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

Theses of PhD dissertation

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1. INTRODUCTION

Powdery mildew (PM) – caused by *Erysiphe necator* Schw. [syn. *Uncinula necator* (Schw.)] Burr. – is one of the most notorious diseases affecting grapevine. Although, numerous studies presented remarkable results concerning the genetic and molecular background of the disease the pathogen still causes severe problems in vineyards. Although growers can control the spread of the fungus by the application of chemicals, the use of fungicides considerably increases the prime cost of production and the risk of potentially harmful impact on the environment. Therefore, studies focusing on the molecular background of plant defense mechanisms and striving to improve the resistance in susceptible varieties are exceptionally conducive. Results of these experiments support the ultimate goal of limiting the use 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 (Fung *et al.*, 2008). 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.

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. MATERIALS AND METHODS

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

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

One-year-old *V. vinifera* L. cv. 'Cabernet Sauvignon' grapevines were mock-inoculated or inoculated with *E. necator* conidia under greenhouse conditions (Figure 1). Three days post inoculation (dpi) all grapevines were transferred to a plant growth chamber. The plants for PM-induction were cultivated in the growth chamber for eight additional days until 11 dpi, when the PM-colonized leaves were harvested for RNA extraction. Plants for SA-induction were cultivated in the growth chamber for seven days, when they were treated with 15 µM of methyl salicylate (MeSA; SA analogue), evaporated in the atmosphere of the growth chamber for 24 hours. SA-induced mock-inoculated leaves were harvested after the 24-hour treatment was completed (11 dpi). Reference plants were also cultivated under identical growth chamber conditions; the mock-inoculated leaves were also harvested at 11 dpi. Three samples were collected for each treatment.

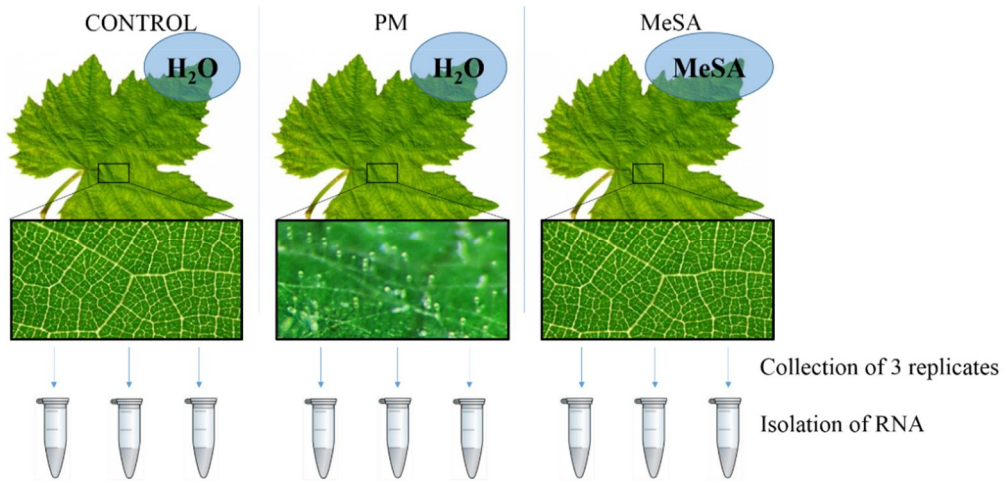

Figure 1. Sample preparation for microarray and qPCR analysis.

RNA isolation

Leaf tissues of the collected samples were ground in liquid nitrogen and homogenized in extraction buffer. After repeated centrifugation the RNA was precipitated and treated with DNase I. Furthermore, RNA was purified using an RNeasy MiniElute Cleanup system.

Microarray analysis

To analyze changes in gene expression patterns in response to PM colonization and SA, the Affymetrix GeneChip *V. vinifera* (Grape) Genome Array was carried out. RNA was used to synthesize double stranded cDNA, then the cDNA was used to produce biotin-labeled cRNA. The labeled cRNA was fragmented before hybridization to GeneChip probes. The prepared chip was then scanned by a GSC3000 laser scanner. The normalized intensity values, as well as raw GeneChip images have been deposited in the Gene Expression Omnibus database in GenBank (accession number: GSE53824). Raw data produced by Affimetrix *Vitis* GeneChip was statistically analyzed. The genes with at least 1.5-fold change in their expression rate compared to the control were selected for further analysis. The annotation of the selected probes was performed by blasting the Affymetrix GeneIDs to the EST database of NCBI GeneBank.

Reverse transcription and qPCR analysis of *NAC042_5*

The expression level of *NAC042_5* measured by microarray analysis was confirmed using the qPCR technique. Therefore, plant material and experimental procedures were applied as previously shown. RNA was also isolated and it was used for cDNA synthesis. Gene specific primers were designed for the grapevine target gene *NAC042_5* and for the reference gene *ACTIN 1*. Relative quantitation of the qPCR was calculated using statistical software. Statistical significance was determined by Student's T-test to compare the treatment-induced response to the control.

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

Promoter isolation of *NAC042_5* gene and construction of binary vector

The *NAC042_5* promoter region was isolated from *V. vinifera* L. cv. 'Cabernet Sauvignon' DNA using gene specific primers. The amplified DNA fragment was cloned into pGWB633 binary vector (Nakamura *et al.*, 2010) using the Gateway® technique. In the pGWB633 the *NAC042_5* promoter id fused to 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 multi-cloning site of the final product binary vector was sequenced and submitted into NCBI GenBank database (GenBank accession number of the promoter sequence: KU297673). The pGWB633 plasmid with the *NAC042_5* promoter construct was transferred into *Agrobacterium tumefaciens*.

The analysis of the promoter was implemented by deletions of the isolated *NAC042_5* promoter. The primer sets were designed to amplify increasingly

shorter fragments of the promoter. These fragments were also cloned into pGWB633 and transferred into *Agrobacterium.*

Transformation of *Arabidopsis thaliana* plants

The analysis of the *NAC042_5* promoter regulation was performed in three different *A. thaliana* lines: a wild type (WT), a salicylic acid signaling deficient (*nim1-1*), and a salicylic acid hydrolase gene containing transgenic (*nahG*) line. The plant transformation was performed using the flower dip method. During this process the flowers of the *Arabidopsis* plants were dipped into the binary vector containing *Agrobacterium* suspension. The seeds developed from the dipped flowers were germinated in soil. Ten days after sowing the seedlings were sprayed with glufosinate-ammonium containing herbicide (Nakamura *et al.*, 2010). The surviving plants were selected and transplanted for further cultivation. The genetically proven transformants were selected to produce the T_2 generation and the transgenic plants were grown till T_3 generation. Those 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*).

Testing the transgenic plants

At least two 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 grown without any environmental challenge. For PM-induced expression the transgenic plants were mock-inoculated or inoculated with *Oidium neolycopersici* conidia under growth chamber conditions.

Spectrophotometric assay

Fourteen days post inoculation six individuals were collected from each line of the PM-inoculated and mock-treated plants. The leaf tissues were ground in extraction buffer and were incubated after addition of 4-nitrophenyl β-D-glucuronide (*p*NPG) substrate (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 wave length 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, and analyzed them using a mixed linear model implemented by the software package SAS.

Histochemical GUS assay

Eleven days post inoculation the plants were investigated by histochemical GUS assay (Jefferson *et al.*, 1987). The leaves were incubated overnight in the assay

solution with X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, cyclohexylammonium salt) substrate. After the staining procedure the chlorophyll content was removed by repeatedly washing the samples with 70% ethanol. In order to stain fungal tissue leaves were dipped into cotton blue solution, rinsed with distilled water, and subsequently investigated using a stereo- and light-microscope.

Bioinformatical analysis of *NAC042_5* promoter sequence

The promoter sequence 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.

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

To test the regulation of the closest ortholog of *Vitis NAC042_5* in *A. thaliana* the promoter of *ANAC042/JUB1* was also isolated using gene specific primers. This fragment was also cloned into the pGWB633 binary vector, and transferred into *Agrobacterium*. This *Agrobacterium* strain was then used for transforming 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. The putative *cis*-regulatory elements were also determined within the sequence.

4. RESULTS AND DISCUSSION

Global expression analysis

In order to distinguish host transcriptome changes triggered exclusively by SA from those induced 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 the 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 the healthy reference leaves. In the second experiment we assayed MeSA-treated grapevine leaves in comparison with the control leaves. We found that 481 genes responded to the MeSA treatment with at least a 1.5 fold-change in their rate of expression and 179 of them constituted a subset of the PM-regulated genes. This suggests that a subset of PM-responsive genes may be regulated via SA signaling.

Genes induced by both MeSA treatment and PM colonization

Among the 179 transcripts that responded to PM and to MeSA inductions in a similar way, we found defense-associated genes that typically function via SA-mediated signaling also shown in other plant species. The group involved genes of receptor-like protein kinases, *EDS1*, *NIMIN1*, *WRKY*, pathogenesis-related (*PR*) proteins, ATP binding cassette transporters, genes of redox regulation, and genes implicated in the regulation of jasmonate, lignin or flavonoid biosynthesis.

Genes induced by PM colonization rather than by SA treatment

Among the PM-regulated genes in grapevine 185 candidates were identified and shown not to be triggered solely by MeSA indicating that elevated SA levels alone were not able to substitute for the regulation by PM. These include numerous genes that are involved in primary metabolism, including the pathways of carbohydrate, protein and fatty acid metabolism. Additionally, in this group we identified all of the photosynthetic genes, *AQUAPORIN TIP1_3*, *PR-10*, genes encoding stilbene synthases, cytochrome P450s, dirigent-like proteins and lipid transfer proteins. These genes are probably independent of SA and likely regulated by other signals.

Quantitative PCR

In the 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 means of microarray technique. The significant fold change was approximately the same measured with the two different techniques.

Analysis of *NAC042_5* regulation

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 similar basal *gusA* expression levels under normal growth conditions. The regulation of *NAC042_5* did not appear to be tissue specific. The promoter activities in transgenic plants were detected in a range of tissues and organs including shoot apical meristem (SAM), young developing shoots and leaves, trichomes, vascular tissues of the leaves, stems, roots, petals and gynoecium, the tips of cotyledon and young leaves in stipules, hydathodes, lateral shoot buds, connective tissue of the anther, style, transmitting tract within the carpel and abscission zone of gynoecium (Figure 2). Promoter activity in this broad variety of organs could be explained by a more general transcriptional regulatory function and suggests that this gene might play a role in organ development.

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) and the synthetic auxin reporter construct *DR5*::*gus* (Ulmasov *et al.*, 1997). The similar expression patterns suggested that *NAC042_5* may be involved in auxin-signaling and may regulate plant development. The unpublished results corroborate this hypothesis, showing that transgenic grape plants overexpressing the *NAC042_5* stayed dwarf and died after a certain period of time, while the plants, in which the *NAC42_5* was silenced grew normally as the WT ones.

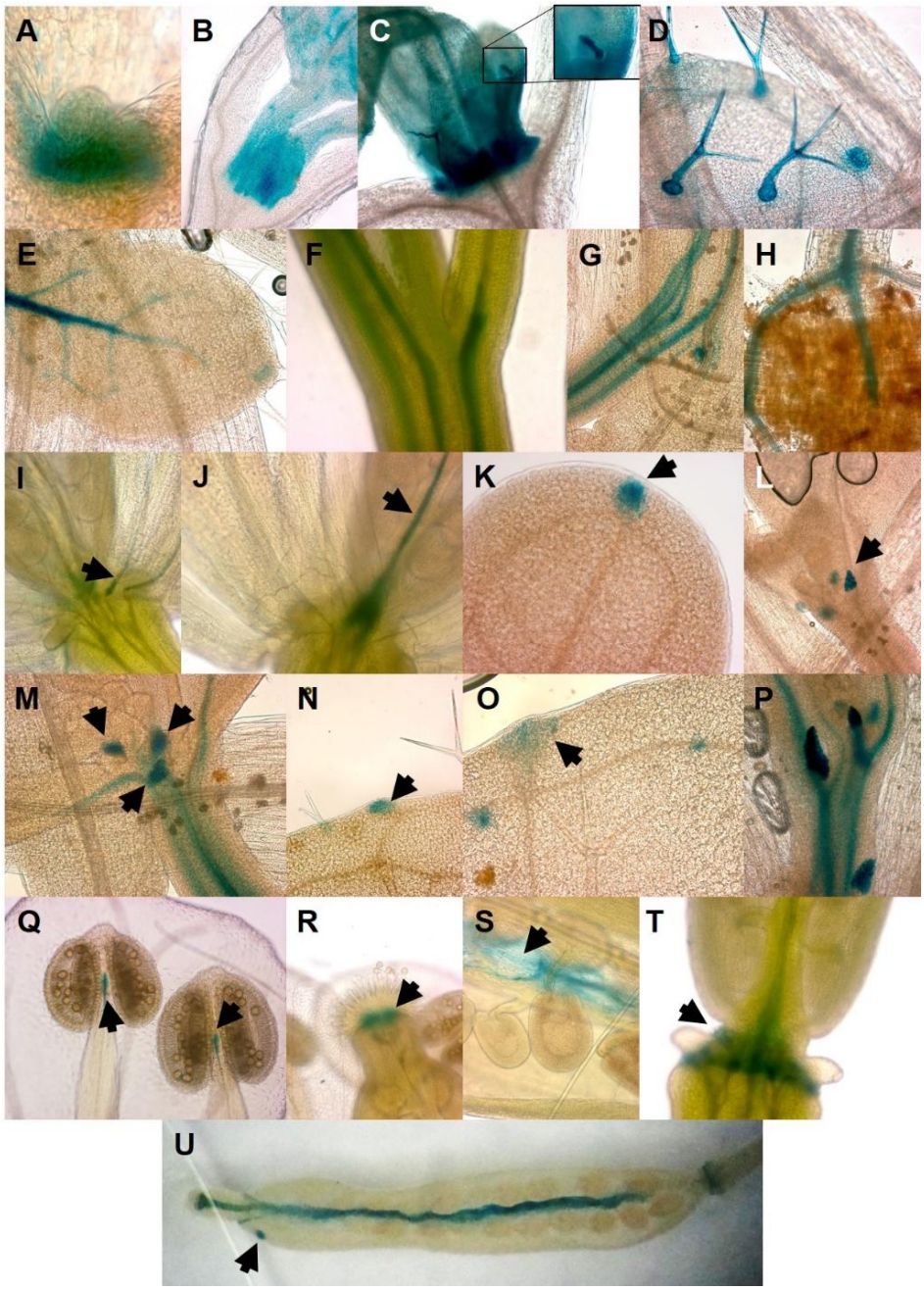

Figure 2. Localization of gusA *expression regulated by the* NAC042_5 *promoter in transgenic* Arabidopsis *plants. (A) SAM, (B) young shoots, (C) developing new leaves (inset: developing trichome) (D) trichomes, (E) vascular tissue (vt) of leaf, (F, G) vt of shoot, (H) vt of root, (I) vt of petals, (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.*

Analysis of PM-induced expression in transgenic plants

After 14 dpi the inoculation has led to fully developed conidium-producing PM colonies in all lines and all mock-treated plants remained PM-free. 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 are significant ($p<0.0001$) and the significance of the PM-infection occurred at 30 min incubation time. Values of plants from the independent lines for each type of transgenics with a similar basal expression are displayed (Figure 3). 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.

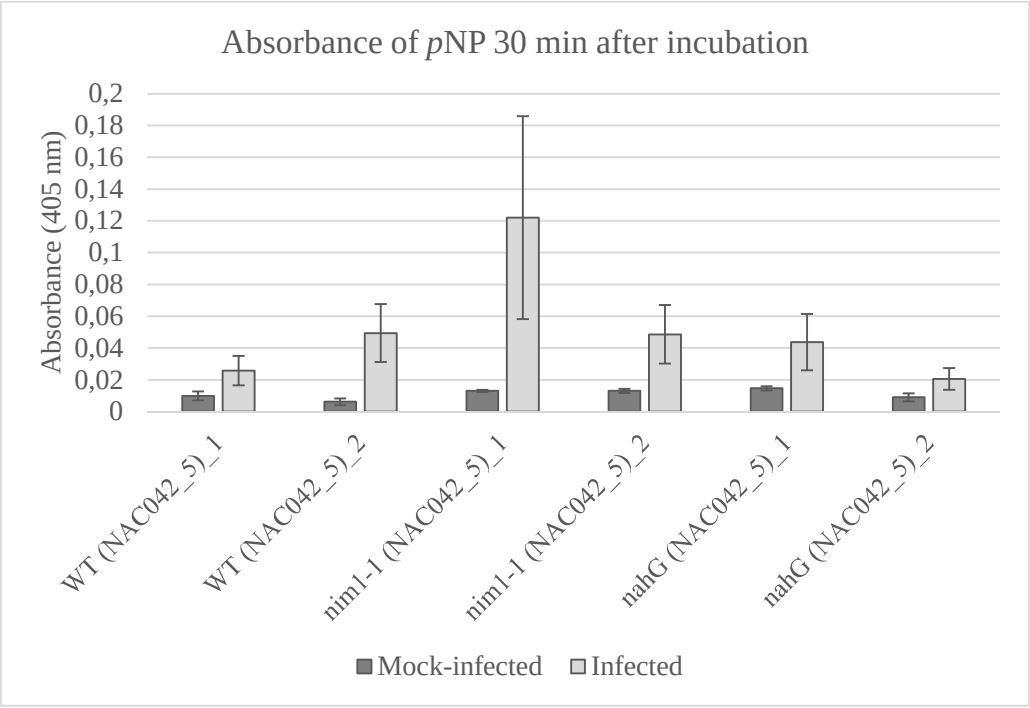

Figure 3. Response of the NAC042_5 *promoter to PM infection in transgenic* Arabidopsis*. GUS quantification based on* pNAC042_5::gusA *activity due to 14 day-old PM colonies on leaves of transgenic* Arabidopsis *with the genetic backgrounds: WT,* nim1-1, *and* nahG *(the columns represent independent lines; each of them with at least 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 4A). In mock-inoculated control leaves GUS-staining was mostly limited to trichomes (Figure 4B). 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 of fungal structures (Figure 4C, D and E) and never occurred in their absence. On mock-inoculated leaves only few confined GUS spots were visible but this was clearly distinguishable from the robust GUS-staining detected at fungal infection sites (Figure 4A, 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 4C, D and E). Higher magnification revealed that *gusA* expression substantially increased mostly in cells, in which the fungus developed haustoria (Figure 4D and E). This PM-dependent increase in *gusA* activity was found in all three types of transgenic plants (WT, *nim1-1*, and *nahG*), which provides further evidence that *NAC042_5* expression does not require SA signaling.

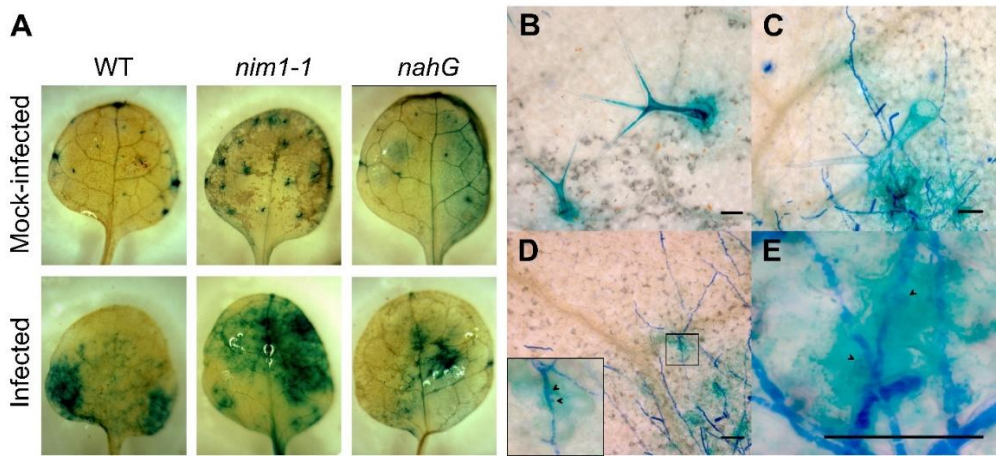

Figure 4. Histochemical staining of pNAC042_5::gusA-*transgenic plants after* O. neolycopersici *inoculation. (A) GUS staining of mock-inoculated control leaves and* O. neolycopersici-*infected leaves (2. rosette leaf) at 11 dpi, (B) microscopic image of mock-inoculated leaf, (C, D and E) microscopic 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 haustorial neck. Scale bars indicate 50 µm.*

If the transcript of *NAC042_5* mediates activation of *PLL* genes, the up-regulation of *NAC042_5* at the site of infection is likely to benefit the pathogen infection. The *PMR6* gene encoding a pectate lyase-like protein was up-regulated at the site of infection and the *pmr6* mutation resulted in an increased resistance against PM (Chandran *et al.*, 2009; 2010). The overexpression of *SND1* (a *NAC* gene) induced expression of a pectate lyase gene and additionally produced stunted phenotype for the transgenic plant (Ko *et al.*, 2007; Hussey *et al.*, 2011), as it was found in the *NAC042_5* overexpressing lines (unpublished data).

Deletional analysis of *NAC042_5* promoter in transgenic plants

In order to test the transcriptional regulation of *NAC042_5* we isolated increasingly shorter fragments of the promoter and fused each one to the *gusA* reporter gene. The *gusA* expression patterns in the various organs were found to be similar in all lines as described earlier for the 3896-bp-long promoter (Figure 2) in sharp contrast to the lines containing a 257-bp-long fragment or the null-promoter controls. After the inoculation of plants we measured the induction levels of *gusA* and found a significant increase in lines containing 3896-bp, 2935-bp, 2456-bp, and 1178-bp-long promoters (p-value < 0.05) in response to PM infection compared to the mock-treatment. No induction was detected in 257-bp or the null promoter controls (Figure 5).

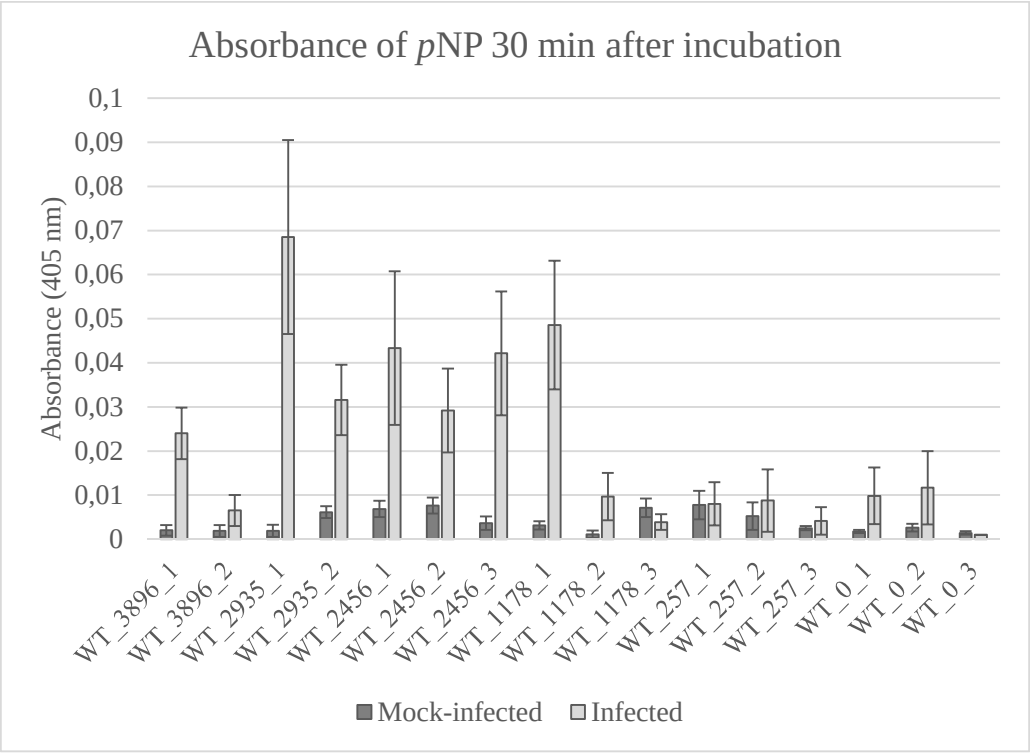

Figure 5. Response of the NAC042_5 *deletional promoters to PM infection in transgenic* Arabidopsis*. GUS quantification based on* pNAC042_5::gusA *activity due to 14 day-old PM colonies on leaves of transgenic* Arabidopsis *with the genetic background of WT (the columns represent independent lines; each of them with at least three biological repeats; bars represent the standard error).*

Bioinformatical analysis of *NAC042_5* sequence

Analyzing the promoter sequence of *NAC042_5* we detected ABA responsive elements, such as ABRE-elements, G-boxes using the *cis*-element databases.

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 also determined growth regulator inducible sites, such as auxin and gibberellin-stimulated elements (auxin: ARF- and BBF1-binding; gibberellin: GARE), which may regulate *NAC042_5* expression during plant development.

Analysis of *ANAC042/JUB1* regulation in transgenic plants

Histochemical staining of non-infected transgenic leaves demonstrated that all three types of plants (WT, *nim1-1* and *nahG*) expressed the *gusA* gene at a basal level similarly to the one regulated by *NAC042_5* promoter. The specific up-regulation was also observed in the stipules, and in the transmitting tract of gynoecium style tissue. However, β-glucuronidase activities were 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. In comparison to the grape *NAC042_5* gene, the *Arabidopsis* ortholog was not expressed specifically in leaf hairs. The differences detected in the regulation of the two promoter may be explained by the lack of CARE (gibberellin (GA)-responsive), CBF-binding (cold-responsive), CPB (cytokinin enhanced protein-binding), DPBF-binding (abscisic acid (ABA)-responsive), TCA1 (SA-responsive) and CGCG-box (calmodulin-binding) elements in the promoter of grape *NAC042_5*. The histochemical staining of *O. neolycopresici*-inoculated 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. The uninfected areas displayed basal expression levels of the *gusA* and were clearly distinguishable from the spots in which induction occurred. The basal and PM-induced expression detected in all three types of transgenic plants (*WT-pJUB1::gusA*, *nim1-1-pJUB1::gusA* and *nahG-pJUB1::gusA*) suggested that SA was probably neither required for basal regulation, nor for induction of *JUB1* by *O. neolycopersici*. Sequence analysis of *JUB1* promoter region revealed the presence of additional hormone- (ethylene/jasmonic acid, SA, GA, ABA, auxin and cytokinin), and elicitor-responsive elements, MYB and WRKY transcription factor-, and calmodulin-binding sites, which might stimulate expression of *JUB1* in response to environmental challenges.

Novel scientific results

I found the following novel scientific results during my research:

T1: 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.

T2: 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*.

T3: The microarray measured gene expression pattern of *NAC transcription factor 42* (*NAC042_5)* was confirmed by quantitative PCR method.

T4: 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.

T5: 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.

T6: 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.

T7: 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.

5. 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.

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