00130
CYP714A1		VIT_13s0067g00110
CYP87A2		VIT_02s0025g04080
-	geraniol 10-hydroxylase	VIT_15s0048g01490
-	dirigent-like protein	VIT_06s0004g01020/ VIT_02s0025g00750/ VIT_06s0004g01010/ VIT_06s0004g00990
-	cinnamoyl-CoA reductase	VIT_02s0012g01570
ACBP3	acyl-CoA-binding domain 3 protein	VIT_07s0129g00430
-	3-oxoacyl-[ACP] reductase	VIT_01s0010g02670
LTP	lipid transfer/binding	VIT_04s0008g05640/ VIT_11s0016g05840/ VIT_04s0008g05640

5.1.3 Confirmation of microarray measured expression of *NAC042_5* in response to PM and MeSA using qPCR

In validation of expression of our gene of interest *NAC042_5* we found that the gene was significantly up-regulated by PM and did not respond to SA, as it was shown by the microarray technique. The significant fold change was approximately the same measured with the two different techniques (Figure 10).

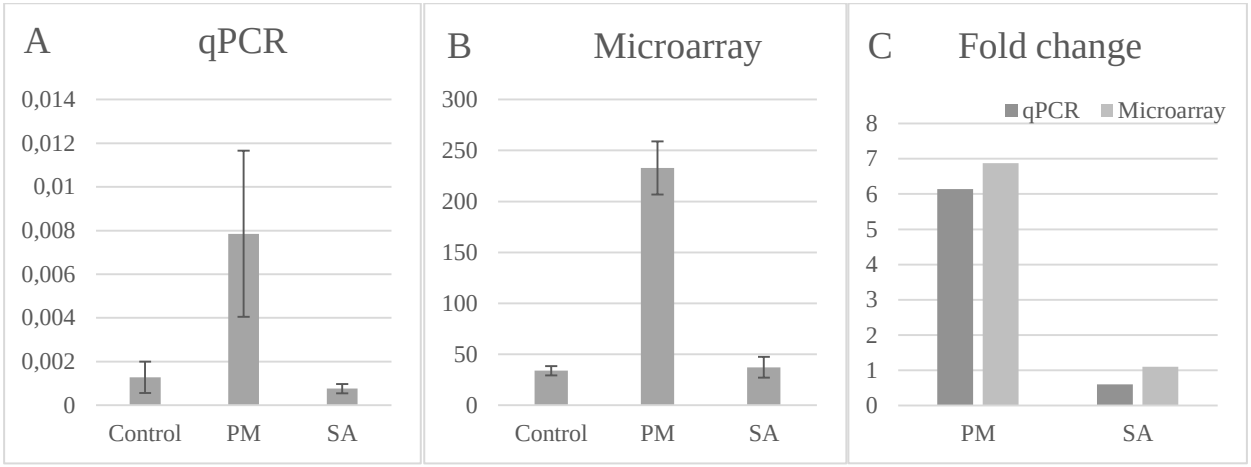


Figure 10. Expression values and fold change chart of NAC transcription factor in response PM or SA compared to the control reference leaves. A) Expression measured using qPCR. B) Expression measured using microarray (linear values). A-B) error bars represent the standard error. C) Fold change in response to the two treatments compared to the control using both techniques.

5.2 Analysis of *NAC042_5* regulation in transgenic plants

Among the genes most dramatically regulated by PM but not by MeSA there is the *NAC042_5* (*NAC-like transcription factor 42*) gene with its 7-fold induction in response to the fungus. To confirm that the transcription of *NAC042_5* gene is indeed SA-independent, a *pNAC042_5::gusA* reporter construct was transferred into Wassilewskija wild type (WT, WS-0), and WS-*nim1-1*, WS-*nahG* mutants of *Arabidopsis thaliana*, where *pNAC042_5* denotes a 3896-bp long stretch sequence of the *pNAC042_5* promoter (GenBank accession number: KU297673).

5.2.1 Analysis of basal expression in transgenic plants

GUS staining of non-inoculated homozygous transgenic plants demonstrated that all three types of transgenic *Arabidopsis* (WS-0, WS-*nim1-1*, and WS-*nahG*) showed a similar basal *gusA* expression independently of PM challenges. Earlier studies demonstrated that members of *Vitis NAC* gene family govern organ development in grapevine species and expression differs in various developmental stages (Le Henanff *et al.*, 2013; Wang *et al.*, 2013). The regulation of *NAC042_5* did not appear to be tissue specific but probably this gene play a role in organ evolution. The promoter activity in transgenic plants was detected in SAM, young developing shoots and leaves, in trichomes, in vascular tissues of the leaves, stems, roots, petals and gynoecium, in the tips of cotyledon, young leaves in stipules, in hydathodes, in lateral shoot buds, in connective tissue of the anther, in style, in transmitting tract within the carpel and in abscission zone of gynoecium (Figure 11). Promoter activity in this broad variety of organs could be explained by a more general transcriptional regulatory function.

In comparison to previous results, the *pNAC042_5* regulated activity showed similar expression patterns to the one regulated by the promoters of *PECTATE LYASE-LIKE (PLL)* gene

family (Sun and van Nocker, 2010). The *PLL* promoters induced *GUS* expression was also identified in the abscission zone of flower organ, in style, septum and transmitting tract, in trichomes, in hydathodes, stipules, and in roots. However, some *PLL* promoters regulated *GUS* expression in root tips, too, and found to be auxin-inducible (Domingo *et al.*, 1998; Laskowski *et al.*, 2006; Palusa *et al.*, 2007). Previous study demonstrated that auxin regulate abscission in flowers; the available IAA stipulates the fixed time of organ shedding (Basu *et al.*, 2013). Furthermore, PLLs catalyze the cleavage of de-esterified pectin within the primary cell wall and their regulation role was explained in abscission of flower petals, anthers, and dehiscence of the siliques (Sun and van Nocker, 2010). Pectate lyases also function in pollen tube emergence, growth and manage degradation of the cell wall of transmitting tissue in the style to promote pollen penetration (Taniguchi *et al.*, 1995; Wu *et al.*, 1996). We found an up-regulation of *NAC042_5* in the style and in the transmitting tract too. Additionally, it was interesting to observe that a pollen grain stuck to the surface of a fertilized gynoecium, and the germination of the pollen induced the expression of *NAC042_5* at that site (Figure 11, arrowhead). Previous results showed that auxin may promote pollen tube growth (Wu *et al.*, 2008), but programmed cell death also occurs in the cells within the transmitting tract during fecundation (Crawford and Yanofsky, 2011).

Auxin is an important phytohormone regulating the development of the *Arabidopsis* gynoecium (Hawkins and Liu, 2014). An auxin maximum is identified in the top ring of the carpel and the level is always associated with the developmental stage of the style and stigmatic tissue. The auxin balance is coordinated by the *SHORT INTERNODE (SHI)/STYLISH (STY)* transcription factor family, members of which induce local synthesis and also transport the auxin away from the site of production (Sohlberg *et al.*, 2006; Alvarez *et al.*, 2009; Trigueros *et al.*, 2009). STY1 induce the YUCCA4 biosynthetic enzyme to produce local auxin in the style (Sohlberg *et al.*, 2006), which then activate *NGATHA (NGA)* genes in the gynoecium apex (Alvarez *et al.*, 2009; Trigueros *et al.*, 2009). *YUCCA4* was found to express in the tip of cotyledon and in the apical meristem (Cheng *et al.*, 2007).

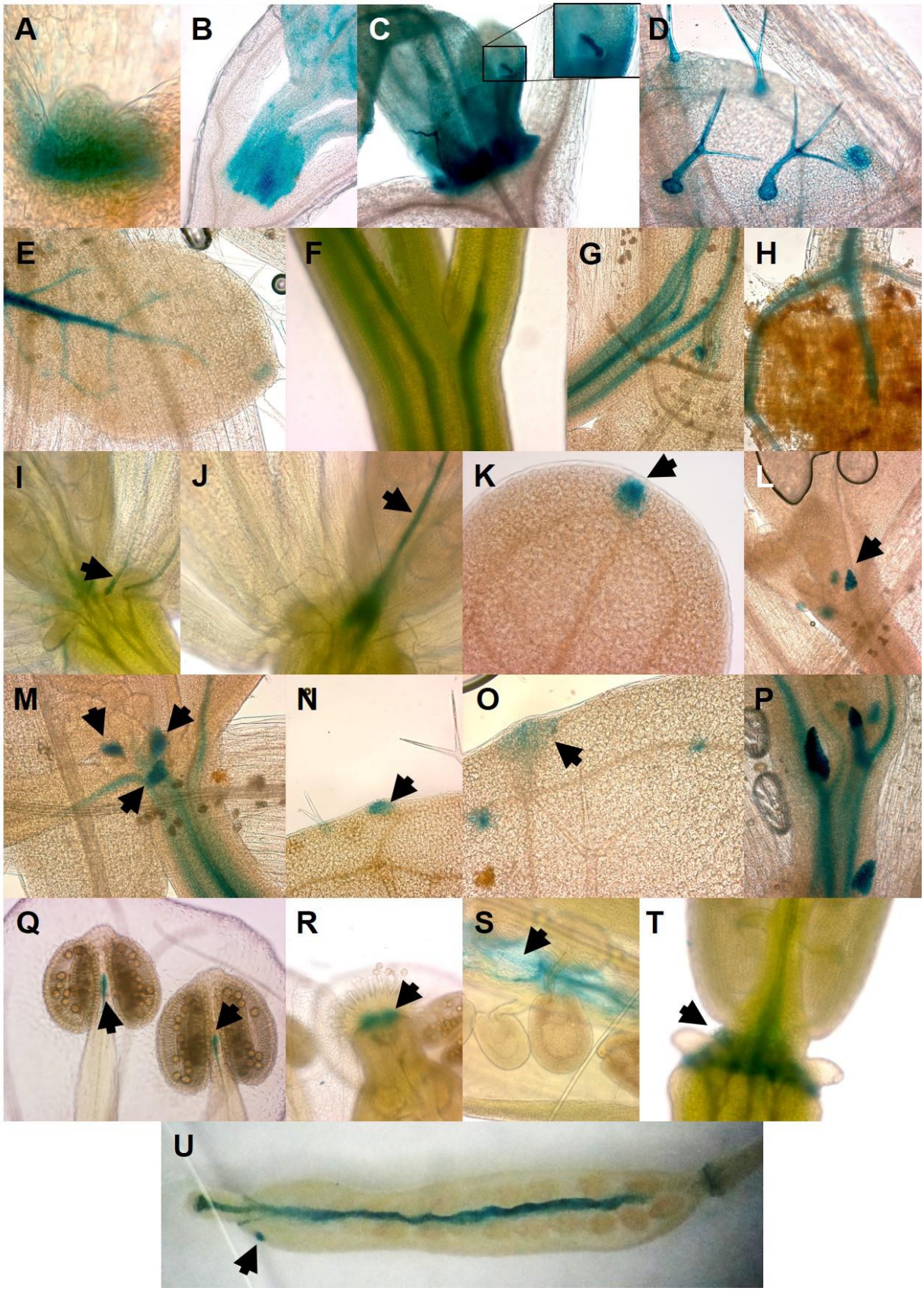


Figure 11. Localization of *gusA* expression regulated by the *NAC042_5* promoter in transgenic *Arabidopsis* plants. (A) SAM, (B) young shoots, (C) developing new leaves (inset: developing trichome) (D) trichomes, (E) vascular tissue (vt) of leaf, (F, G) vt of shoot, (H) vt of root, (I) vt of petals, (J) vt of gynoecium, (K) tip of the cotyledon, (L, M) stipules, (N, O) hydathodes, (P) lateral shoot buds of developing inflorescence, (Q) connective tissue of the anther (R) style (S) transmitting tract of gynoecium (T) abscission zone of gynoecium (U) transmitting tract of the ripen silique

Auxin maximum in the leaf primordia is controlled by its influx carrier (AUXIN RESISTANT/AUX1) and efflux carrier proteins (PIN-FORMED1/PIN1). PIN1 regulates auxin abundancy in the outgrowth lobes of the developed leaves and in the tip of the cotyledon. PIN1 co-regulate leaf serration with the *CUP-SHAPED COTYLEDON2* (*CUC2*) gene (encode a NAC domain). The *CUC2* is expressed in the sinuses of the leaf and the balance in serration is governed by the microRNA, *mir164* (Kalve *et al.*, 2014).

The synthetic auxin reporter construct contains *DR5* promoter (containing response elements such as TGTCTC), which is transcriptionally fused to the *gus* (Ulmasov *et al.*, 1997). This construct was used to detect free auxins in the non-challenged *Arabidopsis* plant (Aloni *et al.*, 2003). The group found that free auxins are abundant in hydathodes, in vascular area, in the tip of the young leaf, at the base of some of the trichomes, at the leaf margin, and in the root tip.

Trichomes are differentiated polyploid cells in the leaf epidermis, and their development is initiated by auxin-dependent transcriptional regulation (Deng *et al.*, 2012). The nuclear ploidy level of these cells is eight or sixteen times higher than the adjacent pavement cells. The trichome precursor within the epidermis multiply its DNA content, therefore able to expand the cell. Both the endoreduplication and the cell elongation are triggered by auxin. A study showed that auxin and cytokinin are required for cell division of single cells but endorduplication only took place if auxin was present and cytokinin was absent in the cells (Valente *et al.*, 1998). After endoreduplication the nucleus migrated to the top of the elongated cell, which was followed by branching of the leafhair (Figure 12) (Hulskamp, 2004). Trichome development is enhanced by gibberellins and jasmonic acids (Kalve *et al.*, 2014) and according to the latest research results cytokinin may also co-regulate the initiation (Sun *et al.*, 2013; Sun *et al.*, 2015). Cytokinin oxidase/dehydrogenase (*CKX*) genes were found to be expressed in the trichomes and in hydoathodes, which enzymes degrade cytokinins (Eckardt, 2003). We found intensive expression in the trichome mostly in the young developing leaves. The not fully expanded trichomes showed higher β-glucuronidase content compared to the full-grown trichomes of the elder leaves, suggesting that elongation of the cells may activate the *NAC042_5* in these cells.

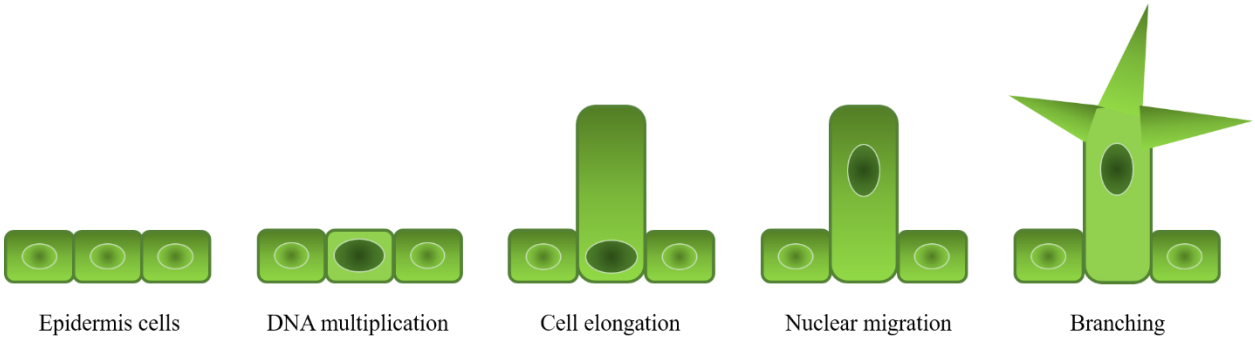


Figure 12. Trichome development in *Arabidopsis thaliana*

The antagonistic relationship of cytokinin and auxin is a well-studied phenomenon in developmental processes. The main organ observed to showh this antagonistic effect in its development is the SAM. Cytokinins maintain stem cells in SAM, which are regulated by the *KNOX1*. If auxin accumulates in the leaf primordia, it inhibits cytokinins by inactivating *KNOX1* (Kalve *et al.*, 2014). Cytokinins may be transported in the vascular system by the purine permeases (PUP). Interestingly, the promoter *PUP1::GUS* fusion also showed hydathode and style expression (Burkle *et al.*, 2003). A NAC transcription factor NTM1 is assumed to participate in cell division; cytokinins regulate activity by controlling the stability of NTM1 (Kim *et al.*, 2006). However, the *ntm1* knock-out plants exhibited reduced growth, suggesting the *NTM1* is a positive regulator of cell growth. In contrast, our co-workers (Edina Novák and Dr. Róbert Oláh) prepared transgenic grape plants overexpressing the *NAC042_5* stayed dwarf and died after a certain period of time. Furthermore, the plants in which the *NAC42_5* was silenced grew normally similarly to the WT ones (unpublished data). This suggests that our identified *NAC042_5* gene may also play a role in plant development, but probably with a negative effect. Other functional studies of NACs showed that up-regulation of the *Arabidopsis ANAC036* gene resulted in a semidwarf phenotype; shorter leaf blades, stems, petioles (Kato *et al.*, 2010). The closest ortholog in rice (ONAC022) also appears to play a role in plant developmental processes, since the up-regulation of *ONAC022* affected plant height in transgenic rice (Hong *et al.*, 2016). Plants overexpressing *ATAF1* or the *Xylem NAC Domain 1* (*XND1*) gene also stayed stunted compared to the wild type (Zhao *et al.*, 2008; Grant *et al.*, 2010). Differentiation of tracheary elements is initiated by vascular cambium formation, cell expansion, secondary cell wall deposition and finally the cell death (Fukuda, 1996), in which auxins and cytokinins probably cooperate (Milioni *et al.*, 2001). The *XND1* was found to negatively regulate PCD in the xylem (Zhao *et al.*, 2008). Contrarily, the *Vascular-Related NAC Domain 6* activates PCD-related and secondary wall formation genes in tracheary elements (Ohashi-Ito *et al.*, 2010).

5.2.2 **Analysis of PM-induced expression in transgenic plants**

To quantify transcriptional activity directed by the *NAC042_5* promoter, we inoculated and mock-inoculated the *pNAC042_5::gusA* transgenic *Arabidopsis* lines with *O. neolycopersici* following the method described by Huibers *et al.* (2013). By 14 dpi, the inoculation has led to fully developed conidium-producing PM colonies in all lines and all mock-treated plants remained PM-free. PM infection advanced apparently faster and produced more extensive colonies in plants with *nim1-1* and *nahG* genetic background than in the wild-type plants, which is likely due to the higher susceptibility of *nim1-1* and *nahG* plants. Leaf tissues with 14 day-old PM colonies and mock-inoculated control leaves were used for a *p*NPG spectrophotometric assay to quantify the gusA activity. Statistical analysis of the GUS assay data revealed that the interaction effect of treatment and assay time is significant ($p<0.0001$), and this significance of the PM-infection occurred at 0 and 30 min dpi. At subsequent time intervals during the spectrophotometric assay the variability of absorbance values increased with time and the absorbance values were also correlated in the PM-infected samples. After adjusting for the dependence and the varying variability, the estimated rate of change is 1.34 times the median, which is significant. The confidence limit for the rate is 1.112 and 1.568 times the median. We also detected a marginally significant ($p=0.0485$) effect of the interaction between the treatment and genetic background which probably reflects the more intense growth of the PM pathogen in the highly susceptible *nim1-1* and *nahG* lines than in the wild-type line (Delaney *et al.*, 1995). Values of plants from the independent lines for each type of transgenics with a similar basal expression are displayed (Figure 13). As SA signaling is abrogated in *nim1-1* and *nahG* plants, these results provide evidence that the *NAC042_5* promoter is responsive to PM infection and independent of SA. Two recent studies presented that another *Vitis* NAC transcription factor gene, *NAC1* expression was activated by *E. necator* along with increased expression of defense-associated genes such as *PDF1.2*, *VSP1*, *PR1*, *PR2*, *PR4* and *PR5* (Zhu *et al.*, 2012b; Le Henanff *et al.*, 2013). However, in contrast to the *NAC042_5* used for our investigations the *NAC1* gene was found to be SA-inducible indicating that the different grapevine *NAC* genes respond to specific triggers.

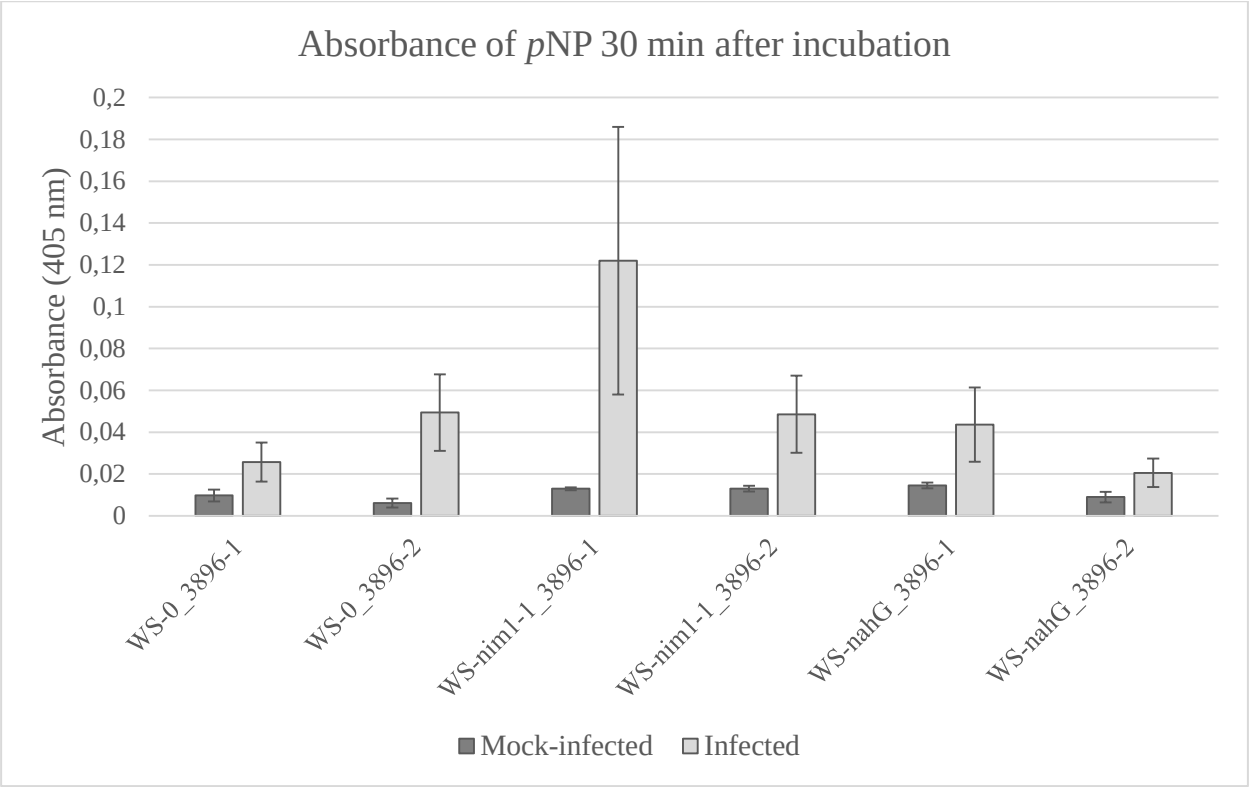


Figure 13. Response of the *NAC042_5* promoter to PM infection in transgenic *Arabidopsis*. GUS quantification based on *pNAC042_5::gusA* activity due to 14 day-old PM colonies on leaves of transgenic *Arabidopsis* with the genetic backgrounds: WT, nim1-1, and nahG (the columns represent independent lines; each of them with six biological repeats; bars represent the standard error).

The *pNAC042_5::gusA* reporter lines were also investigated by histochemical staining in response to PM infection and the staining of these leaves revealed a marked increase in gusA activity at the sites where PM colonies developed (Figure 14A). In mock-inoculated control leaves GUS-staining was mostly limited to trichomes (Figure 14B). To confirm that GUS-staining was indeed caused by the growth of *O. neolycopersici* colonies, we also stained the fungus with cotton blue. Robust GUS-staining was always associated with the presence fungal structures (Figure 14C, D and E), and never occurred in their absence. On mock-inoculated leaves only few confined gusA spots were visible but this was clearly distinguishable from the robust GUS-staining detected at fungal infection sites (Figure 14A, C and D). This indicates that the reporter gene was strongly expressed only in those leaf areas where the pathogen had physical contact with the plant tissue (Figure 14C, D and E). Higher magnification revealed that *gusA* expression severely increased mostly in those cells, in which the fungus developed haustoria (Figure 14D and E). This PM-dependent increase in gusA activity was found in all three types of transgenic plants (with *nim1-1*, *nahG*, and WT background), which provides further evidence that *NAC042_5* expression does not require SA signaling.

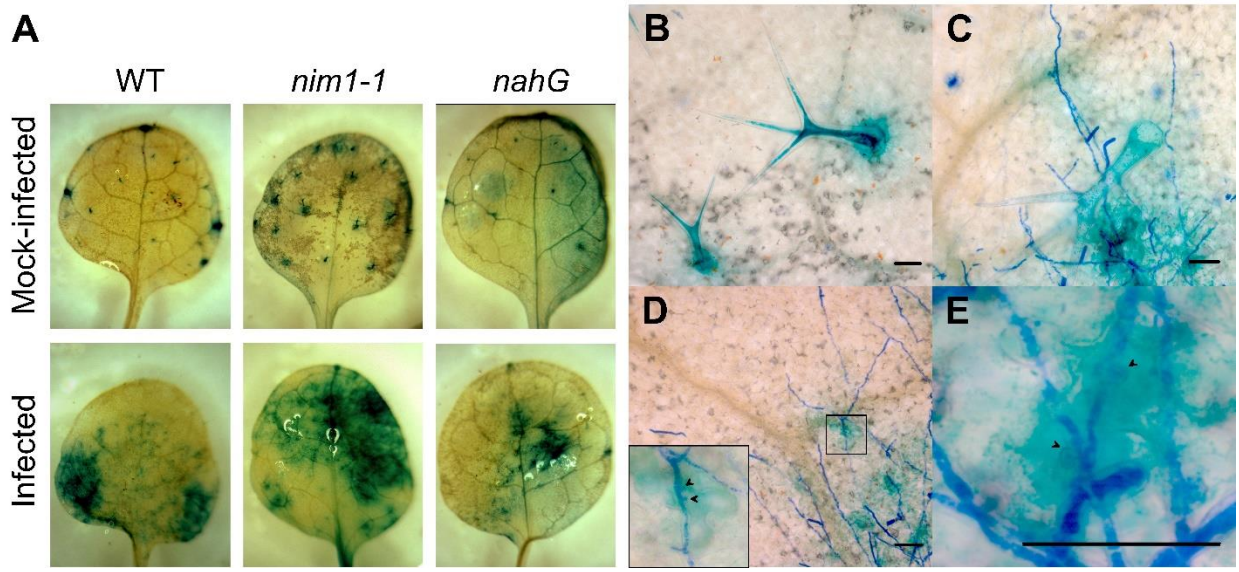


Figure 14. Histochemical staining of *pNAC042_5::gusA*-transgenic plants after *O. neolycopersici* inoculation. (A) GUS staining of mock-inoculated control leaves and *O. neolycopersici*-infected leaves (2. rosette leaf) at 11 dpi, (B) microscopic image of mock-inoculated leaf, (C, D and E) microscopic images of cotton blue-stained PM hyphae (dark blue) on GUS-stained leaf tissue after inoculation. Note the intense GUS staining (turkiz blue) visible in the trichome (B, C) and along the PM hypha (C, D and E). Inset on picture D is an enlargement of an infected epidermis pavement cell, arrowheads indicate the fungal haustoria. Scale bars indicate 50 µm.

Chandran *et al.* (2010) presented that the *NAC042_5* ortholog *ANAC042*/*JUB1* gene is also strongly expressed in the surrounding cells around haustoria and transcripts were not detected in uninfected parts of the leaf. A study investigating the infection site-specific expression of host genes demonstrated that *PR-1* expression was 137-fold up-regulated in *G. orontii-Arabidopsis* interaction only around the haustorium (Chandran *et al.*, 2010). PEN3 ABC transporter, which is known to mediate penetration resistance, also revealed an infection site-specific transcription (Underwood and Somerville, 2013). Similarly, *PUX2* and *DMR6* were up-regulated at the site of infection, although they support mildew development on the plant (Chandran *et al.*, 2010; Zeilmaker *et al.*, 2015). The MYB3R4 and PUX2 transcription factors, as well as the genes *PMR5* and *PMR6* are required for sustaining the accommodation of the fungal haustorium at later stages of colonization but are not involved in SA-mediated defense regulation (Vogel *et al.*, 2002; Vogel *et al.*, 2004). The fact that the PM-responsiveness of these genes is SA-independent suggests that their expression is not modulated by the SA-mediated defense but by other plant signaling pathway or a haustorium-mediated cross-talk between the PM fungus and its host. These genes are also implicated in increasing the ploidy level in mesophyll cells below haustorium-containing epidermal cells in *Arabidopsis* leaves and there was a strong correlation between the increase of ploidy of mesophyll cells and the formation of conidiophores in PM colonies (Chandran *et al.*, 2013). These findings led to the recently presented hypothesis that PM fungi induce

endoreduplication in mesophyll cells at the site of infection to enhance metabolic capacity and this established local sink provides water and nutrients for the fungus (Chandran *et al.*, 2010; Chandran *et al.*, 2013). However, endoreduplication is also known to occur in developing hypocotyl, endosperm, and trichomes (Melaragno *et al.*, 1993). The obvious basal expression in leaf hairs and the PM-dependent expression of *NAC042_5* in infected cells raises the possibility that the expression of this gene may also be associated with endoreduplication or is involved in auxin signaling, which initiates the process. The SA-independent regulation and the relatively late induction of expression (12 hours after infection) of *NAC042_5* gene corroborate this hypothesis.

Auxins may also have signaling functions in pathogen defense (Fu and Wang, 2011) but in numerous cases these phytohormones were shown to benefit infection. Therefore, pathogens are able to induce local auxin biosynthesis, re-route their transport, or alter the auxin-mediated signaling pathways (Chen *et al.*, 2007; Padmanabhan *et al.*, 2008; Grunewald *et al.*, 2009). However, auxin antagonizes cytokinin activity and this interaction enhances defense besides being involved in developmental signaling (Naseem and Dandekar, 2012). Previously cytokinins were believed to provide susceptibility to fungal biotrophs by mediating the well-known 'green island' effect (Walters and McRoberts, 2006). Interestingly, high cytokinin levels resulted in hypersensitive-like responses and increased necrotic lesion formation in tobacco (Novak *et al.*, 2013).

Auxin counteract SA signaling therefore may provide susceptibility to biotrophic pathogens (Wang *et al.*, 2007). The auxin-overproducing lines which were impaired in SA-mediated defenses showed increased susceptibility to *P. syringae* (Mutka *et al.*, 2013).

PLL genes were found to be auxin inducible (Domingo *et al.*, 1998; Laskowski *et al.*, 2006; Palusa *et al.*, 2007) but some responded to H_2O_2 treatment (Palusa *et al.*, 2007). Auxins produced by the infected plant or by the attacker itself promote demethylation of host pectic substances (Agrios, 2005). This fact probably explains the increased membrane permeability in response to auxin treatment (Sacher and Glasziou, 1959). *Erwinia chrysanthemi* is the best known pathogen using its own pectate lyases in infection, which depolymerizes the polygalacturonides of plant cell wall (Barras *et al.*, 1994).

If the *NAC042_5* mediates *PLL* genes activation, then the up-regulation of *NAC042_5* at the site of infection is likely to benefit the pathogen infection. *PMR6* gene encoding a pectate lyase-like protein was up-regulated at the site of infection and *pmr6* mutation resulted in increased resistance against PM. The *SND1* overexpression induced expression of a pectate lyase gene and additionally produced stunted phenotype for the transgenic plant (Ko *et al.*, 2007; Hussey *et al.*, 2011).

5.2.3 **Deletional analysis of *NAC042_5* promoter in transgenic plants**

To test the transcriptional regulation of the *NAC042_5*, we isolated shorter and shorter fragments of the promoter and each fused to the *gusA* reporter gene. The *gusA* expression in the various organs was similar in all lines, as described earlier regarding the 3896 bp long promoter with exception of the lines containing 257 bp fragment or the null-promoter controls. After the inoculation of these plants we measured the *gusA* induction at the site of infection and we found significant increases (p-value < 0.05) in response to PM infection compared to the mock-treatment in transgenic lines containing 3896 bp, 2935 bp, 2456 bp, and 1178 bp long promoter (the induction of *gusA* in WS-0_3896-2 line was not significant) (Figure 15) and not in 257 bp or the null promoter control.

5.2.4 **Promoter analysis of *NAC042_5* sequence**

During analysis of the promoter sequence of *NAC042_5* we detected ABA responsive elements such as ABRE-elements and G-boxes using the PLACE database. Although the *NAC042_5* was not induced by SA treatment, some SA-related (ASF1-motif) elements were also found. Furthermore, we identified ethylene-, jasmonic acid-sensitive boxes (T/G-box, GCC-core sequences) and elicitor-responsive sites, which may also regulate *NAC042_5* in response to PM. We determined growth regulator inducible sites such as auxin and gibberellin-stimulated elements (auxin: ARF- and BBF1-binding; GA: GARE) (Appendix Table 4).

In PlantPAN2.0 database searching for auxin-related transcription factor binding site matrices and motifs we found B3-, ARF-binding elements. In *NAC042_5* sequence specific B3 sites were found at 1059 bp (-), 2558 bp (+), 3001 bp (+), 3055 bp (+), 3057 bp (-), 3058 bp (+), 3059 bp (+), and at 2868 bp (-). BZIP/B3 TF family-related binding sites were found at 268 bp (-), 1733 bp (-), 2777 bp (-), and at 2778 bp (-). The B3 binding domians were detected earlier in *NGATHA1/2/3/4*, *LEAFY COTYLEDON 2*, *VERNALIZATION 1*, *ARF21/31/32*, numerous *REPRODUCTIVE MERISTEM*, *ABSCISIC ACID-INSENSITIVE 3*, or in *FUSCA3* promoters.

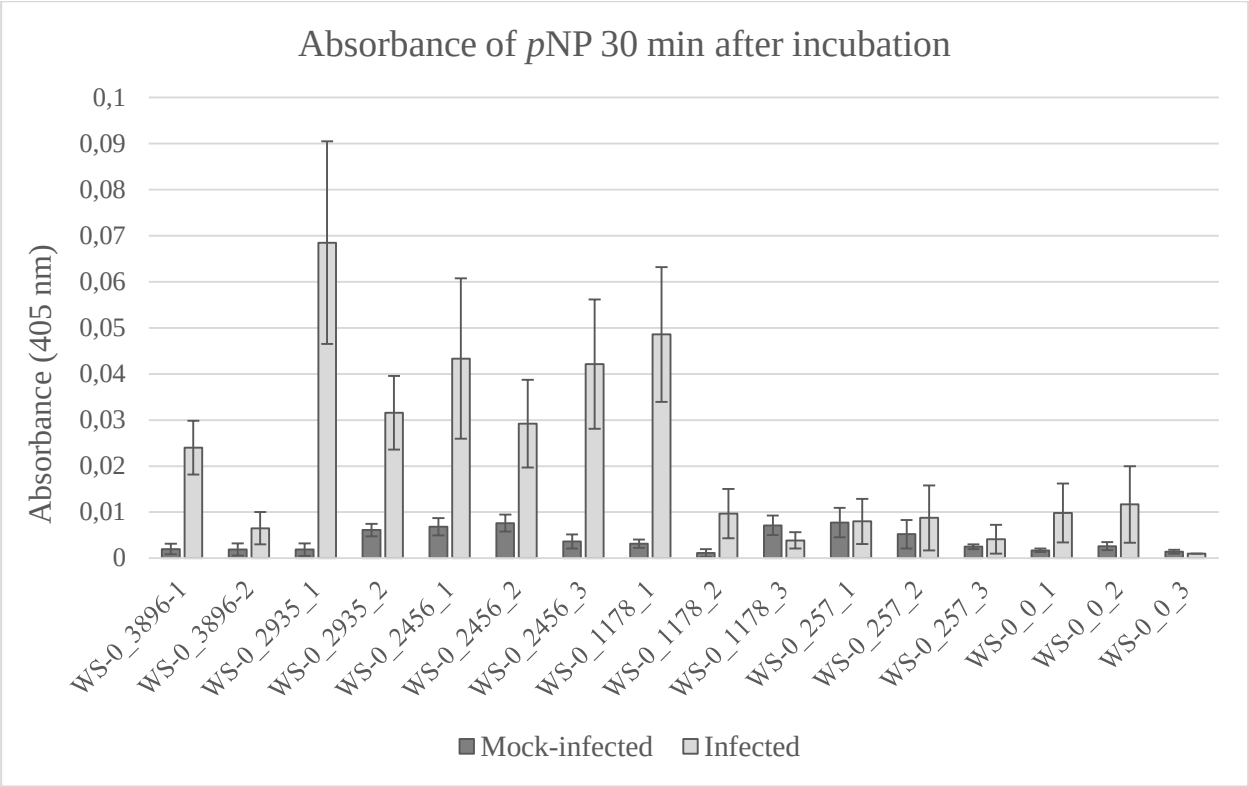


Figure 15. Response of the *NAC042_5* deletional promoters to PM infection in transgenic *Arabidopsis*. GUS quantification based on *pNAC042_5::gusA* activity due to 14 day-old PM colonies on leaves of transgenic *Arabidopsis* with the genetic backgrounds: WT, *nim1-1*, and *nahG* (the columns represent independent lines; each of them with at least three biological repeats; bars represent the standard error).

B3/ARF binding domain matrices were detected at 2922 bp (+) and 2921 bp (-) and a motif at 2975 bp (+) from the translational start and these are also reported in the auxin response factor promoters (*ARF3/ETTIN*, *ARF4*, *ARF5/IAA24*, *ARF6*, *ARF7 ARF8*, *ARF19/IAA22*). The AP2/B3/RAV binding elements were identified in *RAV1* and in *RAV1-like* ethylene-responsive transcription factors and *ARF14* earlier. We also detected elements at the following positions: 759 bp (+,-), 761 bp (+), 992 bp (+,-), 994 bp (-), 994 bp (+), 1342 bp (-), 1342 bp (+), 1402 bp (-), 2058 bp (-), 2058 bp (+), 3083 bp (+,-), 3085 bp (-), 3467 bp (+,-), 3605 bp (+,-).

However we did not detect any auxin-related TF binding site matrix within 268 bp upstream from the translational start. The deletional analysis showing misactivation of *gusA* expression by the 257 bp *NAC042_5* promoter fragment suggests that the required element should be located in the next longest promoter fragment.

5.3 Analysis of *ANAC042/JUB1* regulation in transgenic plants

Histochemical staining of non-infected transgenic leaves demonstrated that all three types of plants (WT-*pJUB1::gusA*, nim1-1-*pJUB1::gusA* and nahG-*pJUB1::gusA*) expressed the β-glucuronidase at a basal level, which was very similar to the expression level in plants with *NAC042_5*. The specific up-regulation was also observed in the stipules and in the vascular region

of gynoecium style tissue. However, the β-glucuronidase was also detected in the root cap (the *gusA* expression was absent in meristematic zone), in the junction of root and hypocotyl and at the margins of leaves (Figure 16). In comparison to the grape *NAC042_5* gene, the *Arabidopsis* ortholog did not express specifically in the leafhairs but at the margin of cotyledons and leaves.

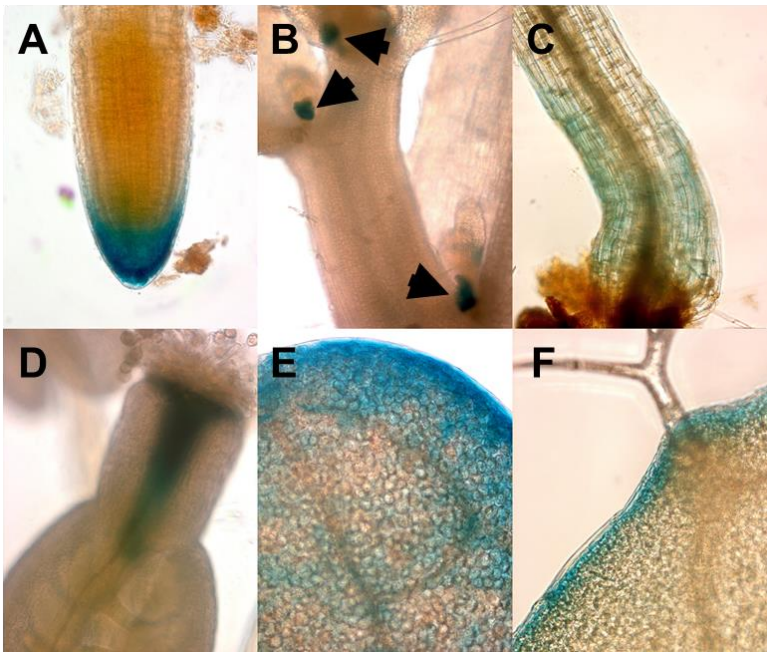


Figure 16. Localization of *gusA* expression regulated by the *JUB1* promoter in transgenic *Arabidopsis* plants. (A) Root apex, (B) stipules, (C) hypocotyl-root junction (D) style, (E) cotyledon (F) margin of leaf.

After the infection of these transgenic plants we observed the colony covered leaves at 14 dai. The *nim1-1* and *nahG* lines were also infected at a higher rate compared to the wild type as it was shown earlier (Gaffney *et al.*, 1993; Delaney *et al.*, 1995). The histochemical staining of these leaves demonstrated that *gusA* expression was induced significantly in the infected plants compared to the mock-infected ones. This phenomenon was detected in all three types of transgenic plants, especially in the plants with *nim1-1* and *nahG* genetic background. During microscopic observation of the infected leaves we detected the induction mostly in areas where the pathogen was in direct contact with the host (Figure 17). The uninfected areas displayed basal level of expression of the *gusA* and were clearly distinguishable from the ones where induction did not occur. The basal and PM-induced expression patterns detected in all three types of transgenic plants (*WT-pJUB1::gusA*, *nim1-1-pJUB1::gusA* and *nahG-pJUB1::gusA*) suggest that SA is probably neither required for basal regulation nor for the induction of *JUB1* by *O. neolycopersici*.

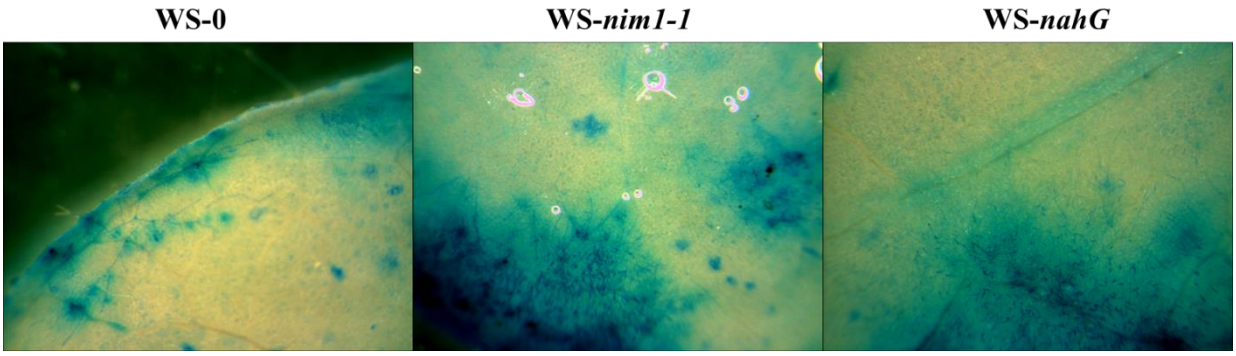


Figure 17. Histochemical staining of *pJUB1::gusA*-transgenic plants with genetic backgrounds of WS-0, WS-*nim1-1*, WS-*nahG* after *O. neolycopersici* inoculation.

Although, the *G. orontii* is also a biotrophic fungus, it was found that the SA biosynthesis mutant *ics1* slightly modulated the induction of *JUB1* in response to *G. orontii* (Chandran *et al.*, 2009). Saga *et al.* (2012) demonstrated that *JUB1* expression was significantly lower in *ein2-1* but not in *sid2-2* (salicylic acid induction–deficient) mutant in response to Flg22 in the primary root apex compared to the wild type. This suggests that *JUB1* may be induced by the attacker via ethylene signaling. Additionally, the *JUB1* responded intensively to *Sclerotinia sclerotiorum* to regulate camalexin against the pathogen but the *coi1-2* mutation did not influence its up-regulation (Stotz *et al.*, 2009). Microscopic observation of the infected leaves showed that the induced expression was mostly around the PM colonies, as it was found in *NAC042_5*, too.

The *JUB1* was found to be H_2O_2 inducible and it functioned in feedback-regulation with the available innate H_2O_2 level (Shahnejat-Bushehri *et al.*, 2012; Wu *et al.*, 2012), which suggests that *JUB1* may respond to *O. neolycopersici* via H_2O_2 homeostasis. Wu et al. (2012) also demonstrated that *JUB1* functions as a regulator of plant longevity. The overexpression of *JUB1* resulted in delayed senescence and increased isopentenyladenosine and zeatin riboside (cytokinins) level, which suggests that this gene may be up-regulated at the site of infection to retard aging of the infected plant cells. Furthermore, the sequence analysis also showed that Cytokinin-enhanced Protein Binding sites are found in the promoter of *JUB1* (Appendix Table 4). Interestingly, the SA level decreased in the *JUB1* overexpressing plants, suggesting that this affect supports PM proliferation. A recent study demonstrated that the highest cytokinin level in the primary root apex was detected in the root cap (Antoniadi *et al.*, 2015), but auxin level is also high in this region. The expression of *JUB1* in the leaf margin suggests that activation of this gene could be mediated by auxin too, since *DR5::GUS* was also detected at the margin of leaves and in the root apex (Aloni *et al.*, 2003). Furthermore, during sequence analysis of *JUB1* promoter region we found hormone (ET/JA, SA, GA, ABA, auxin and cytokinin)-, and elicitor-responsive elements, MYB and WRKY transcription factor-, and calmodulin-binding sites, which may be involved in

stimulating the expression levels of *JUB1* in response to environmental challenges (Appendix Table 4). Although *JUB1* gene was found to play a role in biosynthesis of camalexin and it acts as a defense gene against necrotrophs (Saga *et al.*, 2012) its activation may benefit the biotrophic PM in the host-pathogen interaction.

5.4 Novel scientific results

1. The global expressional analysis revealed that 179 genes responded similarly to both PM and SA treatments compared to the control. Among them we found defense-associated genes that function via SA-mediated signaling such as *EDS1*, *WRKY*, *NIMIN1*, *GST*, *PR* genes.
2. Globally 373 genes altered their rate of expression in response to PM compared to the control. Among them, 185 candidates were found to respond only to PM. This group of genes involved stilbene synthases, dirigent-like proteins, photosynthetic genes, aquaporin, lipid transfer proteins and the *NAC transcription factor 42*.
3. The microarray measured gene expression pattern of *NAC transcription factor 42* (*NAC042_5)* was confirmed by quantitative PCR method.
4. We found basal expression levels of the reporter gene regulated by the *NAC042_5* promoter in a range of tissues and organs including SAM, young developing organs, trichomes, vascular tissues, in the tips of cotyledon, stipules, hydathodes, lateral shoot buds, connective tissue of the anther, style, in transmitting tract within the carpel and abscission zone of gynoecium. This pattern was similar in all transgenic *Arabidopsis* lines (WT, *nim1-1*, *nahG*) proving that the basal regulation is independent of SA.
5. We also proved that the expression of *NAC042_5* gene is independent of SA-signaling in response to PM infection in transgenic *Arabidopsis*. The induced expression of *gusA* was always associated with the presence of fungal structures and severely increased mostly in those cells, in which the fungus developed haustoria.
6. The sequence analysis revealed that hormone- (ABA, auxin, gibberellin, jasmonate/ethylene) and elicitor-responsive elements as well as WRKY- and MYB-binding sites are detected in the 3896-bp-long promoter. Deletional analysis demonstrated that the induction of *gusA* activity could still be shown in plants containing the 1178-bp-long fragment, but it was no longer possible in plants with the 257-bp fragment in transgenic *Arabidopsis*. The responsible *cis*-element should be located within this region, where we found ASF1, ERELEE4, BIHD1-, MYB-binding, SURE2 and W-box elements.
7. The *NAC042_5* ortholog in *Arabidopsis*, *JUB1* promoter regulated expression pattern was similar to the one regulated by *Vitis* promoter. However, several differences could also be shown, since *JUB1* activated *gusA* in the root cap and at the margin of leaves but not in the leaf hairs, which could be explained by the differences in the promoter: the CARE, CPB, CBF-, DPBF-binding, TCA1, and CGCG-box motifs were not found in the *NAC042_5* promoter.

6. CONCLUSION

Earlier studies demonstrated that the levels of salicylic acid in the leaf tissue of *V. vinifera* cv. 'Cabernet Sauvignon' increased in response to *E. necator* infection as a function of time after inoculation. In contrast, the PM-resistant *V. aestivalis* had constitutively high levels of SA, which suggests that the SA is responsible for constitutive resistance in the plant (Fung *et al.*, 2008). Our aim was to test whether there are any differences with regard to the pattern of gene expression between the response to PM infection and the response to exogenous SA application in the susceptible *V. vinifera* cv. 'Cabernet Sauvignon'. The expression changes measured using microarray (Affymetrix Vitis GeneChip) technique revealed that PM colonization triggers changes in the expression of a broad range of genes, many of which were regulated via SA-mediated signaling. Among them we identified numerous genes that are typically related to the defense responses such as *PRs*, *EDS1* and *NIMIN-1*, *WRKY* transcription factor genes, ABC transporters and RLKs. However, some of the PM-responsive genes did not react to the increase in SA levels alone. This suggests that PM colonization activates regulatory networks that are more extensive than the SA-mediated defense system. We also determined defense-associated genes within this group but genes with no known defense-related function were also observed to change their expression patterns. Among them we identified the *PR-10* genes, stilbene synthases, dirigent-like genes, aquaporin *TIP1-3*, cytochrome P450s, and transcription factors, such as the NAC-type transcription factor gene (*NAC042_5*).

The results of global expression analysis contribute to the knowledge acquired in fundamental researches. The PM-induced response was observed at the level of gene expression changes, which may reveal genes functioning in defense mechanisms. The regulation of the *NAC* transcription factor gene observed support this hypothesis and represent a PM-dependent induction in the susceptible grapevine, the function of which is probably operated by the pathogen. Furthermore, the transgenic trial applied in the three *A. thaliana* lines (WT, *nim1-1*-and *nahG*) revealed that the regulation of *NAC042_5* in response to PM is indeed independent of SA-mediated signaling and probably operated by other signals. The PM-triggered induction demonstrated an up-regulation of the reporter gene, which was always associated with the presence of fungal structures. The PM-dependent feature of the *NAC042_5* promoter may allow it to be used in applied researches, in which the goal is to express a transgene solely at the site of infection (for example *R* or *PR* genes). In this way the level of transcript of the transgene is reduced in the entire plant without any decrease in the effectiveness of its function. In addition, if the function of *NAC042_5* is proven to be associated with the auxin-signaling and the regulation at site of infection

is in order to operate endoreduplication process, in that case the NAC transcription factor supports PM proliferation. Therefore, the silencing of *NAC042_5* expression may result in an increase in resistance against PM, as it was shown by the gene of pectate-lyase-like *PMR6*. However, the transgenesis is not the obligate method for execution of silencing; we may acquire the same results with the TALEN and the CRISPR/cas9 techniques, which are not quite the same process as the GMO preparation procedure. The improvement of resistance of the susceptible varieties then would support the goal of reducing the use of chemicals in vineyards.

7. SUMMARY

Numerous studies published results related to the genetic background of pathogen-plant interactions, although there are still several aspects of these processes which are yet to be fully explained. Earlier researches demonstrated that the SA level of *V. vinifera* cv. 'Cabernet Sauvignon' leaf tissue increased in response to *E. necator* infection as a function of time after inoculation. In contrast, the PM-resistant *V. aestivalis* had constitutively high levels of SA, which suggests that the SA is responsible for constitutive resistance in the plant. Our aim was to test whether there are differences in the pattern of gene expression between the response to PM infection and the response to exogenous SA application in the susceptible *V. vinifera* cv. 'Cabernet Sauvignon'. Changes in the pattern of expression were measured by means of microarray (Affymetrix Vitis GeneChip) technique, and the results revealed that SA was able to mimic similar responses in 179 genes to the responses triggered by PM compared to the control. Among these transcripts we found defense-associated genes that function typically via SA-mediated signaling such as *EDS1*, *WRKY*, *NIMIN1*, *GST*, *PR* genes. Globally 373 genes altered their rate of expression in response to PM compared to the control. However, 185 candidates reacted only to PM rather than to SA treatment alone suggesting that these genes are likely regulated via another signaling pathway or respond directly to pathogen effectors. We identified genes encoding stilbene synthases, cytochrome P450s, dirigent-like proteins, PR10s, proteins related to photosynthesis, aquaporin, lipid transfer proteins and transcription factors, among which the NAC transcription factor 42 (*NAC042_5*) was identified. An attempt was made to confirm the microarray data of *NAC042_5* with a real-time qPCR experiment and the results also demonstrated a significant increase in the expression levels of the gene in response to PM infection and not to SA treatment compared to the control.

The expression analysis showed that the expression of the *NAC042_5* gene was induced 7-fold by PM but it did not respond to SA. The results suggested that the regulation of *NAC042_5* gene was either independent of SA or dependent on it but SA alone was insufficient to induce *NAC042_5* expression. To decide which hypothesis is correct we designed a subsequent investigation of NAC transcription factor using a transgenic system in *A. thaliana*. In order to study the regulation of *NAC042_5* gene, the promoter of the *NAC042_5* was fused to the *gusA* reporter gene and the construct was transferred into the genomes of *Arabidopsis* genotypes (WS-0, WS-*nim1-1*, and WS-*nahG*). The transgenic plants were used for testing the induction of *gusA* in response to *O. neolycopersici* PM. Spectrophotometrical measurement showed increased levels of expression of *gusA* in the infected plants compared to the control ones. The up-regulation of the

gene was detected in all transgenic lines irrespectively of their genetic background (WS-0, WS-*nim1-1,* and WS-*nahG*), proving that *NAC042_5* is independently regulated of SA-signaling in response to PM. Furthermore, the SA-independence of the induction process was also confirmed by histochemical GUS assay. The up-regulated expression was always associated with the presence of PM and mostly the cells containing haustoria showed increased levels of *gusA* expression rather than the cells devoid of PM.

The *NAC042_5* promoter sequence was bioinformatically analyzed and the results revealed hormone-sensitive (ET/JA, SA, GA, ABA, and auxin), elicitor-responsive elements, MYB and WRKY transcription factor-binding sites within the 3896-bp-long fragment. The deletional analysis of *NAC042_5* promoter demonstrated that basal and induced expression rates were still found in plants containing the 1178-bp-long promoter but no longer in plants containing the 257-bp-long fragment. The basal expression rates regulated by *NAC042_5* showed similar pattern to the auxin-inducible *PLL* and *DR5* promoters suggesting that the investigated *Vitis* gene may also play a role in auxin-mediated pathway. Interestingly, the PlantPAN2.0 database presented auxin-responsive *cis*-regulatory elements only above 268 bp upstream from the translational start which may explain the lack of activation of the *gusA* by the 257-bp-long promoter.

The promoter analysis of the *Arabidopsis* ortholog *JUB1* showed that the basal and PM-induced expression patterns of transgenic plants were similar to the ones containing *NAC042_5* promoter::*gusA* fusion. This suggests that the *Vitis NAC042_5* may perform analogous function in plant processes similarly to the *Arabidopsis JUB1*.

According to the obvious basal expression rates in developing leaf hairs and site-specific induction of *NAC042_5* in response to PM we can presume that this gene participates in auxin-initiated endoreduplication processes and thus provides susceptibility rather than defense against biotrophic pathogens. The silencing of *NAC042_5* expression may result in an increase in resistance against PM and the improvement of resistance in the susceptible varieties, which would support the goal of reducing the use of chemicals in vineyards

8. APPENDICES

A1. References

Abuqamar, S., Ajeb, S., Sham, A., Enan, M. R. and Iratni, R. (2013) A mutation in the *expansin-like A2* gene enhances resistance to necrotrophic fungi and hypersensitivity to abiotic stress in *Arabidopsis thaliana*. *Mol Plant Pathol*, **14**(8): 813-827.

Aerts, A. M., Francois, I. E., Cammue, B. P. and Thevissen, K. (2008) The mode of antifungal action of plant, insect and human defensins. *Cell Mol Life Sci*, **65**(13): 2069-2079.

Agarwal, P. and Agarwal, P. K. (2013) Pathogenesis related-10 proteins are small, structurally similar but with diverse role in stress signaling. *Mol Biol Rep*, **41**(2): 599-611.

Agrios, G. (2005) How pathogens attack plants. In: D. Dreibelbis and K. D. Sonnack (Editors), Plant Pathology - 5th ed. *Elsevier Academic Press*, Burlington, pp. 196-199.

Aich, S., Delbaere, L. T. and Chen, R. (2001) Continuous spectrophotometric assay for beta-glucuronidase. *Biotechniques*, **30**(4): 846-850.

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H. and Tasaka, M. (1997) Genes involved in organ separation in *Arabidopsis*: an analysis of the *cup-shaped cotyledon* mutant. *Plant Cell*, **9**(6): 841-857.

Aida, M., Ishida, T. and Tasaka, M. (1999) Shoot apical meristem and cotyledon formation during Arabidopsis embryogenesis: interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development*, **126**(8): 1563-1570.

Aloni, R., Schwalm, K., Langhans, M. and Ullrich, C. I. (2003) Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and leaf morphogenesis in *Arabidopsis*. *Planta*, **216**(5): 841-853.

Alvarez, J. P., Goldshmidt, A., Efroni, I., Bowman, J. L. and Eshed, Y. (2009) The *NGATHA* distal organ development genes are essential for style specification in *Arabidopsis*. *Plant Cell*, **21**(5): 1373-1393.

Anderson, J. C. *et al.* (2011) *Arabidopsis MAP kinase phosphatase 1 (AtMKP1)* negatively regulates MPK6-mediated PAMP responses and resistance against bacteria. *Plant J*, **67**(2): 258-268.

Andersson, M. X., Kourtchenko, O., Dangl, J. L., Mackey, D. and Ellerstrom, M. (2006) Phospholipase-dependent signalling during the AvrRpm1- and AvrRpt2-induced disease resistance responses in *Arabidopsis thaliana*. *Plant J*, **47**(6): 947-959.

Antoniadi, I. *et al.* (2015) Cell-type-specific cytokinin distribution within the *Arabidopsis* primary root apex. *Plant Cell*, **27**(7): 1955-1967.

Araya, T., Noguchi, K. and Terashima, I. (2006) Effects of carbohydrate accumulation on photosynthesis differ between sink and source leaves of *Phaseolus vulgaris* L. *Plant Cell Physiol*, **47**(5): 644-652.

Atkinson, N. J. and Urwin, P. E. (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. *J Exp Bot*, **63**(10): 3523-3543.

Avila, C. A. *et al.* (2012) Loss of function of *FATTY ACID DESATURASE7* in tomato enhances basal aphid resistance in a salicylate-dependent manner. *Plant Physiol*, **158**(4): 2028-2041.

Ayliffe, M. A. and Lagudah, E. S. (2004) Molecular genetics of disease resistance in cereals. *Ann Bot*, **94**(6): 765-773.

Barker, C. L. *et al.* (2005) Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. *Theor Appl Genet*, **111**(2): 370-377.

Barras, F., van Gijsegem, F. and Chatterjee, A. K. (1994) Extracellular enzymes and pathogenesis of soft-rot *Erwinia*. *Annu Rev Phytopathol*, **32**(1): 201-234.

Bartels, S. *et al.* (2009) *MAP KINASE PHOSPHATASE1* and *PROTEIN TYROSINE PHOSPHATASE1* are repressors of salicylic acid synthesis and SNC1-mediated responses in *Arabidopsis*. *Plant Cell*, **21**(9): 2884-2897.

Basu, M. M. *et al.* (2013) The manipulation of auxin in the abscission zone cells of *Arabidopsis* flowers reveals that indoleacetic acid signaling is a prerequisite for organ shedding. *Plant Physiol*, **162**(1): 96-106.

Bechtold, U. *et al.* (2010) Constitutive salicylic acid defences do not compromise seed yield, drought tolerance and water productivity in the *Arabidopsis* accession C24. *Plant Cell Environ*, **33**(11): 1959-1973.

Bhuiyan, N. H., Selvaraj, G., Wei, Y. and King, J. (2009) Role of lignification in plant defense. *Plant Signal Behav*, **4**(2): 158-159.

Bienert, G. P. and Chaumont, F. (2014) Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochim Biophys Acta*, **1840**(5): 1596-1604.

Bird, D. *et al.* (2007) Characterization of *Arabidopsis ABCG11/WBC11*, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. *Plant J*, **52**(3): 485-498.

Bohman, S., Staal, J., Thomma, B. P., Wang, M. and Dixelius, C. (2004) Characterisation of an *Arabidopsis-Leptosphaeria maculans* pathosystem: resistance partially requires camalexin biosynthesis and is independent of salicylic acid, ethylene and jasmonic acid signalling. *Plant J*, **37**(1): 9-20.

Borges, A. F., Ferreira, R. B. and Monteiro, S. (2013) Transcriptomic changes following the compatible interaction *Vitis vinifera-Erysiphe necator*. Paving the way towards an enantioselective role in plant defence modulation. *Plant Physiol Biochem*, **68**: 71-80.

Bowling, S. A. *et al.* (1994) A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. *Plant Cell*, **6**(12): 1845-1857.

Brodersen, P. *et al.* (2002) Knockout of *Arabidopsis accelerated-cell-death11* encoding a sphingosine transfer protein causes activation of programmed cell death and defense. *Genes Dev*, **16**(4): 490-502.

Bryan, G. T. *et al.* (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell*, **12**(11): 2033-2046.

Burkle, L. *et al.* (2003) Transport of cytokinins mediated by purine transporters of the *PUP* family expressed in phloem, hydathodes, and pollen of Arabidopsis. *Plant J*, **34**(1): 13-26.

Bushnell, W. R. and Allen, P. J. (1962) Induction of disease symptoms in barley by powdery mildew. *Plant Physiol*, **37**(1): 50-59.

Cenci, A., Guignon, V., Roux, N. and Rouard, M. (2014) Genomic analysis of *NAC* transcription factors in banana (*Musa acuminata*) and definition of *NAC* orthologous groups for monocots and dicots. *Plant Mol Biol*, **85**(1-2): 63-80.

Chaban, C., Waller, F., Furuya, M. and Nick, P. (2003) Auxin responsiveness of a novel cytochrome p450 in rice coleoptiles. *Plant Physiol*, **133**(4): 2000-2009.

Chandran, D. *et al.* (2009) Temporal global expression data reveal known and novel salicylate-impacted processes and regulators mediating powdery mildew growth and reproduction on *Arabidopsis*. *Plant Physiol*, **149**(3): 1435-1451.

Chandran, D., Inada, N., Hather, G., Kleindt, C. K. and Wildermuth, M. C. (2010) Laser microdissection of *Arabidopsis* cells at the powdery mildew infection site reveals site-specific processes and regulators. *Proc Natl Acad Sci U S A*, **107**(1): 460-465.

Chandran, D., Rickert, J., Cherk, C., Dotson, B. R. and Wildermuth, M. C. (2013) Host cell ploidy underlying the fungal feeding site is a determinant of powdery mildew growth and reproduction. *Mol Plant Microbe Interact*, **26**(5): 537-545.

Chen, L. *et al.* (2010a) Analysis of the Rac/Rop small GTPase family in rice: expression, subcellular localization and role in disease resistance. *Plant Cell Physiol*, **51**(4): 585-595.

Chen, L. Q. *et al.* (2010b) Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature*, **468**(7323): 527-532.

Chen, Z. *et al.* (2007) *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. *Proc Natl Acad Sci U S A*, **104**(50): 20131-20136.

Cheng, Y., Dai, X. and Zhao, Y. (2007) Auxin synthesized by the YUCCA flavin monooxygenases is essential for embryogenesis and leaf formation in *Arabidopsis*. *Plant Cell*, **19**(8): 2430-2439.

Choi, D. S., Hwang, I. S. and Hwang, B. K. (2012) Requirement of the cytosolic interaction between PATHOGENESIS-RELATED PROTEIN10 and LEUCINE-RICH REPEAT PROTEIN1 for cell death and defense signaling in pepper. *Plant Cell*, **24**(4): 1675-1690.

Chow, C. N. *et al.* (2016) PlantPAN 2.0: an update of plant promoter analysis navigator for reconstructing transcriptional regulatory networks in plants. *Nucleic Acids Res*, **44**(D1): D1154-1160.

Christ, B. *et al.* (2012) MES16, a member of the methylesterase protein family, specifically demethylates fluorescent chlorophyll catabolites during chlorophyll breakdown in *Arabidopsis*. *Plant Physiol*, **158**(2): 628-641.

Clough, S. J. and Bent, A. F. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J*, **16**(6): 735-743.

Coleman, C. *et al.* (2009) The powdery mildew resistance gene *REN1* co-segregates with an *NBS-LRR* gene cluster in two Central Asian grapevines. *BMC Genet*, **10**: 89.

Collinge, M. and Boller, T. (2001) Differential induction of two potato genes, Stprx2 and StNAC, in response to infection by Phytophthora infestans and to wounding. *Plant Mol Biol*, **46**(5): 521-529.

Collu, G. *et al.* (2001) Geraniol 10-hydroxylase, a cytochrome P450 enzyme involved in terpenoid indole alkaloid biosynthesis. *FEBS Lett*, **508**(2): 215-220.

Consonni, C. *et al.* (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat Genet*, **38**(6): 716-720.

Cosgrove, D. J. (2000) Loosening of plant cell walls by expansins. *Nature*, **407**(6802): 321-326.

Crawford, B. C. and Yanofsky, M. F. (2011) HALF FILLED promotes reproductive tract development and fertilization efficiency in *Arabidopsis thaliana*. *Development*, **138**(14): 2999-3009.

Cui, F., Brosche, M., Sipari, N., Tang, S. and Overmyer, K. (2013) Regulation of ABA dependent wound induced spreading cell death by MYB108. *New Phytol*, **200**(3): 634-640.

Cunningham, F. *et al.* (2015) Ensembl 2015. *Nucl Acids Res*, **43**(D1): D662-D669.

Dai, R., Ge, H., Howard, S. and Qiu, W. (2012) Transcriptional expression of *Stilbene synthase* genes are regulated developmentally and differentially in response to powdery mildew in Norton and Cabernet Sauvignon grapevine. *Plant Sci*, **197**: 70-76.

Dangl, J. L. and Jones, J. D. (2001) Plant pathogens and integrated defence responses to infection. *Nature*, **411**(6839): 826-833.

Davin, L. B. and Lewis, N. G. (2000) Dirigent proteins and dirigent sites explain the mystery of specificity of radical precursor coupling in lignan and lignin biosynthesis. *Plant Physiol*, **123**(2): 453-462.

De Bruyne, L., Hofte, M. and De Vleesschauwer, D. (2014) Connecting growth and defense: the emerging roles of brassinosteroids and gibberellins in plant innate immunity. *Mol Plant*, **7**(6): 943-959.

De Sa, M. E. *et al.* (2012) Transcriptome analysis of resistant soybean roots infected by *Meloidogyne javanica*. *Genet Mol Biol*, **35**(1 (suppl)): 272-282.

De Zelicourt, A. *et al.* (2007) Ha-DEF1, a sunflower defensin, induces cell death in *Orobanche* parasitic plants. *Planta*, **226**(3): 591-600.

Deák, T. *et al.* (2014) Marker assisted selection for seedlessness in a multiresistant table grape hybrid family. In: B. I. Reisch and J. Londo (Editors), X International Conference on Grapevine Breeding and Genetics Acta Horticulturae, Geneva, NY, USA.

Delaney, T. P. *et al.* (1994) A central role of salicylic acid in plant disease resistance. *Science*, **266**(5188): 1247-1250.

Delaney, T. P., Friedrich, L. and Ryals, J. A. (1995) *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. *Proc Natl Acad Sci U S A*, **92**(14): 6602-6606.

Delessert, C. *et al.* (2005) The transcription factor ATAF2 represses the expression of pathogenesis-related genes in *Arabidopsis*, Plant J, England, pp. 745-757.

Deng, W. *et al.* (2012) The tomato SlIAA15 is involved in trichome formation and axillary shoot development. *New Phytol*, **194**(2): 379-390.

Deslandes, L. *et al.* (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 U S A*, **100**(13): 8024-8029.

DeYoung, B. J. and Innes, R. W. (2006) Plant NBS-LRR proteins in pathogen sensing and host defense. *Nat Immunol*, **7**(12): 1243-1249.

Domingo, C. *et al.* (1998) A pectate lyase from *Zinnia elegans* is auxin inducible. *Plant J*, **13**(1): 17-28.

Donald, T. M. *et al.* (2002) Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. *Theor Appl Genet*, **104**(4): 610-618.

Du, L. *et al.* (2009) Ca(2+)/calmodulin regulates salicylic-acid-mediated plant immunity. *Nature*, **457**(7233): 1154-1158.

Duval, M., Hsieh, T. F., Kim, S. Y. and Thomas, T. L. (2002) Molecular characterization of *AtNAM*: a member of the *Arabidopsis NAC* domain superfamily. *Plant Mol Biol*, **50**(2): 237-248.

Eckardt, N. A. (2003) A new classic of cytokinin research: cytokinin-deficient *Arabidopsis* plants provide new insights into cytokinin biology. *Plant Cell*, **15**(11): 2489-2492.

Eichmann, R., Schultheiss, H., Kogel, K. H. and Huckelhoven, R. (2004) The barley apoptosis suppressor homologue BAX inhibitor-1 compromises nonhost penetration resistance of barley to the inappropriate pathogen *Blumeria graminis* f. sp. *tritici*. *Mol Plant Microbe Interact*, **17**(5): 484-490.

Eichmann, R. *et al.* (2010) *BAX INHIBITOR-1* is required for full susceptibility of barley to powdery mildew. *Mol Plant Microbe Interact*, **23**(9): 1217-1227.

Ellis, C. and Turner, J. G. (2001) The *Arabidopsis* mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. *Plant Cell*, **13**(5): 1025-1033.

Ellis, C., Karafyllidis, I., Wasternack, C. and Turner, J. G. (2002) The *Arabidopsis* mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. *Plant Cell*, **14**(7): 1557-1566.

Eulgem, T. (2005) Regulation of the *Arabidopsis* defense transcriptome. *Trends Plant Sci*, **10**(2): 71-78.

Fan, J., Hill, L., Crooks, C., Doerner, P. and Lamb, C. (2009) Abscisic acid has a key role in modulating diverse plant-pathogen interactions. *Plant Physiol*, **150**(4): 1750-1761.

Fang, L. *et al.* (2016) Expression of Vitis amurensis NAC26 in Arabidopsis enhances drought tolerance by modulating jasmonic acid synthesis. *Journal of Experimental Botany*, **67**(9): 2829-2845.

Favel, A. *et al.* (1994) *In vitro* antifungal activity of triterpenoid saponins. *Planta Med*, **60**(1): 50-53.

Feechan, A., Jermakow, A. M., Torregrosa, L., Panstruga, R. and Dry, I. B. (2008) Identification of grapevine *MLO* gene candidates involved in susceptibility to powdery mildew. *Funct Plant Biol*, **35**(12): 1255-1266.

Feng, H. *et al.* (2014) The target gene of tae-miR164, a novel *NAC* transcription factor from the *NAM* subfamily, negatively regulates resistance of wheat to stripe rust. *Mol Plant Pathol*, **15**(3): 284-296.

Fodor, J. *et al.* (1997) Local and systemic responses of antioxidants to tobacco mosaic virus infection and to salicylic acid in tobacco (role in systemic acquired resistance). *Plant Physiol*, **114**(4): 1443-1451.

Friedrich, L. *et al.* (1996) A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Plant J*, **10**(1): 61-70.

Frye, C. A., Tang, D. and Innes, R. W. (2000) Negative regulation of defense responses in plants by a conserved MAPKK kinase. *Proc Natl Acad Sci U S A*, **98**(1): 373-378.

Fu, J. and Wang, S. (2011) Insights into auxin signaling in plant-pathogen interactions. *Front Plant Sci*, **2**: 74.

Fujita, M. *et al.* (2004) A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. *Plant J*, **39**(6): 863-876.

Fukuda, H. (1996) Xylogenesis: initiation, progression, and cell death. *Annu Rev Plant Physiol Plant Mol Biol*, **47**: 299-325.

Fukushima, E. O. *et al.* (2011) CYP716A subfamily members are multifunctional oxidases in triterpenoid biosynthesis. *Plant Cell Physiol*, **52**(12): 2050-2061.

Fung, R. W. *et al.* (2008) Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. *Plant Physiol*, **146**(1): 236-249.

Gadoury, D. M. *et al.* (2012) Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Mol Plant Pathol*, **13**(1): 1-16.

Gaffney, T. *et al.* (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. *Science*, **261**(5122): 754-756.

Gao, F. *et al.* (2010) A functional *EDS1* ortholog is differentially regulated in powdery mildew resistant and susceptible grapevines and complements an *Arabidopsis eds1* mutant. *Planta*, **231**(5): 1037-1047.

Gao, X. *et al.* (2007) Disruption of a maize 9-lipoxygenase results in increased resistance to fungal pathogens and reduced levels of contamination with mycotoxin fumonisin. *Mol Plant Microbe Interact*, **20**(8): 922-933.

Gao, X. *et al.* (2009) Inactivation of the lipoxygenase *ZmLOX3* increases susceptibility of maize to *Aspergillus* spp. *Mol Plant Microbe Interact*, **22**(2): 222-231.

Gille, S. and Pauly, M. (2012) O-acetylation of plant cell wall polysaccharides. *Front Plant Sci*, **3**.

Gilmartin, P. M. and Bowler, C. (2002) Molecular Plant Biology. A Practical Approach, 2. *Oxford University Press*, Oxford, OX2 6DP, 368 pp.

Glazebrook, J. (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu Rev Phytopathol*, **43**: 205-227.

Grant, E. H., Fujino, T., Beers, E. P. and Brunner, A. M. (2010) Characterization of NAC domain transcription factors implicated in control of vascular cell differentiation in *Arabidopsis* and *Populus*. *Planta*, **232**(2): 337-352.

Grunewald, W., Cannoot, B., Friml, J. and Gheysen, G. (2009) Parasitic nematodes modulate PIN-mediated auxin transport to facilitate infection. *PLoS Pathog*, **5**(1): e1000266.

Guo, W. *et al.* (2014) The chloride channel family gene *CLCd* negatively regulates pathogen-associated molecular pattern (PAMP)-triggered immunity in *Arabidopsis*. *J Exp Bot*, **65**(4): 1205-1215.

Guo, Y. and Gan, S. (2006) AtNAP, a NAC family transcription factor, has an important role in leaf senescence. *Plant J*, **46**(4): 601-612.

Gupta, D. and Tuteja, N. (2011) Chaperones and foldases in endoplasmic reticulum stress signaling in plants. *Plant Signal Behav*, **6**(2): 232-236.

Gururania, M. A. *et al.* (2012) Plant disease resistance genes: Current status and future directions. *Physiol Mol Plant Pathol*, **78**: 51–65.

Hansjakob, A., Riederer, M. and Hildebrandt, U. (2011) Wax matters: abscence of very-long-chain aldehydes from the leaf cuticular wax of the *glossy11* mutant of maize compromises the prepenetration proccesses of *Blumria graminis*. *Plant Pathology*, **60**(6): 1151-1161.

Hawkins, C. and Liu, Z. (2014) A model for an early role of auxin in *Arabidopsis* gynoecium morphogenesis. *Front Plant Sci*, **5**: 327.

He, X. J. *et al.* (2005) AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. *Plant J*, **44**(6): 903-916.

Hegedus, D. *et al.* (2004) Molecular characterization of *Brassica napus* NAC domain transcriptional activators induced in response to biotic and abiotic stress. *Plant Mol Biol*, **53**(3): 383-397.

Hegedüs, N. and Marx, F. (2013) Antifungal proteins: More than antimicrobials? *Fungal Biol Rev*, **26**(4): 132-145.

Hermann, M. *et al.* (2013) The *Arabidopsis* NIMIN proteins affect *NPR1* differentially. *Front Plant Sci*, **4**: 88.

Hernandez-Blanco, C. *et al.* (2007) Impairment of cellulose synthases required for *Arabidopsis* secondary cell wall formation enhances disease resistance. *Plant Cell*, **19**(3): 890-903.

Herrera-Vasquez, A., Salinas, P. and Holuigue, L. (2015) Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. *Front Plant Sci*, **6**: 171.

Herrmann, K. M. (1995) The shikimate pathway: early steps in the biosynthesis of aromatic compounds. *Plant Cell*, **7**(7): 907-919.

Hibara, K., Takada, S. and Tasaka, M. (2003) *CUC1* gene activates the expression of SAM-related genes to induce adventitious shoot formation. *Plant J*, **36**(5): 687-696.

Hickman, R. *et al.* (2013) A local regulatory network around three NAC transcription factors in stress responses and senescence in *Arabidopsis* leaves. *Plant J*, **75**(1): 26-39.

Higo, K., Ugawa, Y., Iwamoto, M. and Korenaga, T. (1999) Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Res*, **27**(1): 297-300.

Hoffmann, S. *et al.* (2008) Resistance to *Erysiphe necator* in the grapevine 'Kishmish vatkana' is controlled by a single locus through restriction of hyphal growth. *Theor Appl Genet*, **116**(3): 427-438.

Holl, J. *et al.* (2013) The R2R3-MYB transcription factors MYB14 and MYB15 regulate stilbene biosynthesis in *Vitis vinifera*. *Plant Cell*, **25**(10): 4135-4149.

Hong, Y., Zhang, H., Huang, L., Li, D. and Song, F. (2016) Overexpression of a stress-responsive NAC transcription factor gene *ONAC022* improves drought and salt tolerance in rice. *Front Plant Sci*, **7**: 4.

Hu, H. *et al.* (2006) Overexpressing a *NAM, ATAF,* and *CUC (NAC)* transcription factor enhances drought resistance and salt tolerance in rice. *Proc Natl Acad Sci U S A*, **103**(35): 12987-12992.

Hu, H. *et al.* (2008) Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. *Plant Mol Biol*, **67**(1-2): 169-181.

Hu, L., Smith, T. F. and Goldberger, G. (2009) *LFG*: a candidate apoptosis regulatory gene family. *Apoptosis*, **14**(11): 1255-1265.

Hu, R. *et al.* (2010) Comprehensive analysis of *NAC* domain transcription factor gene family in *Populus trichocarpa*. *BMC Plant Biol*, **10**: 145.

Hu, X. *et al.* (2003) Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. *Plant Physiol*, **133**(1): 170-181.

Hubbard, T. *et al.* (2002) The Ensembl genome database project. *Nucl Acids Res*, **30**(1): 38-41.

Huckelhoven, R., Dechert, C. and Kogel, K. H. (2003) Overexpression of barley *BAX inhibitor 1* induces breakdown of *mlo*-mediated penetration resistance to *Blumeria graminis*. *Proc Natl Acad Sci U S A*, **100**(9): 5555-5560.

Huibers, R. P. *et al.* (2013) Powdery mildew resistance in tomato by impairment of *SlPMR4* and *SlDMR1*. *PLoS One*, **8**(6): e67467.

Hulskamp, M. (2004) Plant trichomes: a model for cell differentiation. *Nat Rev Mol Cell Biol*, **5**(6): 471-480.

Humphry, M., Consonni, C. and Panstruga, R. (2006) *Mlo*-based powdery mildew immunity: silver bullet or simply non-host resistance? *Mol Plant Pathol*, **7**(6): 605-610.

Hussey, S. G. *et al.* (2011) *SND2*, a *NAC* transcription factor gene, regulates genes involved in secondary cell wall development in *Arabidopsis* fibres and increases fibre cell area in *Eucalyptus*. *BMC Plant Biol*, **11**: 173.

Jacobs, A. K. *et al.* (2003) An *Arabidopsis* callose synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell*, **15**(11): 2503-2513.

Jefferson, R. A., Kavanagh, T. A. and Bevan, M. W. (1987) *GUS* fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *Embo J*, **6**(13): 3901-3907.

Jensen, M. K. *et al.* (2007) The *HvNAC6* transcription factor: a positive regulator of penetration resistance in barley and *Arabidopsis*. *Plant Mol Biol*, **65**(1-2): 137-150.

Jensen, M. K. *et al.* (2008) Transcriptional regulation by an NAC (NAM-ATAF1,2-CUC2) transcription factor attenuates ABA signalling for efficient basal defence towards *Blumeria graminis* f. sp. *hordei* in *Arabidopsis*. *Plant J*, **56**(6): 867-880.

Jensen, M. K., Kjaersgaard, T., Petersen, K. and Skriver, K. (2010) *NAC* genes: time-specific regulators of hormonal signaling in *Arabidopsis*. *Plant Signal Behav*, **5**(7): 907-910.

Jeong, J. S. *et al.* (2010) Root-specific expression of *OsNAC10* improves drought tolerance and grain yield in rice under field drought conditions. *Plant Physiol*, **153**(1): 185-197.

Jin, H., Xu, G., Meng, Q., Huang, F. and Yu, D. (2013) GmNAC5, a NAC transcription factor, is a transient response regulator induced by abiotic stress in soybean. *Sci World J*, **2013**: 768972.

Johal, G. S. and Briggs, S. P. (1992) Reductase activity encoded by the *HM1* disease resistance gene in maize. *Science*, **258**(5184): 985-987.

Jones, D. A., Thomas, C. M., Hammond-Kosack, K. E., Balint-Kurti, P. J. and Jones, J. D. (1994) Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science*, **266**(5186): 789-793.

Jørgensen, J. H. (1976) Identification of powdery mildew resistant barley mutants and their allelic relationship. In: H. Gaul (Editor), 3. International Barley Genetics Symposium. Barley Genetics III. Verlag Karl Thiemig, München, pp. 446-455.

Jung, Y. H. *et al.* (2006) Functional characterization of OsRacB GTPase–a potentially negative regulator of basal disease resistance in rice. *Plant Physiol Biochem*, **44**(1): 68-77.

Kalve, S., De Vos, D. and Beemster, G. T. (2014) Leaf development: a cellular perspective. *Front Plant Sci*, **5**: 362.

Kanehisa, M. and Goto, S. (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*, **28**(1): 27-30.

Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M. and Tanabe, M. (2016) KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res*, **44**(D1): D457-462.

Kato, H., Motomura, T., Komeda, Y., Saito, T. and Kato, A. (2010) Overexpression of the *NAC* transcription factor family gene *ANAC036* results in a dwarf phenotype in *Arabidopsis thaliana*. *J Plant Physiol*, **167**(7): 571-577.

Katsir, L., Schilmiller, A. L., Staswick, P. E., He, S. Y. and Howe, G. A. (2008) COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proc Natl Acad Sci U S A*, **105**(19): 7100-7105.

Katula-Debreceni, D. *et al.* (2010) Marker-assisted selection for two dominant powdery mildew resistance genes introgressed into a hybrid grape population. *Sci Hortic*, **126**(4): 448–453.

Kay, S., Hahn, S., Marois, E., Hause, G. and Bonas, U. (2007) A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science*, **318**(5850): 648-651.

Kazan, K. and Manners, J. M. (2009) Linking development to defense: auxin in plant-pathogen interactions. *Trends Plant Sci*, **14**(7): 373-382.

Keller, B., Feuillet, C. and Messmer, M. (2000) Genetics of Disease Resistance – Basic concepts and ist application in resistance breeding. In: A. J. Slusarenko, R. S. Fraser and L. C. Van Loon (Editors), Mechanisms of Resistance to Plant Diseases. *Kluwer Academic Publishers*, The Netherlands.

Kikuchi, K. *et al.* (2000) Molecular analysis of the *NAC* gene family in rice. *Mol Gen Genet*, **262**(6): 1047-1051.

Kim, H. S. *et al.* (2012) A NAC transcription factor and SNI1 cooperatively suppress basal pathogen resistance in *Arabidopsis thaliana*. *Nucleic Acids Res*, **40**(18): 9182-9192.

Kim, Y. S. *et al.* (2006) A membrane-bound NAC transcription factor regulates cell division in *Arabidopsis*. *Plant Cell*, **18**(11): 3132-3144.

Király, Z., Barna, B. and Érsek, T. (1972) Hypersensitivity as a consequence, not the cause, of plant resistance to infection. *Nature*, **239**: 456-458.

Ko, J. H., Yang, S. H., Park, A. H., Lerouxel, O. and Han, K. H. (2007) *ANAC012*, a member of the plant-specific *NAC* transcription factor family, negatively regulates xylary fiber development in *Arabidopsis thaliana*. *Plant J*, **50**(6): 1035-1048.

Kotak, S., Port, M., Ganguli, A., Bicker, F. and von Koskull-Doring, P. (2004) Characterization of C-terminal domains of *Arabidopsis* heat stress transcription factors (Hsfs) and identification of a new signature combination of plant class A Hsfs with AHA and NES motifs essential for activator function and intracellular localization. *Plant J*, **39**(1): 98-112.

Kozma, P., Hoffmann, S. and Cindric, P. (2014) New generation of resistant table grape cultivars. In: B. I. Reisch and J. Londo (Editors), X International Conference on Grapevine Breeding and Genetics Acta Horticulturae, Geneva, NY, USA pp. 41-48.

König, S. *et al.* (2012) *Arabidopsis* mutants of sphingolipid fatty acid alpha-hydroxylases accumulate ceramides and salicylates. *New Phytol*, **196**(4): 1086-1097.

Lahaye, T. (2002) The *Arabidopsis RRS1-R* disease resistance gene – uncovering the plant's nucleus as the new battlefield of plant defense? *Trends Plant Sci*, **7**(10): 425-427.

Laskowski, M., Biller, S., Stanley, K., Kajstura, T. and Prusty, R. (2006) Expression profiling of auxin-treated *Arabidopsis* roots: toward a molecular analysis of lateral root emergence. *Plant Cell Physiol*, **47**(6): 788-792.

Le Henanff, G. *et al.* (2013) Grapevine NAC1 transcription factor as a convergent node in developmental processes, abiotic stresses, and necrotrophic/biotrophic pathogen tolerance. *J Exp Bot*, **64**(16): 4877-4893.

Lee, S. W. *et al.* (2009) A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity. *Science*, **326**(5954): 850-853.

Liu, J. Z. *et al.* (2011) Soybean homologs of *MPK4* negatively regulate defense responses and positively regulate growth and development. *Plant Physiol*, **157**(3): 1363-1378.

Liu, X., Bai, X., Wang, X. and Chu, C. (2006) OsWRKY71, a rice transcription factor, is involved in rice defense response. *J Plant Physiol*, **164**(8): 969-979.

Liu, Y., Schiff, M., Marathe, R. and Dinesh-Kumar, S. P. (2002) Tobacco *Rar1*, *EDS1* and *NPR1/NIM1* like genes are required for *N*-mediated resistance to tobacco mosaic virus. *Plant J*, **30**(4): 415-429.

Louie, G. V. *et al.* (2010) Structure-function analyses of a caffeic acid O-methyltransferase from perennial ryegrass reveal the molecular basis for substrate preference. *Plant Cell*, **22**(12): 4114-4127.

Lu, D. *et al.* (2010) A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proc Natl Acad Sci U S A*, **107**(1): 496-501.

Lu, P. L. *et al.* (2007) A novel drought-inducible gene, *ATAF1*, encodes a NAC family protein that negatively regulates the expression of stress-responsive genes in *Arabidopsis*. *Plant Mol Biol*, **63**(2): 289-305.

Lumbreras, V. *et al.* (2010) MAPK phosphatase MKP2 mediates disease responses in *Arabidopsis* and functionally interacts with MPK3 and MPK6. *Plant J*, **63**(6): 1017-1030.

Ma, S., Quist, T. M., Ulanov, A., Joly, R. and Bohnert, H. J. (2004) Loss of *TIP1;1* aquaporin in *Arabidopsis* leads to cell and plant death. *Plant J*, **40**(6): 845-859.

Ma, W., Smigel, A., Tsai, Y. C., Braam, J. and Berkowitz, G. A. (2008) Innate immunity signaling: cytosolic Ca2+ elevation is linked to downstream nitric oxide generation through the action of calmodulin or a calmodulin-like protein. *Plant Physiol*, **148**(2): 818-828.

Mao, C. *et al.* (2007) Overexpression of a NAC-domain protein promotes shoot branching in rice. *New Phytol*, **176**(2): 288-298.

Mao, X., Chen, S., Li, A., Zhai, C. and Jing, R. (2014) Novel NAC transcription factor TaNAC67 confers enhanced multi-abiotic stress tolerances in *Arabidopsis*. *PLoS One*, **9**(1): e84359.

Marin-Rodriguez, M. C., Orchard, J. and Seymour, G. B. (2002) Pectate lyases, cell wall degradation and fruit softening. *J Exp Bot*, **53**(377): 2115-2119.

Matsushima, N. and Miyashita, H. (2012) Leucine-rich repeat (LRR) domains containing intervening motifs in plants. *Biomolecules*, **2**(2): 288-311.

Mauch-Mani, B. and Métraux, J. P. (1998) Salicylic acid and systemic acquired resistance to pathogen attack. *Ann Bot*, **82**(5): 535-540.

Mazarei, M. *et al.* (2011) Gene expression profiling of resistant and susceptible soybean lines infected with soybean cyst nematode. *Theor Appl Genet*, **123**(7): 1193-1206.

McCabe, D. E., Swain, W. F., Martinell, B. J. and Christou, P. (1988) Stable transformation of soybean (*Glycine max*) by particle acceleration. *Nat Biotechnol*, **6**(8): 923-926.

Melaragno, J. E., Mehrotra, B. and Coleman, A. W. (1993) Relationship between endopolyploidy and cell size in epidermal tissue of *Arabidopsis*. *Plant Cell*, **5**(11): 1661-1668.

Merz, P. R. *et al.* (2014) The transcription factor VvWRKY33 is involved in the regulation of grapevine (*Vitis vinifera*) defense against the oomycete pathogen *Plasmopara viticola*. *Physiol Plant*, **153**(3): 365-380.

Métraux, J. P. (2001) Systemic acquired resistance and salicylic acid: Current state of knowledge. *Eur J Plant Pathol*, **107**(1): 13-18.

Milioni, D. *et al.* (2001) Differential expression of cell-wall-related genes during the formation of tracheary elements in the *Zinnia* mesophyll cell system. *Plant Mol Biol*, **47**(1-2): 221-238.

Mitsuda, N., Seki, M., Shinozaki, K. and Ohme-Takagi, M. (2005) The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulate secondary wall thickenings and are required for anther dehiscence. *Plant Cell*, **17**(11): 2993-3006.

Miziorko, H. M. (2011) Enzymes of the mevalonate pathway of isoprenoid biosynthesis. *Arch Biochem Biophys*, **505**(2): 131-143.

Morishita, T. *et al.* (2009) *Arabidopsis* NAC transcription factor, ANAC078, regulates flavonoid biosynthesis under high-light. *Plant Cell Physiol*, **50**(12): 2210-2222.

Mur, L. A., Sturgess, F. J., Farrell, G. G. and Draper, J. (2004) The *AoPR10* promoter and certain endogenous *PR10* genes respond to oxidative signals in *Arabidopsis*. *Mol Plant Pathol*, **5**(5): 435-451.

Mutka, A. M., Fawley, S., Tsao, T. and Kunkel, B. N. (2013) Auxin promotes susceptibility to *Pseudomonas syringae* via a mechanism independent of suppression of salicylic acid-mediated defenses. *Plant J*, **74**(5): 746-754.

Nakamura, S. *et al.* (2010) Gateway binary vectors with the bialaphos resistance gene, *bar*, as a selection marker for plant transformation. *Biosci Biotechnol Biochem*, **74**(6): 1315-1319.

Nakashima, K. *et al.* (2007) Functional analysis of a *NAC*-type transcription factor *OsNAC6* involved in abiotic and biotic stress-responsive gene expression in rice. *Plant J*, **51**(4): 617-630.

Naseem, M. and Dandekar, T. (2012) The role of auxin-cytokinin antagonism in plant-pathogen interactions. *PLoS Pathog*, **8**(11): e1003026.

Ndamukong, I. *et al.* (2007) SA-inducible *Arabidopsis* glutaredoxin interacts with TGA factors and suppresses JA-responsive *PDF1.2* transcription. *Plant J*, **50**(1): 128-139.

Neill, S., Desikan, R. and Hancock, J. (2002) Hydrogen peroxide signalling. *Curr Opin Plant Biol*, **5**(5): 388-395.

Nicaise, V., Roux, M. and Zipfel, C. (2009) Recent advances in PAMP-triggered immunity against bacteria: pattern recognition receptors watch over and raise the alarm. *Plant Physiol*, **150**(4): 1638-1647.

Nie, H. *et al.* (2012) SR1, a calmodulin-binding transcription factor, modulates plant defense and ethylene-induced senescence by directly regulating NDR1 and EIN3. *Plant Physiol*, **158**(4): 1847-1859.

Nielsen, M. E. and Thordal-Christensen, H. (2013) Transcytosis shuts the door for an unwanted guest. *Trends Plant Sci*, **18**(11): 611-616.

Nishimura, M. T. *et al.* (2003) Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science*, **301**(5635): 969-972.

Novak, J. *et al.* (2013) High cytokinin levels induce a hypersensitive-like response in tobacco. *Ann Bot*, **112**(1): 41-55.

Nuruzzaman, M. *et al.* (2010) Genome-wide analysis of *NAC* transcription factor family in rice. *Gene*, **465**(1-2): 30-44.

Oh, C. S. and Martin, G. B. (2011) Effector-triggered immunity mediated by the Pto kinase. *Trends Plant Sci*, **16**(3): 132-140.

Ohashi-Ito, K., Oda, Y. and Fukuda, H. (2010) *Arabidopsis* VASCULAR-RELATED NAC-DOMAIN6 directly regulates the genes that govern programmed cell death and secondary wall formation during xylem differentiation. *Plant Cell*, **22**(10): 3461-3473.

Ohnishi, T. *et al.* (2005) *OsNAC6*, a member of the *NAC* gene family, is induced by various stresses in rice. *Genes Genet Syst*, **80**(2): 135-139.

Oliveira, T. M. *et al.* (2011) Analysis of the *NAC* transcription factor gene family in citrus reveals a novel member involved in multiple abiotic stress responses. *Tree Genet Genomes*, **7**(6): 1123-1134.

Olsen, A. N., Ernst, H. A., Leggio, L. L. and Skriver, K. (2005) NAC transcription factors: structurally distinct, functionally diverse. *Trends Plant Sci*, **10**(2): 79-87.

Ooka, H. *et al.* (2003) Comprehensive analysis of *NAC* family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Res*, **10**(6): 239-247.

Padmanabhan, M. S., Kramer, S. R., Wang, X. and Culver, J. N. (2008) Tobacco mosaic virus replicase-auxin/indole acetic acid protein interactions: reprogramming the auxin response pathway to enhance virus infection. *J Virol*, **82**(5): 2477-2485.

Palusa, S. G., Golovkin, M., Shin, S.-B., Richardson, D. N. and Reddy, A. S. N. (2007) Organ-specific, developmental, hormonal and stress regulation of expression of putative pectate lyase genes in *Arabidopsis*. *New Phytol*, **174**(3): 537-550.

Papadopoulou, K., Melton, R. E., Leggett, M., Daniels, M. J. and Osbourn, A. E. (1999) Compromised disease resistance in saponin-deficient plants. *Proc Natl Acad Sci U S A*, **96**(22): 12923-12928.

Park, C. Y. *et al.* (2005) WRKY group IId transcription factors interact with calmodulin. *FEBS Lett*, **579**(6): 1545-1550.

Park, S. W., Kaimoyo, E., Kumar, D., Mosher, S. and Klessig, D. F. (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science*, **318**(5847): 113-116.

Pathuri, I. P. *et al.* (2008) Constitutively activated barley *ROPs* modulate epidermal cell size, defense reactions and interactions with fungal leaf pathogens. *Plant Cell Rep*, **27**(12): 1877-1887.

Pathuri, I. P., Reitberger, I. E., Huckelhoven, R. and Proels, R. K. (2011) *Alcohol dehydrogenase 1* of barley modulates susceptibility to the parasitic fungus *Blumeria graminis* f.sp. *hordei*. *J Exp Bot*, **62**(10): 3449-3457.

Pauquet, A., Bouquet, A., This, P. and Adam-Blondon, A. F. (2001) Establishment of a local map of AFLP markers around the powdery mildew. *Theor Appl Genet*, **103**(8): 1201-1210.

Pauwels, L. and Goossens, A. (2011) The JAZ proteins: a crucial interface in the jasmonate signaling cascade. *Plant Cell*, **23**(9): 3089-3100.

Pearson, R. C. and Gadoury, D. M. (1992) Powdery Mildew of Grape. In: J. Kumar, H. S. Chaube, U. S. Singh and A. N. Mukhopadhyay (Editors), Plant Diseases of International Importance: Diseases of fruit crops. *Prentice Hall*, Englewood Cliffs, NJ, USA, pp. 129-146.

Peirson, S. N., Butler, J. N. and Foster, R. G. (2003) Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. *Nucleic Acids Res*, **31**(14): e73.

Pieterse, C. M., van Wees, S. C., Hoffland, E., van Pelt, J. A. and van Loon, L. C. (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell*, **8**(8): 1225-1237.

Pieterse, C. M., Van der Does, D., Zamioudis, C., Leon-Reyes, A. and Van Wees, S. C. (2012) Hormonal modulation of plant immunity. *Annu Rev Cell Dev Biol*, **28**: 489-521.

Pieterse, C. M. J., Leon-Reyes, A., Van der Ent, S. and Van Wees, S. C. M. (2009) Networking by small-molecule hormones in plant immunity. *Nat Chem Biol*, **5**(5): 308-316.

Pinheiro, G. L. *et al.* (2009) Complete inventory of soybean NAC transcription factors: sequence conservation and expression analysis uncover their distinct roles in stress response. *Gene*, **444**(1-2): 10-23.

Qi, Y., Tsuda, K., Glazebrook, J. and Katagiri, F. (2011) Physical association of pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) immune receptors in *Arabidopsis*. *Mol Plant Pathol*, **12**(7): 702-708.

Rahantaniaina, M.-S., Tuzet, A., Mhamdi, A. and Noctor, G. (2013) Missing links in understanding redox signaling via thiol/disulfide modulation: How is glutathione oxidized in plants? *Front Plant Sci*, **4**: 477.

Ridout, C. J. (2001) Powdery Mildews, Encyclopedia of Life Sciences. *John Wiley & Sons, Ltd*, Norwich.

Riechmann, J. L. *et al.* (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science*, **290**(5499): 2105-2110.

Robert-Seilaniantz, A., Grant, M. and Jones, J. D. (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annu Rev Phytopathol*, **49**: 317-343.

Rowland, O. *et al.* (2005) Functional analysis of *Avr9/Cf-9 rapidly elicited* genes identifies a protein kinase, ACIK1, that is essential for full *Cf-9*-dependent disease resistance in tomato. *Plant Cell*, **17**(1): 295-310.

Ryals, J. A. *et al.* (1996) Systemic acquired resistance. *Plant Cell*, **8**(10): 1809-1819.

Sablowski, R. W. and Meyerowitz, E. M. (1998) A homolog of *NO APICAL MERISTEM* is an immediate target of the floral homeotic genes *APETALA3/PISTILLATA*. *Cell*, **92**(1): 93-103.

Sacher, J. A. and Glasziou, K. T. (1959) Effects of auxins on membrane permeability and pectic substances in bean endocarp. *Nature*, **183**(4663): 757-758.

Saga, H. *et al.* (2012) Identification and characterization of *ANAC042*, a transcription factor family gene involved in the regulation of camalexin biosynthesis in *Arabidopsis*. *Mol Plant Microbe Interact*, **25**(5): 684-696.

Sagaram, U. S., Pandurangi, R., Kaur, J., Smith, T. J. and Shah, D. M. (2011) Structure-activity determinants in antifungal plant defensins MsDef1 and MtDef4 with different modes of action against *Fusarium graminearum*. *PLoS One*, **6**(4): e18550.

Salzman, R. A. *et al.* (2005) Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. *Plant Physiol*, **138**(1): 352-368.

Sang-Keun, O. *et al.* (2014) Expression of cucumber *LOX* genes in response to powdery mildew and defense-related signal molecules. *Can J Plant Sci*, **94**(5): 845-850.

Schaefer, H. M., Rentzsch, M. and Breuer, M. (2008) Anthocyanins reduce fungal growth in fruits. *Nat Prod Commun*, **3**(8): 1267-1272.

Schmidt, S. M. *et al.* (2014) Interaction of a *Blumeria graminis* f. sp. *hordei* effector candidate with a barley ARF-GAP suggests that host vesicle trafficking is a fungal pathogenicity target. *Mol Plant Pathol*, **15**(6): 535-549.

Schoonbeek, H. J. *et al.* (2015) *Arabidopsis* EF-Tu receptor enhances bacterial disease resistance in transgenic wheat. *New Phytol*, **206**(2): 606-613.

Schornack, S. *et al.* (2004) The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. *Plant J*, **37**(1): 46-60.

Schön, M. *et al.* (2013) Analyses of *wrky18 wrky40* plants reveal critical roles of SA/EDS1 signaling and indole-glucosinolate biosynthesis for *Golovinomyces orontii* resistance and a loss-of resistance towards *Pseudomonas syringae* pv. *tomato AvrRPS4*. *Mol Plant Microbe Interact*, **26**(7): 758-767.

Schultheiss, H., Dechert, C., Kogel, K. H. and Huckelhoven, R. (2002) A small GTP-binding host protein is required for entry of powdery mildew fungus into epidermal cells of barley. *Plant Physiol*, **128**(4): 1447-1454.

Schulze-Lefert, P. and Vogel, J. (2000) Closing the ranks to attack by powdery mildew. *Trends Plant Sci*, **5**(8): 343-348.

Seki, H., Tamura, K. and Muranaka, T. (2015) P450s and UGTs: Key players in the structural diversity of triterpenoid saponins. *Plant Cell Physiol*, **56**(8): 1463-1471.

Seo, P. J. *et al.* (2010) Cold activation of a plasma membrane-tethered NAC transcription factor induces a pathogen resistance response in *Arabidopsis*. *Plant J*, **61**(4): 661-671.

Shah, J. (2003) The salicylic acid loop in plant defense. *Curr Opin Plant Biol*, **6**(4): 365-371.

Shahnejat-Bushehri, S., Mueller-Roeber, B. and Balazadeh, S. (2012) *Arabidopsis* NAC transcription factor JUNGBRUNNEN1 affects thermomemory-associated genes and enhances heat stress tolerance in primed and unprimed conditions. *Plant Signal Behav*, **7**(12): 1518-1521.

Shen, Q. H. *et al.* (2003) Recognition specificity and RAR1/SGT1 dependence in barley *Mla* disease resistance genes to the powdery mildew fungus. *Plant Cell*, **15**(3): 732-744.

Shulaev, V., Silverman, P. and Raskin, I. (1997) Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature*, **385**(6618): 718-721.

Silva, H., Yoshioka, K., Dooner, H. K. and Klessig, D. F. (1999) Characterization of a new *Arabidopsis* mutant exhibiting enhanced disease resistance. *Mol Plant Microbe Interact*, **12**(12): 1053-1063.

Sohlberg, J. J. *et al.* (2006) STY1 regulates auxin homeostasis and affects apical-basal patterning of the *Arabidopsis* gynoecium. *Plant J*, **47**(1): 112-123.

Song, S. *et al.* (2013) The bHLH subgroup IIId factors negatively regulate jasmonate-mediated plant defense and development. *PLoS Genet*, **9**(7): e1003653.

Song, S. Y., Chen, Y., Chen, J., Dai, X. Y. and Zhang, W. H. (2011) Physiological mechanisms underlying *OsNAC5*-dependent tolerance of rice plants to abiotic stress. *Planta*, **234**(2): 331-345.

Song, W. Y. *et al.* (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science*, **271**(5243): 1804-1806.

Souer, E., van Houwelingen, A., Kloos, D., Mol, J. and Koes, R. (1996) The *no apical meristem* gene of *Petunia* is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell*, **85**(2): 159-170.

Stergiopoulos, I. *et al.* (2010) Tomato Cf resistance proteins mediate recognition of cognate homologous effectors from fungi pathogenic on dicots and monocots. *Proc Natl Acad Sci U S A*, **107**(16): 7610-7615.

Stotz, H. U., Spence, B. and Wang, Y. (2009) A defensin from tomato with dual function in defense and development. *Plant Mol Biol*, **71**(1-2): 131-143.

Sun, L. and van Nocker, S. (2010) Analysis of promoter activity of members of the *PECTATE LYASE-LIKE (PLL)* gene family in cell separation in *Arabidopsis*. *BMC Plant Biol*, **10**: 152.

Sun, L. *et al.* (2015) *GLABROUS INFLORESCENCE STEMS3 (GIS3)* regulates trichome initiation and development in *Arabidopsis*. *New Phytol*, **206**(1): 220-230.

Sun, L. L. *et al.* (2013) GLABROUS INFLORESCENCE STEMS regulates trichome branching by genetically interacting with *SIM* in *Arabidopsis*. *J Zhejiang Univ Sci B*, **14**(7): 563-569.

Svalheim and Robertsen, B. (1993) Elicitation of H2O2-production in cucumber hypocotyl segments by oligo-1,4-α-D-galacturonides and an oligo-β-glucan preparation from cell walls of *Phythophthora megasperma* f. sp. *glycinea*. *Physiol Plant*, **88**(4): 675-681.

Szabo, L. J. and Bushnell, W. R. (2001) Hidden robbers: the role of fungal haustoria in parasitism of plants. *Proc Natl Acad Sci U S A*, **98**(14): 7654-7655.

Takada, S., Hibara, K., Ishida, T. and Tasaka, M. (2001) The *CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. *Development*, **128**(7): 1127-1135.

Takasaki, H. *et al.* (2010) The abiotic stress-responsive NAC-type transcription factor OsNAC5 regulates stress-inducible genes and stress tolerance in rice. *Mol Genet Genomics*, **284**(3): 173-183.

Tang, D., Christiansen, K. M. and Innes, R. W. (2005) Regulation of plant disease resistance, stress responses, cell death, and ethylene signaling in *Arabidopsis* by the EDR1 protein kinase. *Plant Physiol*, **138**(2): 1018-1026.

Taniguchi, Y. *et al.* (1995) Cry j I, a major allergen of Japanese cedar pollen, has pectate lyase enzyme activity. *Allergy*, **50**(1): 90-93.

Tao, Z. *et al.* (2009) A pair of allelic *WRKY* genes play opposite roles in rice-bacteria interactions. *Plant Physiol*, **151**(2): 936-948.

Thibaud, M. C., Gineste, S., Nussaume, L. and Robaglia, C. (2004) Sucrose increases pathogenesis-related *PR-2* gene expression in *Arabidopsis thaliana* through an SA-dependent but NPR1-independent signaling pathway. *Plant Physiol Biochem*, **42**(1): 81-88.

Thimm, O. *et al.* (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J*, **37**(6): 914-939.

Thomma, B. P., Nürnberger, T. and Joosten, M. H. (2011) Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *Plant Cell*, **23**(1): 4-15.

Thordal-Christensen, H., Zhang, Z., Wei, Y. and Collinge, D. B. (1997) Subcellular localization of H2O2 in plants. H2O2 accumulation in papillae and hypersensitive response during the barley—powdery mildew interaction. *Plant J*, **11**(6): 1187-1194.

Thordal-Christensen, H. (2003) Fresh insights into processes of nonhost resistance. *Curr Opin Plant Biol*, **6**(4): 351-357.

Toth, Z. *et al.* (2016) Expression of a grapevine *NAC* transcription factor gene is induced in response to powdery mildew colonization in salicylic acid-independent manner. *Sci Rep*, **6**: 30825.

Tran, L. S. *et al.* (2004) Isolation and functional analysis of *Arabidopsis* stress-inducible NAC transcription factors that bind to a drought-responsive cis-element in the *early responsive to dehydration stress 1* promoter. *Plant Cell*, **16**(9): 2481-2498.

Trigueros, M. *et al.* (2009) The *NGATHA* genes direct style development in the *Arabidopsis* gynoecium. *Plant Cell*, **21**(5): 1394-1409.

Tsuba, M., Katagiri, C., Takeuchi, Y., Takada, Y. and Yamaoka, N. (2002) Chemical factors of the leaf surface involved in the morphogenesis of *Blumeria graminis*. *Physiol Mol Plant Pathol*, **60**(2): 51-57.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A. and Dubcovsky, J. (2006) A *NAC* Gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science*, **314**(5803): 1298-1301.

Uknes, S. *et al.* (1992) Acquired resistance in *Arabidopsis*. *Plant Cell*, **4**(6): 645-656.

Uknes, S. *et al.* (1993) Biological induction of systemic acquired resistance in Arabidopsis. *Mol Plant Microbe Interact*, **6**(6): 692-698.

Ulmasov, T., Murfett, J., Hagen, G. and Guilfoyle, T. J. (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell*, **9**(11): 1963-1971.

Underwood, W. and Somerville, S. C. (2013) Perception of conserved pathogen elicitors at the plasma membrane leads to relocalization of the *Arabidopsis* PEN3 transporter. *Proc Natl Acad Sci U S A*, **110**(30): 12492-12497.

Uppalapati, S. R. *et al.* (2012) Loss of abaxial leaf epicuticular wax in *Medicago truncatula irg1/palm1* mutants results in reduced spore differentiation of anthracnose and nonhost rust pathogens. *Plant Cell*, **24**(1): 353-370.

Usadel, B. *et al.* (2005) Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of corresponding genes, and comparison with known responses. *Plant Physiol*, **138**(3): 1195-1204.

Valente, P., Tao, W. and Verbelen, J.-P. (1998) Auxins and cytokinins control DNA endoreduplication and deduplication in single cells of tobacco. *Plant Sci*, **134**(2): 207-215.

Van Schie, C. C. and Takken, F. L. (2014) Susceptibility genes 101: how to be a good host. *Annu Rev Phytopathol*, **52**: 551-581.

Vernooij, B. *et al.* (1994) Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *Plant Cell*, **6**(7): 959-965.

Vernooij, B. *et al.* (1995) 2,6-Dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. *Mol Plant Microbe Interact*, **8**(2): 228-234.

Veronese, P. *et al.* (2004) The *BOS* loci of *Arabidopsis* are required for resistance to *Botrytis cinerea* infection. *Plant J*, **40**(4): 558-574.

Veronese, P. *et al.* (2006) The membrane-anchored BOTRYTIS-INDUCED KINASE1 plays distinct roles in *Arabidopsis* resistance to necrotrophic and biotrophic pathogens. *Plant Cell*, **18**(1): 257-273.

Vlot, A. C., Dempsey, D. A. and Klessig, D. F. (2009) Salicylic acid, a multifaceted hormone to combat disease. *Annu Rev Phytopathol*, **47**: 177-206.

Voegele, R. T., Struck, C., Hahn, M. and Mendgen, K. (2001) The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proc Natl Acad Sci U S A*, **98**(14): 8133-8138.

Vogel, J. P., Raab, T. K., Schiff, C. and Somerville, S. C. (2002) *PMR6*, a pectate lyase-like gene required for powdery mildew susceptibility in *Arabidopsis*. *Plant Cell*, **14**(9): 2095-2106.

Vogel, J. P., Raab, T. K., Somerville, C. R. and Somerville, S. C. (2004) Mutations in *PMR5* result in powdery mildew resistance and altered cell wall composition. *Plant J*, **40**(6): 968-978.

Voitsik, A. M., Muench, S., Deising, H. B. and Voll, L. M. (2013) Two recently duplicated maize *NAC* transcription factor paralogs are induced in response to *Colletotrichum graminicola* infection. *BMC Plant Biol*, **13**: 85-100.

Vroemen, C. W., Mordhorst, A. P., Albrecht, C., Kwaaitaal, M. A. and de Vries, S. C. (2003) The *CUP-SHAPED COTYLEDON3* gene is required for boundary and shoot meristem formation in *Arabidopsis*. *Plant Cell*, **15**(7): 1563-1577.

Walters, D. R. and McRoberts, N. (2006) Plants and biotrophs: a pivotal role for cytokinins? *Trends Plant Sci*, **11**(12): 581-586.

Wang, D., Amornsiripanitch, N. and Dong, X. (2006) A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *PLoS Pathog*, **2**(11): e123.

Wang, D., Pajerowska-Mukhtar, K., Culler, A. H. and Dong, X. (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Curr Biol*, **17**(20): 1784-1790.

Wang, E. *et al.* (2012a) A common signaling process that promotes mycorrhizal and oomycete colonization of plants. *Curr Biol*, **22**(23): 2242-2246.

Wang, G. L., Song, W. Y., Ruan, D. L., Sideris, S. and Ronald, P. C. (1996) The cloned gene, *Xa21*, confers resistance to multiple *Xanthomonas oryzae* pv. *oryzae* isolates in transgenic plants. *Mol Plant Microbe Interact*, **9**(9): 850-855.

Wang, H. *et al.* (2011) Overexpression of *Brassica juncea* wild-type and mutant *HMG-CoA synthase 1* in *Arabidopsis* up-regulates genes in sterol biosynthesis and enhances sterol production and stress tolerance. *Plant Biotechnol J*, **10**(1): 31-42.

Wang, K., Senthil-Kumar, M., Ryu, C. M., Kang, L. and Mysore, K. S. (2012b) Phytosterols play a key role in plant innate immunity against bacterial pathogens by regulating nutrient efflux into the apoplast. *Plant Physiol*, **158**(4): 1789-1802.

Wang, K. L., Li, H. and Ecker, J. R. (2002) Ethylene biosynthesis and signaling networks. *Plant Cell*, **14**: 131-151.

Wang, N., Zheng, Y., Xin, H., Fang, L. and Li, S. (2013) Comprehensive analysis of *NAC* domain transcription factor gene family in *Vitis vinifera*. *Plant Cell Rep*, **32**(1): 61-75.

Wang, Z., Rashotte, A., Moss, A. and Dane, F. (2014) Two NAC transcription factors from *Cytrullus colocynthis*, CcNAC1, CcNAC2 implicated in multiple stress responses. *Acta Physiol Plant*, **36**(3): 621-634.

Ward, E. R. *et al.* (1991) Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell*, **3**(10): 1085-1094.

Waters, B. M., Uauy, C., Dubcovsky, J. and Grusak, M. A. (2009) Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *J Exp Bot*, **60**(15): 4263-4274.

Weigel, R. R., Pfitzner, U. M. and Gatz, C. (2005) Interaction of NIMIN1 with NPR1 modulates *PR* gene expression in *Arabidopsis*. *Plant Cell*, **17**(4): 1279-1291.

Weir, I. *et al.* (2004) *CUPULIFORMIS* establishes lateral organ boundaries in *Antirrhinum*. *Development*, **131**(4): 915-922.

Weymann, K. *et al.* (1995) Suppression and restoration of lesion formation in *Arabidopsis lsd* mutants. *Plant Cell*, **7**(12): 2013-2022.

Wildermuth, M. C., Dewdney, J., Wu, G. and Ausubel, F. M. (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*, **414**(6863): 562-565.

Willemsen, V. *et al.* (2008) The NAC domain transcription factors FEZ and SOMBRERO control the orientation of cell division plane in *Arabidopsis* root stem cells. *Dev Cell*, **15**(6): 913-922.

Wu, A. *et al.* (2012) JUNGBRUNNEN1, a reactive oxygen species-responsive NAC transcription factor, regulates longevity in *Arabidopsis*. *Plant Cell*, **24**(2): 482-506.

Wu, J. Z., Lin, Y., Zhang, X. L., Pang, D. W. and Zhao, J. (2008) IAA stimulates pollen tube growth and mediates the modification of its wall composition and structure in *Torenia fournieri*. *J Exp Bot*, **59**(9): 2529-2543.

Wu, Y., Qiu, X., Du, S. and Erickson, L. (1996) *PO149*, a new member of pollen pectate lyase-like gene family from alfalfa. *Plant Mol Biol*, **32**(6): 1037-1042.

Xia, N. *et al.* (2010) Characterization of a novel wheat *NAC* transcription factor gene involved in defense response against stripe rust pathogen infection and abiotic stresses. *Mol Biol Rep*, **37**(8): 3703-3712.

Xiao, S. *et al.* (2005) The atypical resistance gene, *RPW8*, recruits components of basal defence for powdery mildew resistance in *Arabidopsis*. *Plant J*, **42**(1): 95-110.

Xiao, S. and Chye, M. L. (2011) Overexpression of *Arabidopsis ACBP3* enhances NPR1-dependent plant resistance to *Pseudomonas syringae* pv *tomato* DC30001. *Plant Physiol*, **156**(4): 2069-2081.

Xie, Q., Sanz-Burgos, A. P., Guo, H., Garcia, J. A. and Gutierrez, C. (1999) GRAB proteins, novel members of the NAC domain family, isolated by their interaction with a geminivirus protein. *Plant Mol Biol*, **39**(4): 647-656.

Xie, Q., Frugis, G., Colgan, D. and Chua, N. H. (2000) *Arabidopsis* NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. *Genes Dev*, **14**(23): 3024-3036.

Xiong, L. and Yang, Y. (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *Plant Cell*, **15**(3): 745-759.

Xu, R. and Li, Q. Q. (2008) Protocol: Streamline cloning of genes into binary vectors in *Agrobacterium* via the Gateway® TOPO vector system. *Plant Methods*, **4**(1): 4.

Xu, X., Chen, C., Fan, B. and Chen, Z. (2006) Physical and functional interactions between pathogen-induced *Arabidopsis* WRKY18, WRKY40, and WRKY60 transcription factors. *Plant Cell*, **18**(5): 1310-1326.

Yang, Y., Shah, J. and Klessig, D. F. (1997) Signal perception and transduction in plant defense responses. *Genes Dev*, **11**(13): 1621-1639.

Yang, Z. T. *et al.* (2014) The membrane-associated transcription factor NAC089 controls ER-stress-induced programmed cell death in plants. *PLoS Genet*, **10**(3): e1004243.

Yao, D. *et al.* (2012) Comparative genomic analysis of NAC transcriptional factors to dissect the regulatory mechanisms for cell wall biosynthesis. *BMC Bioinformatics*, **13 (Suppl 15)**: S10.

Yuan, M., Chu, Z., Li, X., Xu, C. and Wang, S. (2010) The bacterial pathogen *Xanthomonas oryzae* overcomes rice defenses by regulating host copper redistribution. *Plant Cell*, **22**(9): 3164-3176.

Zeilmaker, T. *et al.* (2015) *DOWNY MILDEW RESISTANT 6* and *DMR6-LIKE OXYGENASE 1* are partially redundant but distinct suppressors of immunity in *Arabidopsis*. *Plant J*, **81**(2): 210-222.

Zhai, W. *et al.* (2002) Breeding bacterial blight-resistant hybrid rice with the cloned bacterial blight resistance gene *Xa21*. *Mol Breeding*, **8**(4): 285-293.

Zhang, K., Halitschke, R., Yin, C., Liu, C. J. and Gan, S. S. (2013a) Salicylic acid 3-hydroxylase regulates *Arabidopsis* leaf longevity by mediating salicylic acid catabolism. *Proc Natl Acad Sci U S A*, **110**(36): 14807-14812.

Zhang, Y., Lubberstedt, T. and Xu, M. (2013b) The genetic and molecular basis of plant resistance to pathogens. *J Genet Genomics*, **40**(1): 23-35.

Zhao, C., Craig, J. C., Petzold, H. E., Dickerman, A. W. and Beers, E. P. (2005) The xylem and phloem transcriptomes from secondary tissues of the *Arabidopsis* root-hypocotyl. *Plant Physiol*, **138**(2): 803-818.

Zhao, C., Avci, U., Grant, E. H., Haigler, C. H. and Beers, E. P. (2008) XND1, a member of the NAC domain family in *Arabidopsis thaliana*, negatively regulates lignocellulose synthesis and programmed cell death in xylem. *Plant J*, **53**(3): 425-436.

Zhu, T., Nevo, E., Sun, D. and Peng, J. (2012a) Phylogenetic analyses unravel the evolutionary history of NAC proteins in plants. *Evolution*, **66**(6): 1833-1848.

Zhu, Y., Nam, J., Carpita, N. C., Matthysse, A. G. and Gelvin, S. B. (2003) *Agrobacterium*-mediated root transformation is inhibited by mutation of an *Arabidopsis* cellulose synthase-like gene. *Plant Physiol*, **133**(3): 1000-1010.

Zhu, Z., Shi, J., He, M., Cao, J. and Wang, Y. (2012b) Isolation and functional characterization of a transcription factor *VpNAC1* from Chinese wild *Vitis pseudoreticulata*. *Biotechnol Lett*, **34**(7): 1335-1342.

Zipfel, C. *et al.* (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell*, **125**(4): 749-760.

Zipfel, C. and Robatzek, S. (2010) Pathogen-associated molecular pattern-triggered immunity: veni, vidi...? *Plant Physiol*, **154**(2): 551-554.

Zou, C. *et al.* (2011) Cis-regulatory code of stress-responsive transcription in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A*, **108**(36): 14992-14997.

A2. Supplemental data

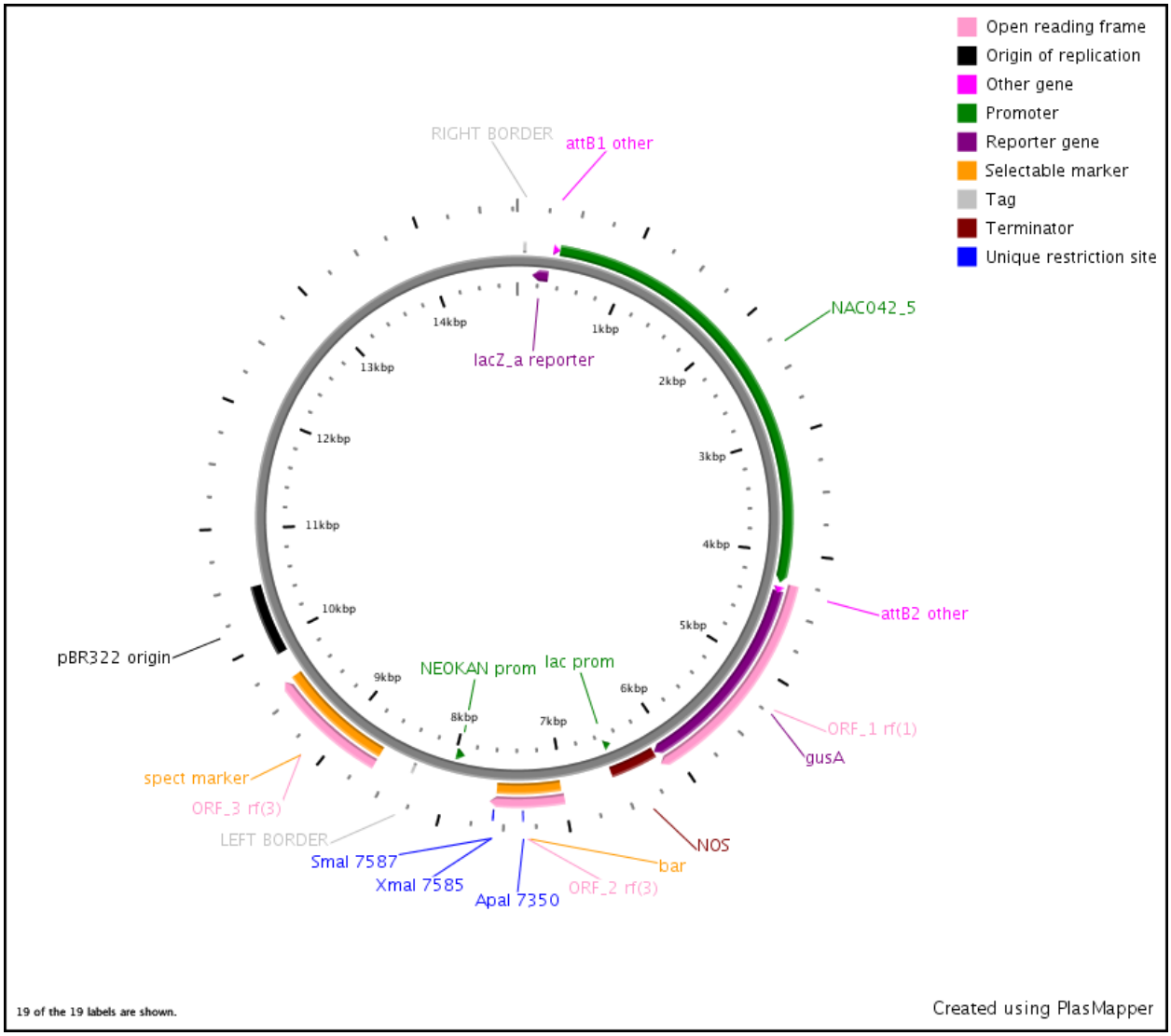


Figure 18. Plasmid map of pGWB633 containing the *NAC042_5* promoter (3896 bp).

Table 3. Primer sequences applied in this study

		Forward	Reverse
qPCR-sets	*NAC042_5*	TGGTTGTCGGCCAAATGAG	CAGTCATAAACATGAGGTGG
	ACTIN 1	CCCCACCTCAACACATCTCC	TCCATTGTCCACAGGAAGTGC
Primers used for amplification of *NAC042_5* promoter fragments	*NAC042_5* 3896 bp	CACCTCAATCACACTCAAAAACCA	AGTGCTAGTCTTCTCCACCTCCAT
	NAC042_5 2935 bp	CACCCTATGACCCGACATGAATTT	
	NAC042_5 2456 bp	CACCGGCTGACTGAAAACAAAAAC	
	NAC042_5 1178 bp	CACCGAGGAAAGGAAGATGAAAGA	
	NAC042_5 257 bp	CACCTTTTGCCTTCCAAGTTCCAC	
Primers used for amplification of *AtNAC* promoter	*ANAC042* 3814 bp	CACCTTACAGCGAGGGAGATAATGA	TCGATCTCTTTAGAACACCAATCA
Primers for checking the cloned fragment in pENTR	Full-size	CGGGCCCCAAATAATGAT	CCTGTTCGTTGCAACAAA
	Orientation	GAATCAATATCACAGCCTTG	
Primers for checking the cloned fragment in T-DNA of pGWB633, and also were used for confirmation of positive transgenic plants	Full-size	TCCACCATGAACCTTTATG	AGTTTTCGCGATCCAGA
	Orientation	GAATCAATATCACAGCCTTG	

Table 4. Putative *cis*-regulatory elements identified in the sequences of *NAC042_5* and *JUB1* using the PLACE databas

Cis-element	Sequence	Function	Location of the *cis*-element upstream of translational start	
			NAC042_5	*JUB1*
ABRE	YACGTGGC	ABA-responsive		3754(-)
	RYACGTGGYR			3753(-)
	ACGTG		1732(+), 2781(+)	788(-), 789(+), 3365(+), 3471(-), 3756(-), 3757(+)
	MACGYGB			788(+), 3755(-), 3756(+)
ARF	TGTCTC	Auxin-response factor binding	2975(+)	
ASF1	TGACG	SA-responsive, WRKY-bZIP-binding	270(+), 1735(+), 2603(-), 2779(+)	3485(+), 3516(-)
ATHB6-binding	CAATTATTA	Active in hormone responses		3152(-)
BBF1	ACTTTA	Auxin-inducible	2245(+)	443(+)

BIHD1-binding	TGTCA	Regulate in defense response	486(-), 755(-), 1574(-), 2094(+), 2102(-), 2575(-)	1415(-), 1632(-), 2594(-), 2828(+), 2868(+), 3592(-), 3839(+)
Box-C	CTCCCAC	Small nucleolar RNA-bnding	1787(+)	
BS1	AGCGGG	Vascular expression	17(-)	2754(+)
CARE	CAACTC	Gibberellin-responsive		1332(+), 3916(-), 3969(-)
CArG	CWWWWWWWWG	Flowering-regulated	657(+,-), 883(+,-), 1044(+,-), 1466(+,-), 2005(+,-), 2606(+,-), 2639(+,-), 3368(+,-), 3394(+,-)	85(+,-), 334(+,-), 816(+,-), 1265(+,-), 3151(+,-),
	CCWWWWWWGG		3831(+,-)	
CBF-binding	RYCGAC	Cold-responsive		1527(+,-), 2251(-), 2331(+), 3555(+)
CGCG-box	VCGCGB	Calmodulin-binding		2895(+,-), 3106(+,-)
CPB	TATTAG	Cytokinin-enhanced Protein-binding		1220(+), 1558(-), 3151(-), 3256(+)
CRT/DRE	GTCGAC	Low-temperature-responsive, found in CBF2 promoter		1527(+,-)
DRE1	ACCGAGA			3100(+)
DRE/CRT	RCCGAC			2251(-), 2331(+)
DPBF-core	ACACNNG	bZIP transcription factors, DPBF-1 and 2-binding, ABA-response		160(+), 3756(-)
E-box	CANNTG	Enhancer-box	162(+,-), 962(+,-), 1121(+,-), 1791(+,-), 1853(+,-), 2476(+,-), 2537(+,-), 2847(+,-),	
EECCRCAH1	GANTTNC	Two enhancer element	372(+), 392(-), 844(-), 1232(+), 1722(+), 2512(-), 3353(+)	175(+), 653(-), 2216(+), 2229(+), 3317(-)
ELRE-core	TTGACC	Elicitor-responsive	99(-), 959(-), 1698 (-), 3235(+)	126(-), 2951(-)
ERELEE4	AWTTCAAA	Ethylene-responsive	493(-), 1008(-), 2509(-), 2903(+), 3410(+), 3518(+)	655(+), 2937(-), 3768(+)
G-box	CACGTG	GBF4-binding, ABA-,UV-responsive		788(+,-), 3756(+,-)
GA-down	ACGTGTC	Gibberellin-responsive		3757(+)
GARE	TAACAAR		2351(-), 3505(-)	293(+), 1651(+), 3460(+)
GCC-core	GCCGCC	JA/ET-responsive	1293(-)	2320(+)

L1-box	TAAATGYA	Required for L1 epidermal layer specific expression	3062(-), 3480(-)	2220(-)
LTRE1	CCGAAA	Low-temperature element	2964(+)	1049(+)
LTRE-core	CCGAC		2924(-), 2947 (-)	2251(-), 2332(+)
MYB1	WAACCA	MYB-binding	207(-), 2937(+), 3015(-), 3174(-), 3200(+), 3550(-), 3629(-), 3684(-), 3877(-)	238(-), 1694(-), 1911(+), 2704(+), 2969(+), 3563(+) 3651(+)
	GTTAGTT			3292(-)
MYB2	TAACTG		2775(+), 3009(+)	2273(-), 3295(+)
	YAACKG			988(+), 1341(-), 2273(-), 2853(-), 3295(+)
MYB-core MYB-core	CNGTTR		313(+), 1363(-), 2059(+), 2476(+), 2734(+)	988 (-), 1039 (+), 1136 (-), 1341 (+), 1877 (+), 2270 (-), 2273 (+), 2282 (-), 2853 (+), 2878 (+), 3170 (-), 3295 (-), 3787 (+)
	AACGG		1749(+)	2886(-)
	TAACAAA		2351(-), 3505(-)	293(+), 1651(+), 3460(+)
	CCWACC		1131(+), 1387(+), 2696(+), 2747(-), 3783(+)	793(-)
	GGATA		1506(+), 2693(-), 3383(+), 3403(+), 3439(+), 3475(+)	877(+), 2030(-), 3116(+), 3427(+)
MYC-site	CATGTG/CACATG	MYC-binding, cold-, dehydration-response	2537(+,-), 2847(+,-)	3010(+)
MYC-consensus	CANNTG		162(+,-), 962(+,-), 1121(+,-), 1791(+,-), 1853(+,-), 2476(+,-), 2537(+,-), 2847(+,-)	788(+,-), 988(+,-), 1165(+,-), 1341(+,-), 1823(+,-), 1840(+,-), 2733(+,-), 2853(+,-), 3010(+,-), 3709(+,-), 3756(+,-)
PRE (HSP70)	SCGAYNRNNNNNNNNNNNNNNHD	Plastid response element, required for HSP70 induction		322(+), 1347(+), 2332(+), 3664(-)
SEBF (PR-10)	YTGTCWC	Silencing Element Binding Factor, found in PR-10 promoter		2827(+), 3838(+)
SRE	TTATCC	Sugar-repressive element	1506(-), 3383(-)	877(-), 3427(-)
SURE1	AATAGAAAA	Sucrose responsive	3774(-)	

SURE2	AATACTAAT		853(+)	
SURE-core	GAGAC		157(-), 220(-), 653(+), 2976(-), 3791(-)	2369(-), 3103(+), 3940(-)
SV40-core	GTGGWWHG	SV40-core enhancer	3014(+)	2760(-)
T/G-box	AACGTG	Jasmonate-responsive	1731(+)	3364(+)
TCA1-motif	TCATCTTCTT	Salicylic acid-responsive		3881(+)
UP1	GGCCCAWWW	Required for axillary bud outgrowth		2451(+)
W-box	TTTGACY	WRKY-binding, pathogen-responsive	1312(-), 1698(-), 3234(+)	143(+), 1872(+), 2014(-), 2951(-)
	TTGAC		100(-), 485(+), 754(+), 960(-), 1313(-), 1573(+), 1699(-), 2101(+), 2604(-), 2786(+), 3235(+), 3609(+)	127 (-), 144 (+), 1294 (+), 1873 (+), 2015 (-), 2589 (+), 2952 (-), 3484 (+), 3517 (-), 3591 (+)
	TGACT		345(+), 748(+), 847(-), 1312(-), 2787(+), 3191(-), 3610(+)	35 (-), 139 (+), 145 (+), 785 (-), 1295 (+), 1439 (-), 1555 (+), 1874 (+), 2014 (-), 2590 (+)
	CTGACY		2449(-), 3808(-)	1439(-)
	TGACY		99(-), 345(+), 748(+), 847(-), 959(-), 1312(-), 1698(-), 2449(-), 2787(+), 2928(-), 2951(-), 3191 (-), 3236 (+), 3610 (+), 3808(-)	35 (-), 126 (-), 139 (+), 145 (+), 785 (-), 1295 (+), 1439 (-), 1555 (+), 1835 (+),1874 (+), 2014 (-), 2590 (+), 2951 (-)
WRKY71-binding	TGAC	WRKY71-binding	100(-), 270(+), 345(+), 486(+), 748(+), 755(+), 848(-), 960(-), 1313(-), 1574(+), 1699(-), 1735(+), 2095(-), 2102(+), 2450(-), 2575(+), 2604(-), 2779(+), 2787(+), 2929(-), 2952(-), 3192(-), 3236(+), 3610(+), 3809(-)	36 (-), 127 (-), 139 (+), 145 (+), 786 (-), 1295 (+), 1415 (+), 1440 (-), 1555 (+), 1632 (+), 1835 (+), 1874 (+), 2015 (-), 2590 (+), 2594 (+), 2829 (-), 2869 (-), 2952 (-), 3485 (+), 3517 (-), 3592 (+), 3840 (-)
XYLAT	ACAAAGAA	Xylem-core	3656(-)	2148(+)

9. ACKNOWLEDGEMENTS

First and foremost, I would like to say thanks to my supervisor Dr. Erzsébet Kiss for assisting my studies in the Institute of Genetics and Biotechnology of Szent István University. I would like to thank her especially for her most valuable professional help, patience, and her countless useful suggestions during my PhD program and thereafter. I would also like to thank her for having trust in my competence, for working together for so many years and helping me achieve prominent and outstanding results.

I would like to say thanks to Dr. Laszlo Kovacs for proposing his idea to be the topic of my PhD study, providing the opportunity for me to perform my research in his lab, equipping me with numerous useful pieces advice and supporting me in the USA.

I would also like to thank the present and previous Head of the Plant Science PhD School Dr. Lajos Helyes and Dr. László Heszky, respectively, for allowing the execution of PhD studies and special thanks go to Dr. László Heszky for all the help he provided me with to solve the problems emerging during my PhD studies.

I would also like to thank Dr. László Varga, the Director of the Institute of Genetics and Biotechnology for supporting my research work and studies in the Institution. I also thank him for his support during the completion of my dissertation.

Special thanks to Dr. Sandor Fekete for helping me during the preparation of my dissertation.

Special thanks go to my co-workers, Dr. Patrick Winterhagen, Dr. Yingcai Su, Balázs Kalapos, Dr. Zoltán Szabó, Dr. Róbert Oláh, Edina Novák, Dániel Pap, Alyssa Higgins, and for all the colleagues in the Institute of Genetics and Biotechnology and at Biology Department of Missouri State University for helping me and suggesting solutions for the emerging problems during my research.

I would also like to thank my students, Solomon Abera, and Bianka Brassányi for their participation in the project.

I would like to thank Nakagawa Laboratory in Japan and the Institute of Plant Biology and Zürich-Basel Plant Science Centre of University of Zürich for providing the binary vectors.

I would like to thank Dr. Walter Gassman (University of Missouri, Columbia) for providing seeds of Wassilewszkija WT and mutant *(nim1-1, nahG)* lines.

Special thanks for the collaboration between Missouri State University and Szent Istvan University, which was orchestrated by Dr. Erzsébet Kiss, Dr. Laszlo Kovacs Dr. Frank Einhellig (Former Dean of MSU) and Michael T. Nietzel (Former President of MSU). Many thanks for the

cooperation, in the framework of which I could conduct my experiments and acquire the technical support required for my studies both at the Biology Department of MSU and the Institute of Genetics and Biotechnology of SZIE. I would also like to say thanks for the Missouri Life Science Trust Fund Award 13243-2007 provided by MSU and for the Grants of Hungarian Ministry of Human Capacities; NTP-EFÖ-P-15 ("Scholarship for the Nation's Young Excellences") and TÁMOP-4.2.2.B-10/1 project ("Development of a complex educational assistance system for talented students and prospective researchers at the Szent István University") to supporting me during my PhD studies.

I thank the funds for financially supporting the research; the USDA CSREES Federal Grant 2010-38901-20939, the Hungarian Scientific Research Fund (OTKA 77867) and the "Research Centre of Excellence"–9878-3/2016/FEKUT Grants.

I would like to extend my sincerest thanks and appreciation for the patient soul of my husband Mark Makovecz and my family during the PhD studies.