Transcriptome and DNA methylome studies in *Arabidopsis* during TuMV infection

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Abbreviations

A – Adenine

ABA – Abscisic acid

BR – Brassinosteroids

C – Cytosine

CK – Cytokinin

dai – days after infection

DEG – Differential expressed gene

DNA – Deoxyribonucleic acid

DMR – Differential methylated region

ET – Ethylene

ETI – Effector triggered immunity

G – Guanine

GA – Gibberellic Acid

GFP – Green fluorescent protein

GO – Gene Ontology

HR – hypersensitive reaction

JA – Jasmonic acid

KEGG - Kyoto Encyclopedia of Genes and Genomes

lnRNA – long non-coding RNA

Log2FC – Fold change as the logarithm to the base 2

mRNA – messenger RNA

PTI – PAMP triggered immunity

PAMP – pathogen-associated molecular patterns

PR – pathogenesis-related

qPCR – quantitative polymerase chain reaction

R – resistance

RC – Read count

RNA – Ribonucleic acid

ROS- reactive oxygen species

SA – Salicylic acid

SAR – systemic acquired resistance

siRNA – small interfering RNA

T – Thymine

TE – Transposable Elements

TF – Transcription factor

TuMV – Turnip Mosaic Virus

UV - Ultraviolet

WGBS – Whole Genome Bisulfite Sequencing

1 Introduction

Studying and understanding the health of plants has become one of the major challenges in agriculture throughout the last decades. The sessile lifestyle humans developed thousands of years ago has lead to a dependance on cultivated plants. The need for healthy plants to produce the acquired amount of harvest was determining the difference between life and death of human populations. Abiotic stresses like drought, flood or heat had been known to cause loss of harvests resulting in mass starvations and famines in the history of humans. In the last centuries humans discovered and learned more and more about biotic stresses such as bacteria and viruses. Not only can they be life-threatening to the human organism, but they also as endanger our livestock and crops. Scientists are studying these topics for centuries now and are able to understand more and more of the complex details. Viruses, in comparison to other biotic or abiotic stresses, have long been somewhat of a mystery to researchers. Especially plant viruses, although one of the first biotic stresses to be detected, are still one of the least researched. One of the reasons for this can be found in the virus itself. Viruses have a very simple structure combined with very effective, long evolved mechanisms of infection. Counter-measurements by the host are difficult as the simplicity of the viral structure does not leave much room for weak points. All of this combined leads to an ever-evolving battle between host and pathogen, described in the hypothesis of "Red Queens' race". Understanding more about virus-host interactions is crucial not only for researchers. It also effects humankind in a world where the number of earth inhabitants grows every day, resources becoming more and more precious and climate change tipping the scales against us. A profound background knowledge of infections would give us an advantage in the "Red Queens' race". It would allow for quick counter-measurements to keep plants and crops healthy while exposed to increasingly harsh biotic and abiotic stresses.

This study focused on shedding more light on virus-plant interactions down to the molecular level. The infection was studied on the example of *Arabidopsis thaliana* plants (a prominent model organisms closely related to many crop species) infected with the Turnip Mosaic virus (part of the potyvirus family which is causing much of the damage in crop species). The transcriptome and DNA methylome of infected plants as well as their next generation was investigated in next-generation-sequencing experiments. A small side project analysed certain phenotypic changes after virus infection.

1.1 The Turnip Mosaic Virus – an introduction

The Turnip Mosaic Virus (from here on TuMV), first discovered in the host *Brassica rapa* in 1921 (Gardner, 1921) is one of the oldest known plant viruses. It originated around a millennium ago from European orchids and researchers see a correlation between virus emergence and beginning expansion of agriculture, thus making the research of its origin an important topic for prevention of virus spread in modern agriculture (Nguyen *et al.*, 2013; Yasaka *et al.*, 2017). Currently it has a world wide distribution and is one of the most important plant viruses in agriculture as it infects many field-grown vegetables, e.g. *Brassica* crops (Tomlinson, 1987). TuMV belongs to the potyvirus family, in which it has the widest range of hosts, including 318 species in 156 genera from 43 families (Nellist *et al.*, 2022). As it easily infects *Arabidopsis thaliana,* the interaction of TuMV and *A. thaliana* has become a strongly studied topic. Symptoms of TuMV disease are various and depend on the host organism, virus strain and abiotic factors. It can include necrosis, plant stunting, plant death and the mosaic patterns from which the virus got its name (Nellist *et al.*, 2022). Two of the most important strains UK1 and JPN1, result in different plant infection phenotypes (Sanchez *et al.*, 2015), in this thesis the strain UK1 was used. Infection of plant hosts happens mostly via aphids, so far 89 species of aphids showed function as TuMV vectors and infection is fast and robust (Edwardson & Christie, 1991).

Recently researchers (Nellist *et al.*, 2022) summed up very nicely some general information about this virus: The Turnip Mosaic virus is a positive, single stranded RNA virus (+ssRNA) with a genome of approx. 9,833 nucleotides (UK1 isolate). Attached to the 5'end is a single genome-linked viral protein (VPg), on the 3' end a polyA tail of various lengths. The genome can be translated into a polyprotein, which will be cut further into the following viral proteins (see Figure 1): The protein 1 (P1), a helper component proteinase protein (HC-Pro), the protein 3 (P3), the 6 kDa1 protein (6K1) the cylindrical inclusion protein (CI), the 6 kDa 2 protein (6K2), the genome-linked viral protein (VPg), a nuclear inclusion a-proteinase protein (NIa-Pro), the nuclear inclusion b protein (Nib) and finally the coat protein (CP). A single viral genome is enclosed in a virion, a non-membranous structure, made of more than 2,000 copies of the coat protein (see Figure 2).

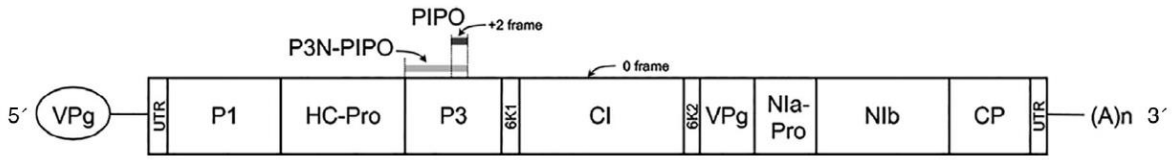


Figure 1 TuMV Genome *Schematic overview of genes encoded in the TuMV genome (Nellist et al., 2022)*

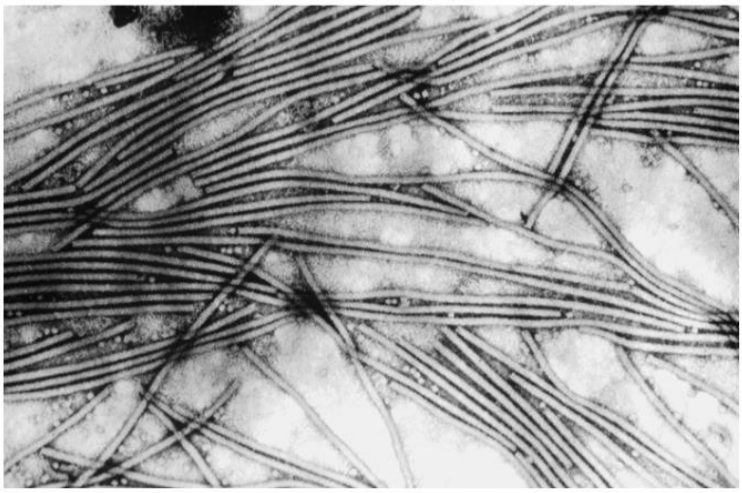


Figure 2 TuMV virions *Picture of TuMV virions in an electron micrograph (Walsh, 2002)*

Once inside the plant cell the Turnip Mosaic Virus is able to move intra- and intercellular using phloem and xylem (Nellist *et al.*, 2022). This happens in form of ribonucleic protein complexes or virions but the exact mechanics are still unclear (Wan *et al.*, 2015).

The TuMV is able to spread to the next generation through seeds; however researchers found that this is dependent on virus isolate and host and chances in *Arabidopsis* plants with the UK1 isolate are slim (Nellist *et al.*, 2022). Still, transgenerational effects of pathogen infections (including virus infection) have been described with DNA methylation being one of the proposed mechanisms (Boyko & Kovalchuk, 2011; Boyko, 2010).

1.2 General defence mechanisms after virus infection in plants

The history between viral pathogens and plants has been long and multispectral as each pathogen-host combination can have different models of interaction. Especially changes in plant phenotype resulting in crop loss are a large problem in agriculture. This drives the interest of understanding the terms of infection. To this day, around 450 plant-pathogenic viruses have been found (Soosaar *et al.*, 2005). The situation becomes more severe as climate change forces even more stress on plants which tips the scale in the fight between plants and pathogens.

During centuries of co-evolution, plants have developed several resistance mechanisms against viruses. Some of them will be presented in the following, a more thorough overview can be seen in Figure 3. One main mechanisms is the innate immunity. This starts already on the outside of plant cells, in the form of pattern recognition receptors (PRRs). Here, mainly two classes of receptors are active: plasma membrane localized receptor kinases (RKs) and receptor-like proteins (RLPs). They can identify pathogen-associated molecular patterns (PAMPS), resulting in PAMP-triggered immunity (PTI). Although this is mostly known for pathogen types like fungi or bacteria (Boller, 1995; Franco-Orozco *et al.*, 2017), new studies suggest that it might also play a role during virus infection (Wu *et al.*, 2019).

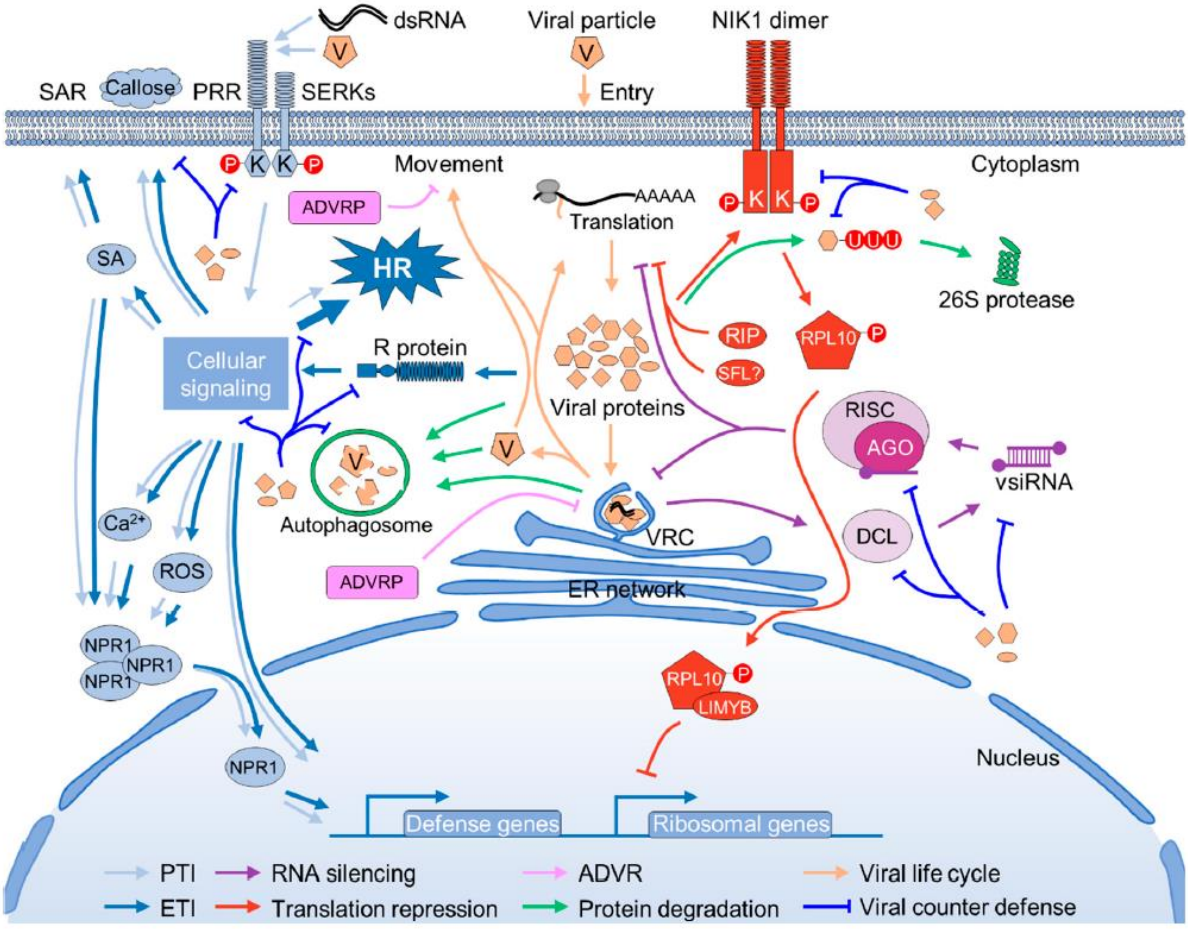


Figure 3 Overview of mechanisms during virus infection *Host and virus mechanisms throughout the cell during virus infection are depicted, important reactions such as PTI and ETI are marked with colored arrows (see legend in the bottom of the picture) (Wu et al., 2019)*

To enforce infection, pathogens have developed so-called effector proteins, undermining the plants PTI reaction. This was countered again by the evolution of resistance (R) genes of the host, leading to the effector-triggered immunity (ETI). During ETI, plants monitor the presence of pathogen effector proteins with intracellular receptors, the R genes. Effectors and R genes

are very specific for each pathogen and host; for viral infection several R genes and their corresponding effector, also called AVR (avirulence factor), have been studied (Fraile, 2010). The pathogen-specific, host-expressed R genes act mostly via two distinct outcomes. They can induce a hypersensitive reaction (HR) in the organism, leading to cell death in many cases. The other outcome is the systemic acquired resistance (SAR), promoting response reactions in cells distant from the infection center to achieve immunity (Soosaar *et al.*, 2005). SAR is a key mechanism during defence and can be induced by ETI and PTI (Fu & Dong, 2013). The main inducer of SAR is the phytohormone salicylic acid (SA) (Gao *et al.*, 2015).

1.3 Overview of phytohormones in stress signaling during virus infection in plants

Phytohormones (like salicylic acid) play a key role in plant signaling, development and more, thus they are prominent factors in plant defence. The interplay between phytohormones are numerous during stresses such as virus infection. Although there is still a lot of research needed to understand the crosstalk, some hints to the functions of hormones during virus infection have been discovered. A good review about this was made by the working group around Zhao (Zhao & Li, 2021) and can be seen in Figure 4. In the following some of the main important relations, as mentioned in their paper, will be presented.

The phenolic compound salicylic acid (SA) induces the hypersensitive reaction (HR) as well as local and systemic-acquired resistance (SAR) (Gao *et al.*, 2015). Its expression can induce pathogenesis-related (PR) genes, reactive oxygen species (ROS) and callose deposition; it was shown that SA mainly affects the viruses intercellular trafficking, its long-distance movement and virus replication (Zhao & Li, 2021). SA and JA have a prominent antagonisms to each other while regulating defence gene expression. The relations of jasmonic acid (JA) and viral infection is highly ambiguous, as some studies point into a positive effect in defence, some have discovered infection promoting properties. JA and SA both have complex signaling pathways and their crosstalk is very important for plant defence. SA-mediated defence is mostly activated for biotrophic pathogens and JA-mediated defence after necrotrophic pathogens. The role of the phytohormone Abscisic Acid (ABA) in virus infection is still in discussion, as some studies showed a regulation after infection, others reported no changes. However, as ABA can regulate callose production, which in turn can vary PD conductivity and thus virus spread, a role in virus defence is possible and is being investigated (Zhao & Li, 2021). The gas molecule ethylene (ET)

has functions in senescence and necrotrophic pathogen defence. It was found to be induced by virus infection and has a proposed role in defence showing restricting and promoting effects like the previously mentioned JA. ABA can, in high concentrations, inhibit ET production and researchers proposed an antagonistic behavior between these two phytohormones, but there is still little known about ABA/ET crosstalk. For ET/JA crosstalk, it is known that they regulate together induced systemic resistance (ISR). ET is needed for SAR establishment and might induce in the following SA and SAR. Virus infection can also impact the phytohormones Auxin and Gibberellic Acid (GA), which mostly results in phenotypic changes in infected plants, as both hormones are controlling, amongst other areas, plant growth and development. Studies show a repression and a promotion of auxin pathway genes and a general repression of GA by suppressing GA biosynthesis and inducing GA inactivation (Pan *et al.*, 2021).

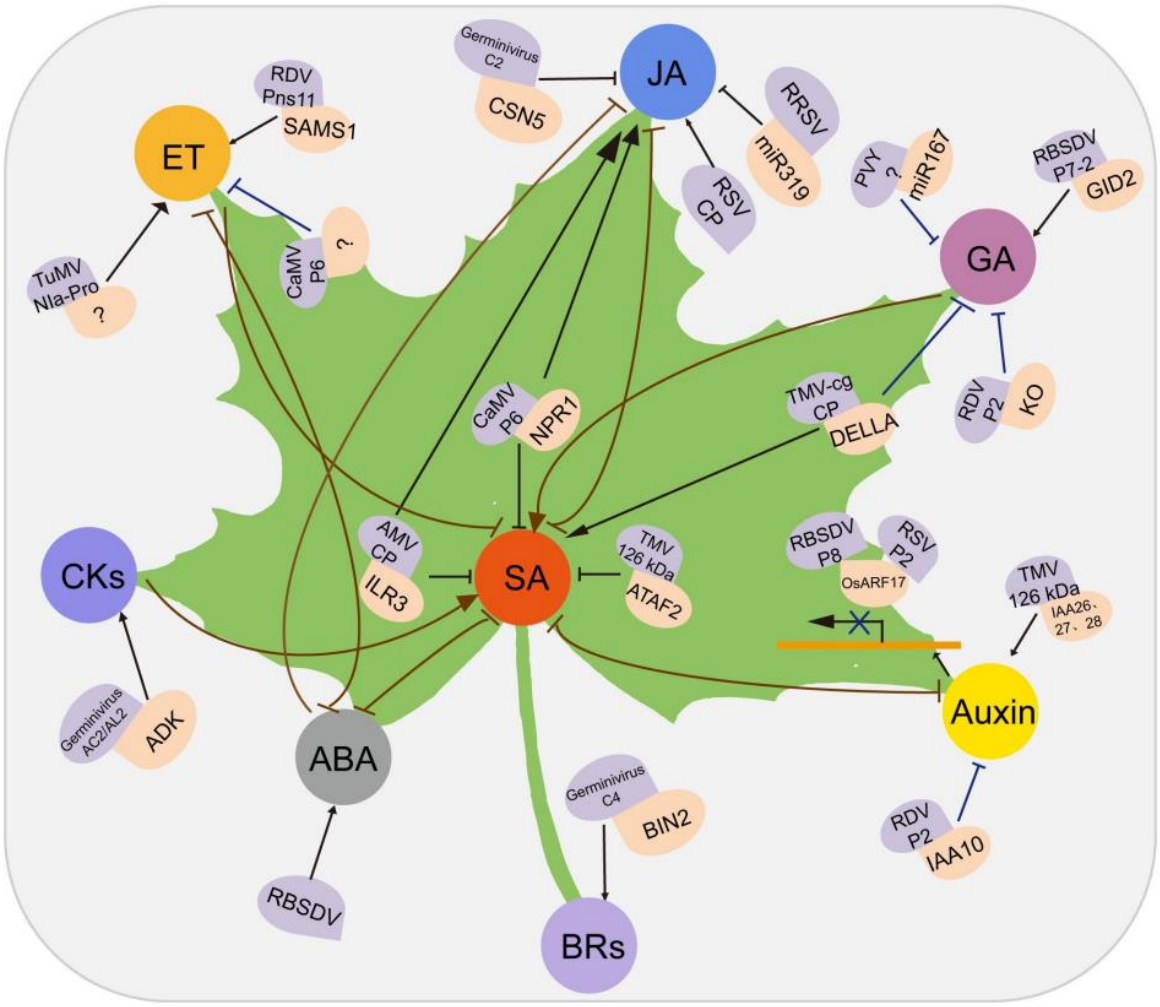


Figure 4 Interplay of phytohormones during virus infection *The graphic depicts the communication of important phytohormones, related genes are included (Zhao & Li, 2021)*

Furthermore, preliminary research was done on the effect of virus infection on the long-range and local signal cytokinin (CK). It was found, that virus infection could decrease active CKs and induce CK-mediated genes, however, there are still a lot of unanswered question (Zhao & Li, 2021). Lastly, the plant steroid hormones Brassinosteroids (BRs) should be mentioned, as they have the ability to promote resistance in plants (Nakashita, 2003).

1.4 DNA methylation as an epigenetic marker

Cytosine methylation, also called DNA methylation, is a flexible and strong mechanism of a plant to control its genome. This 5-methylcytosine (m^5C) was found by researchers throughout most organisms: plants, vertebrates, fungi, invertebrates and some bacteria (Goll & Bestor, 2005).

Methylated cytosines appear in three contexts in the genetic code: CG, CHG or CHH with the H representing either a G, T or A. The frequency of the contexts varies between groups of organisms; for example between vertebrates and plants. In vertebrates methylated cytosine is most commonly found in the CG context (also called CpG dinucleotide); CGs tend to cluster as CpG island, however then mostly unmethylated (Tajima, 1998). Researchers have indications that in humans approximately 70% of promoter regions co-align with CpG islands (Saxonov, 2006). In plants DNA methylation is present in all three contexts: CG, CHG and CHH (Niederhuth & Schmitz, 2017).

DNA methylation is part of a mechanism to regulate access to the genetic information while maintaining genome stability. A eukaryotic genome is stored highly condensed in the nucleus. The DNA strands are tightly wrapped around histone proteins to allow fitting into the relatively small nucleus. Plasticity of this packaging can be achieved via histone modifications. Methylation, acetylation and ubiquitination of the plant histone protein tails play a regulatory role in the expression of genes. For example the methylation set at a specific amino acid, like the lysine in position 27 of the histone 3 (also called H2K27), allows for repression of certain genes (Ramirez-Prado *et al.*, 2018). The more condensed the DNA is packed, the less accessible genes are for the transcriptional machinery, and vie versa. Another layer in this fine-tuning of accessible genes is added by changing the DNA methylation status.

De novo DNA methylation can be established in plants via the RNA-directed DNA methylation pathway (RdDM) including siRNAs, scaffold-RNAs and proteins like RNA-dependent polymerases, dicer-like proteins, argonautes and other proteins (Zhang *et al.*, 2018). Changes in

methylation status are realized by different proteins and siRNAs and researchers have determined in mammals and plants an impressive overview of responsible effector proteins and tools for targeted (de-)methylation via siRNAs and programmable DNA-binding proteins (Gallego-Bartolome, 2020).

DNA methylation is inheritable in plants. This is in contrast to other organisms, epigenetic inheritance is very uncommon in mammals for example partly because epigenetic information is erased during germline reprogramming (Calarco *et al.*, 2012). Plants were found to maintain CG and CHG methylation in the germline and also have a mechanism to restore CHH methylation (Calarco *et al.*, 2012). A transgenerational epigenetic memory is discussed among researchers and might be a combination between DNA methylation and Histone modifications (Miryeganeh & Saze, 2019).

There are mainly two roles of DNA methylation. The first function is fine-tuning gene expression. The methylation of cytosines at various genome sites, like promoters, introns, exons and also non-coding areas can influence the outcome of transcription efficiency. Not only the loci, but also the context of DNA methylation is having an effect on the effect on gene expression (Niederhuth & Schmitz, 2017). Especially methylation in the promoter region is discussed to influence the binding of transcription activators or repressors which would normally regulate transcription. Researchers assume that DNA methylation in promoter regions would generally inhibit gene transcription; however the exact mechanisms and the counterpart of DNA methylation inducing gene expression are unfortunately still largely unclear (Zhang *et al.*, 2018). A second function is the protection of the genome from the actions of repetitive elements like transposable elements (TEs). TEs are highly mobile and can interfere with the stability of the genome, thus the primary mechanism is the inhibition via DNA methylation (Goll & Bestor, 2005).

In plants, DNA methylation is involved in various areas: Imprinting and seed development, vegetative growth and pattern formation and fruit ripening (Zhang *et al.*, 2018). Researchers also found that environmental stimuli like abiotic and biotic stress can influence the DNA methylation pattern in plants (Dowen *et al.*, 2012; Santos *et al.*, 2017). DNA methylation furthermore plays a major role in plant immunity in various ways (Deleris *et al.*, 2016). It is additionally discussed to have a major role in transgenerational effects of pathogen infection (including virus infection) (Boyko & Kovalchuk, 2011).

1.7 Bioinformatic – an overview of RNA-Seq and WGBS

The field of bioinformatics gained greatly from the rapid improvements of computers. Sequencing of whole genomes and transcriptomes is nowadays fast and cost-efficient. Transcriptome analysis is a very commonly used tool in various areas of biology, a whole-genome-bisulfite-sequencing (WGBS), is much less common. The sequencing and analysis of RNA (further on called RNA-Seq) and WGBS can be subdivided into different steps which are presented shortly in the following.

1.7.1 RNA-Seq

A very nice and detailed review of RNA-sequencing was given by Kukurba and Montogomery in 2015 (Kukurba & Montgomery, 2015). The most important steps (loosely following their review) will be explained in this chapter, for more valuable information please refer to the publication. RNA has to be prepared in several steps before sequencing and a bioinformatical analysis can occur. The type of RNA to be studied determines the procedure. Harvested total RNA (which is used in many studies) consist to a large proportion of ribosomal RNA (Kukurba & Montgomery, 2015). For mRNA (which will be the example further on) a polyA-enrichment method is needed. Poly-d(T) primer are complementing only mRNA due to its polyA-tail. Complementary DNA (cDNA) is gained in a reverse transcription PCR (polymerase-chain-reaction). For other types of RNA other methods are chosen, for example random primers for long-noncoding RNA (lncRNA). Next, the DNA derived from RNA is used to build libraries for sequencing. Please find an overview of the next steps in Figure 5. The DNA is fragmented to allow for library strings of the same length. Sequencing primers and unique adapters are cloned on the ends of each DNA strand.

The next step after library preparation is the clustering and sequencing of the libraries. For clustering, a cell coated with adapters is used – the adapter sequence of the single DNA library string binds to the unique adapter on the flow cell. The single string is amplified in various Bridge PCR steps and clusters are created for each unique library. Afterward sequencing proceeds with primers binding to the primer regions inside the library strings (between adapters and insert DNA). Via an enzymatic reaction the single DNA strand is complemented nucleotide by nucleotide. A specific light burst originating from the engineered, fluorescent nucleotides determines the type of nucleotide implemented in each step. The detection allows for the creation of virtual sequences, the so called reads. Different read lengths are possible for

different projects (determined by the fragmentation step), one standard with mRNA is 150 base pairs (bp). Depending on the number of primers that were added during library creation, the sequencing can produce single-end (SE) reads (only sequenced into the read from one side) or paired-end (PE) reads (sequencing is done from both ends into the inserted DNA). PE reads are a standard for mRNA projects and contain more information than SE reads. Depending on the size chosen for fragmentation, an overlap of both reads can be achieved and the sequencing is more thorough.

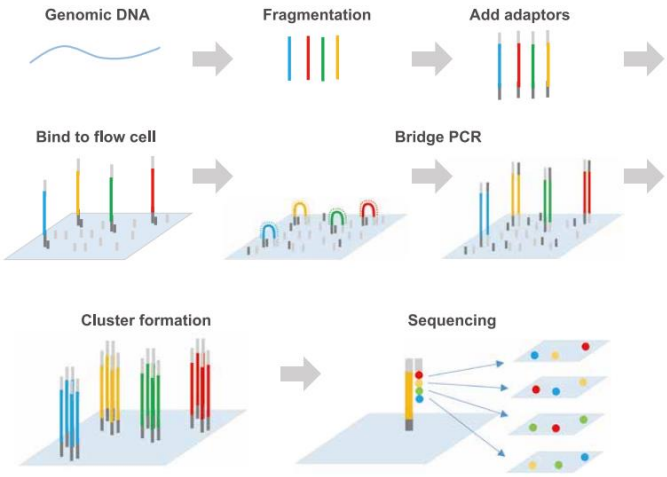


Figure 5 Overview of Library creation and sequencing *The process of library creation starts with the fragmentation of genomic DNA (or cDNA derived from RNA) and the addition of adapters (and primers sequences). The adapters can bind to complementary DNA fragments on a flow cell. Clusters are formed via a Bridge PCR. By using fluorescent nucleotides in the sequencing step the exact arrangement of the DNA can be detected (GenScript, 2002-2022)*

The production of reads via sequencing is followed by a step-by-step bioinformatic analysis. First a quality control (QC) of the produced reads is made. This includes several steps for example everything that is not the desired sequence (f.e. used primers) is trimmed. Typically information like number of reads, number of clean bases, error rates and GC content is determined.

In the next step the loci of the reads within the genome is determined, the so called mapping. For this a bioinformatic program has transformed a reference genome to allow for a quick comparison of each read sequence to the sequence of the genome to find a fitting match. Certain programs include a mathematical solution to skip introns found in the reference genome for analyzing RNA-Seq data. Once the loci is found, the number of transcripts for each gene can be determined for each sample. For certain downstream analysis the read number is usually normalized to gene size, for example in the form of fragments-per-kilobase-million (FPKM). A

comparison between uniquely and multiple mapped reads offers valuable information, most times the analysis proceeds with uniquely mapped reads. Several options for analysis are possible for the data at this stage. Some of the more common once include a visualization of read loci via programs like the IGV viewer, an overview of gene expression distribution, Venn diagrams or Pearson correlations (for projects with several samples).

A further step in projects aiming at a comparison between samples is the differential gene expression analysis. The read strength of genes is compared between samples using a mathematical algorithm in a bioinformatic program. The most prominent outcome of many programs is a table of genes including the read numbers found in each sample, the expression strength difference (Log2FoldChange) and basic information about the reads (length, loci, details about the gene). Overviews of the data in form of volcano plots depicting up-, down- and unregulated genes, or cluster analysis in forms of heat maps are very common. Lastly, a functional analysis is often attached. Two of the most often used evaluations are the Gene Ontology (GO) term enrichment and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment.

Many more bioinformatic analysis are possible and included into projects, adapted to the specific needs.

1.7.2 WGBS

Regarding Whole-genome-bisulfite-sequencing (WGBS) a good review was recently published by Omony and other researchers (Omony *et al.*, 2020). This chapter presents shortly the major steps. Preparations for and the analysis of a WGBS data set are similar to RNA-Seq. The aim is to detect methylated cytosines in the genome and analyse their distribution. The process starts directly with the genomic DNA. To mark the methylation status of cytosines for further analysis, an enzymatic reaction replaces all unmethylated cytosines with uracil nucleotides. This is called bisulfite conversion. The process is continued by library preparation and sequencing as presented previously for the RNA-Seq.

The bioinformatical analysis is in some parts rather different from what is described for RNA-Seq. The first steps, creating a quality control for the data, is similar. Among other things reads are cleaned and typically overview details of read numbers are created. Since the sequence information is kind of distorted due to the bisulfite conversion, several additional steps have to be taken by a bioinformatical program to ensure a reliable mapping onto the reference genome

(for example using the Bismarck Alignment Strategy). After successful mapping, several important details can be examined like number of mapped reads, read coverage and coverage of cytosine sites. An important following step detects methylated sites using common algorithms (for example BS-Seq) to create a methylation profile. Further on in the analysis many approaches can be chosen. Methylation sites can be (comparable to RNA-Seq) made visible using software like IGV. Detailed graphs showing the methylation level of various areas of interested (for example promoters) can be produced. A Pearson correlation, a Principle-Component-Analysis (PCA) or a heatmap could be created to investigate further the data of each sample. The next step for projects with multiple samples is a comparison of methylation levels between samples. For this a differential methylation analysis is made (again in form of a table), containing information like loci of methylation site, the mean methylation level of each sample as well as the detected different methylation level, the methylation context, region information (promoter, intron, etc.) and details about the gene. Each subunit is called differential methylation region (DMR). To visualize DMRs between samples a variety of graphs can be produced, fitting to the specific research interest. Lastly, a functional analysis could be attached using a similar approach as in the RNA-Seq analysis.

2 Material and methods

2.1 Work with plants

2.1.1 Strains

The origin of the organisms used in this thesis are listed in table 1.

Table 1 Organisms and origin

Organism	Origin
***Arabidopsis thaliana* Ecotype Col-0**	AG Hoth, Universität Hamburg
Turnip Mosaic Virus (TuMV) Isolate UK1 (NCBI Accession: EF028235; UniProt ID: Q5TLC8)	Manfred Heinlein (Université de Strasbourg, CNRS; Institut de biologie moléculaire des plantes)
***Brassica napus* cv. Drakkar**	AG Kehr, Universität Hamburg

2.1.2 Growth of plant material

Arabidopsis thaliana (Col-0) seeds were sawn on soil and incubated for stratification at 4°C without light. Afterwards pots were transferred to *Arabidopsis* incubation chambers from Percival Scientific and grown with 14/20h light-dark cycle and 25°C.

2.1.3 Plant material from second generation

Some of the plants grown for the main comparison of wildtype and virus infected plants were not harvested. After flowering and ripe production, the plants were not watered and seeds were harvested once the plants were ready. Seeds were sterilized with bleach, transferred to MS media plates and incubated in growth chambers at 25°C and a 16/18 light cycle. Seedlings were transferred to soil after 7 days and grown for 7 more days before harvest.

2.1.4 TuMV infection

Fresh leaf material from TuMV infected *Brassica napus* plants was used for infection. For this, 400 mg ground, infected leaf material was mixed with 1 ml sodium phosphate Buffer (10 mM). *A. thaliana* plants were infected at 21 days after germination. Two leaves of each plant (preferably one older and one younger leaf) were rub-inoculated. For this, Carborundum powder was sprinkled onto the leaves and the infection solution was rubbed into the leaves with a finger (gloves were worn) until the first cell layers were disturbed. Leaves were rinsed with tap water

after 45 minutes to get rid of infection material. Plants were transferred back to former incubation conditions and grown for two more weeks before harvest.

2.1.5 Verification of TuMV infection via GFP

The used TuMV UK1 strain encodes a soluble GFP protein which can be detected via UV light. For this, plants were analysed in a dark room with a strong UV lamp (UVP UV-Lampe B-100AP, 100W 365nm LAMP; Fisher Scientific) and plants/leaves/tissue showing fluorescence was marked for harvest.

2.2 Molecular methods

For the sequenced material (RNA-Seq) the reads were also aligned to the virus genome (source) (link to further on table)

2.2.1 Protein extraction

For each 100 mg plant material, previously ground in liq. N_2, 800 µl of extraction buffer (50 mM MOPS pH 7.5, 5% v/v Glycerol, 0.55% Polyvinylpolypyrrolidone and 0.5% Nonidet P-40, solved in ddH_2O), with freshly added 10µl 500 mM L-Ascorbic acid, 5 µl 1 M DTT and 5 µl Protease inhibitor solution, was added and the solution centrifuged for 15 minutes at 14,000 g and 4°C. Supernatant containing the extracted proteins was pipetted to a new reaction tube.

2.2.2 SDS PAGE

For protein analysis sodium-dodecyl-sulfate (SDS) – polyacrylamide gel electrophoresis (PAGE) (SDS-PAGE) with 15% polyacrylamide was used. Gels were pipetted according to table below (Table 2). Samples were mixed with 6x Loading Dye prior to gel loading and cooked for 5 min. at 95°C. The PAGE Rule Prestained Protein Ladder Plus from ThermoFisher served as a size marker, loaded (2 µl) in a separate lane next to the samples. PAGE was done with 120 V and 60mA per Gel for 60 minutes using a Mini-PROTEAN Tetra Vertical Electrophoresis Cell (Bio-Rad) and a PowerPac™ Power Supply (Bio-Rad).

Table 2 Components of SDS-PAGE

Component	Volumen in separation gel	Volume in stacking gel
H_2O	1.2 ml	1.4 ml
1.5 M Tris (pH 8.8)	1.3 ml	-
1 M Tris (pH 6.8)	-	250 µl
10% SDS	50 µl	20 µl

30% Acrylamid	2.5 ml	330 µl
10% APS	50 µl	20 µl
TEMED	2 µl	2 µl

2.2.3 Western Blot

Protein analysis after SDS-PAGE was done via Western Blot. A standard protocol was used for stacking the Western Blot. Blotting was done via the Biometra Fastblot (analytikjena) with 3.5 mA/cm^2 gel, 50 V and 8 W for 30 minutes. Washing was also according to standard protocols. The following antibodies were used: primary α-GFP (mouse) with 1mg/ml, DIA-1500 Clone GF28R (Dianova), primary α -TuMV (DSMZ) and two secondary peroxidase conjugated AffiniPure Goat Anti-Mouse-IgG antibodies (Jackson Immunoresearch Laboratories Inc.) α-mouse and α-rabbit with 0.8 mg/ml. Chemiluminescence was detected using the Clarity Western ECL Substrate (Bio-Rad) and the ChemiDoc Imaging System (Bio-Rad).

2.2.4 RNA Extraction

For RNA isolation the kit innuprep Plant RNA Kit (analytikjena) was used and samples were treated according to the manual. The used TuMV infected *Arabidopsis thaliana* plant material was harvested 35 days after germination and 14 days after infection into liquid nitrogen and stored at -80°C.

Plant material was taken from -80°C storage and ground extensively with a mortar and pestle and cooled by liquid nitrogen during the whole procedure. Plant material was split into ~150 mg aliquots for further RNA isolation. Caution was taken that samples do not thaw. Lysis solution RL was used from the kit and RNA was eluted in 30 µl DEPC-treated ddH_2O. Samples were kept on ice while checking concentrations and purities on the NanoDrop™ One/OneC (ThermoFisher) and finally stored at -80°C until further use.

2.2.5 Genomic DNA Extraction

For isolation of genomic DNA, the kit NucleoSpin® Plant II (Macherey-Nagel) was used. Several changes to the manual were implemented. TuMV infected *A. thaliana* plant material was harvested 35 days after germination and 14 days after infection into liquid nitrogen and stored at -80°C.

Plant material was taken from -80°C storage and ground extensively with a mortar and pestle and cooled by liquid nitrogen during the whole procedure. Plant material was split into ~200 mg

aliquots for further DNA isolation. Caution was taken that samples do not thaw. Changes to the Kit manual were done as explained in the following: Step 2b: 450 µl PL2 buffer and 15 µl RNAse A were used and incubation was done for 30 min and 500 rpm at 65°C. 112 µl PL3 was used. Step 3: Before filtration trough the violet NucleoSpin® Filter the suggested alternative centrifugation of the crude lysate was done and the supernatant was used further on. Step 4: 675 µl PC buffer was used. Step 5: Centrifugation was done at 8000 x g. Step 7: The elution was done both times with 25 µl buffer PE, resulting in 50 µl eluted volume.

Samples were kept on ice while checking concentrations and purities on the NanoDrop™ One/OneC (ThermoFisher) and finally stored at -20°C until further use.

2.2.6 Agarose Gel Electrophoresis

To analyse PCR products, 1% agarose gels (1x TAE-Buffer, 1 % (w/v) LE Agarose and 5 % (v/v) GelRed®) were created following a standard protocol. As a size marker served the Gene Ruler 1kb Plus DNA Ladder (ThermoFisher). Loaded gels were run for 1 h with 100 mA and 150 W using a standard agarose gel electrophoresis chamber and power supply.

2.2.7 cDNA synthesis

A DNA digestion (see Table 3) was performed for all RNA samples, incubation was done for 30 minutes at 37°C, 1 µl EDTA was added afterwards and the DNAse was inactivated for 10 minutes at 65°C. The equivalent of 1 µg RNA in volume was used for each reaction.

Table 3 DNA digestion

Component	Volume (total 10µl)
RNA	x µl (1µg)
10x Buffer for DNAse I (+MgCl2)	1 µl
DNAse I (1U/µl)	1 µl
RNase-free water	x µl

After DNAse digestion a RT (reverse transcriptase) reaction (see Table 4) followed, samples were incubated for 60 minutes at 42°C and denaturized for 10 minutes at 70°C

Table 4 Reverse transcriptase reaction

Component	Volume (20µl)
DNAse digestion	11 µl
Oligo-(dT)-Primer (0.5µg/µl)	1µl

5x reaction buffer for RT	4 µl
RiboLock RNase Inhibitor (20 U/µl)	0.5 µl
10 mM dNTP-Mix	2 µl
Reverse Transcriptase enzyme	1 µl
RNase-free water	0.5 µl

2.2.8 Primer design

Gene sequences were taken from TAIR (https://www.arabidopsis.org/) and can be found in the supplementary data. All primers were designed using the Primer Design Tool from NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primers used in this thesis (listed in Table 5) follow the outlay necessary for qPCR: a size up to 200 bp and a similar melting temperature, taking the used polymerase in mind. It was not possible to create qPCR primers that span over exon-intron junctions on these genes. *A. thaliana* house-keeping gene *actin8* was chosen as a control.

Table 5 Designed qPCR primers to investigate phenotype changes

Name	Sequence (5' to 3')	Product length	Tm
FLA11_for	CCCTTCAGGTCCAACGAACA	188	59,89
FLA11_rev	AGGTTCCGGATTTGAGGCTG		60,04
FLA12_for	CCTCGTTCACCGGTCTCAAA	141	59,97
FLA12_rev	CCTGAGTCCGGAGAGGGTTA		60,03
FLA16_for	TGGCTACAACGAGATGGCTG	145	60,11
FLA16_rev	GCTAAGCTGGTCCGTTGTGA		60,32
GA20OX1_for	TGTGGAAAATCAATGGCGCTC	87	59,80
GA20OX1_rev	TCGTTCGATAGAGCCATGAAAGT		59,87
GA20OX2_for	AGGACCTCATTGTGATCCAAGTT	157	59,67
GA20OX2_rev	CCCGTTCGATAGAGCCATGA		59,33
GA20OX3_for	GAGTCTTGGGGTCGAGAGGA	180	60,32
GA20OX3_rev	AAAACTTGCAGACCGCCAAC		59,90
GAI_for	GGTTTGCGGCTGCACATATT	168	59,83
GAI_rev	TGGTGGAGAGTTTCCAAGCC		59,89
AtHB12_for	CTAGGGCTGCAACCAAGACA	169	59,96

AtHB12_rev	GCAGCTCAGAGACCAGAGAT		58,89
Actin8_for	CCATGACGGGATCACATTTC	108	60,00
Actin8_rev	CAAACGCTGTAACCGGAAAG		60,00

RT-PCR Primers (listed in Table 6) were designed following the same principles mentioned above, to be useful for both RT-PCR and qPCR.

Table 6 RT-PCR primers for detection of GFP and TuMV RNA

Name	Sequence	Product length [bp]	Tm
GFP_for	TGAGGGATACGTGCAGGAGAG	353	55,1
GFP_rev	GGGCAGATTGTGTGGACAGG		55,1
NIa_for	CACAACTCAGCTACACTTGCTACC	288	55,8
NIa_rev	AGTATTTTCCCGTCTTTCGTGCTC		55,5
Actin12_for	TGAAAAATGGCGGACGGTGAAG	238	56,1
Actin12_rev	TCCCATTCCAACCATCACTCCC		56,0

2.2.9 Polymerase-Chain-Reaction (PCR)

Reagents for polymerase-chain-reaction (Table 7) were first mixed as a master mix for all samples, excluding primers and cDNA, which were added in the final reaction tube accordingly. The reaction took place in a Thermocycler T3000 (Biometra) following the protocol in Table 8, with the PCR cycle of step 2-4 for 40 cycles.

Table 7 Reaction components of PCR

Component	Volume [µl]
10x Taq-Buffer + $(NH_4)2SO_4$, - $MgCl_2$	1
50 mM $MgCl_2$	0.4
10 mM dNTP-Mix	0.2
Taq-Polymerase (1U/µl)	0.25
Primer for (1:10)	1 µl
Primer rev (1:10)	1 µl
RNAse-free water	2 µl
cDNA or ddH_2O	1 µl

ddH_2O	5.15

Table 8 PCR program

Step	Temperature	Time
Initial denaturation	95°C	30 sec
Denaturation	95°C	30 sec
Annealing	60°C	30 sec
Extension	72°C	30 sec
Final Extension	68°C	10 min
Holding	4°C	∞

2.2.10 Quantitative Polymerase-Chain-Reaction (qPCR)

The general component mixtures was the same for all investigated samples (see Table 9).

Table 9 qPCR mixture

Component	Volume
2x QuantiFast SYBR Green PCR Master Mix	5 µl
Primer for	1 µl
Primer rev	1 µl
RNAse-free water	2 µl
cDNA or water	1 µl

All qPCR reactions were done with the QuantStudio 6 (ThermoFisher) using 96 well plates and the PCR program in **Table 8**. To create comparable conditions, one sample was tested with all primers in one reaction.

For all shown graphs, qPCR results were analysed using the comparative CT method (Schmittgen and Livak 2008) and Fold Changes were calculated with the following formula:

$$Fold\ Change = 2^{-\Delta\Delta C_T}$$
$$= [(C_T \text{ gene of interest} - C_T \text{ gene of internal control}) \text{ treated sample} - (C_T \text{ gene of interest} - C_T \text{ gene of internal control}) \text{ untreated sample}]$$

For samples in which the first ΔC_T is greater than the second ΔC_T, the negative inverse of the result of the above formula was taken, following the instructions of Schmittgen and Livak (Schmittgen & Livak, 2008)

2.2.11 Determining Primer Efficiency

To ensure a comparability of qPCR results from different samples, e.g. potentially different amounts of cDNA, primers were tested for reliability at various target amounts. For this, the protocol from Schmittgen and Livak (Schmittgen & Livak, 2008) was followed and the information from Stephen Bradburn on toptipbio.com (Bradburn, 2021)

2.3 Sequencing

Sequencing was outsourced to the company Novogene.

2.3.1 Samples QC RNA and DNA

The purity of the RNA and DNA samples for sequencing was tested at Novogene. RNA purity checked via Agarose Gel Electrophoresis and on the Bioanalyser 2100 (Agilent). RNA integrity was tested with the Bioanalyser 2100 (Agilent). For DNA, the degradation as well as RNA contamination was tested via Agarose Gel Electrophoresis.

2.3.2 RNA library construction and sequencing

For each sample 1 µg of RNA was used as input material. The NEB Next Ultra RNA Library Prep Kit for Illumina was used for generating sequencing libraries, according to manufacturer's recommendations. To add attribute sequences to each sample, index codes were added. For purifying mRNA from the send total RNA poly-T attached magnetic beads were used. In NEBNext First Strand Synthesis Reaction Buffer (5x) the fragmentation was done by divalent cations under elevated temperature. Further on, random hexamer primer and M-MuL V Reverse Transcriptase RNaseH were used for synthesizing first strand cDNA. The second strand cDNA synthesis was afterwards made vua DNA Polymerase I and RNaseH.

2.3.3 DNA library construction and sequencing

For library construction lambda DNA was used as a negative control and samples were fragmented into sizes of 200 to 400bp using the S220 Focused-ultrasonicator (Covaris). Next fragments were repaired, dA-tailed and ligated to sequencing adaptors with methylated cytosines. The EZ DNA Methylation Gold Kit (Zymo Research) was further on used for Bisulfite treatment of the DNA fragments and final library was gained by size selection and PCR

amplification. Before sequencing a QC of the library was done. For this, the concentration of the library sample was set to 1 ng/l and initial quantification was done with Qubit 2.0 (ThermoFisher). Insertion size of the library was determined via Bioanalyser 2100 (Agilent). Finally accurate concentration of library samples were quantified by qPCR. Sequencing was done on Illumina HiSeq/NovaSeq platform.

2.4 Bioinformatic methods

The whole bioinformatic analysis was outsourced to Novogene.

2.4.1 RNA-Seq Quality control of reads

Quality control was done with fastp (Chen *et al.*, 2018), clean reads were gained by removing reads containing adapter and poly-N sequences as well as reads with low quality. Q20, Q30 and GC content were calculated and can be found in supplementary files.

2.4.2 RNA-Seq Mapping, Novel Gene prediction, Quantification, Enrichment Analysis, Differential gene expression analysis, Alternative splicing and SNP/InDel Analysis

Mapping of high quality reads was done with HISAT2 (Kim *et al.*, 2019) in version v2.0.5 with the parameter --dta --phred33 against the *Arabidopsis thaliana* reference genome TAIR10. Novel gene prediction was with StringTie (please refer to chapter 8 for availability) in v1.3.3 with default parameters to assemble transcripts and GffCompare in v0.10.6 in default parameters to compare transcriptomes. Next, reads were quantified with FeatureCounts (Liao *et al.*, 2013)in version v1.5.0-p3 with default parameters. A differential analysis was finally made with DESeq2 (Love *et al.*, 2014), v1.20.0 with a padj<=0.05. Further on, the enrichment analysis was investigated with ClusterProfiler (Yu *et al.*, 2012), v3.8.1 and padj < 0.05 and GSEA Analysis with gsea (Mootha *et al.*, 2003; Subramanian *et al.*, 2005), v3.0. NCBI BLAST (Sayers, 2022) was used for a protein-protein interaction analysis, in v2.5.0 and an e-value = 1e-10 using blast and string database. Alternative splicing was detected by rMATS (Shen, 2014), v3.2.5 with default parameters. Finally, SNP/InDel analysis was done with GATK4 (Van der Auwera & O'Connor, 2020) in v4.1.0.0 with the parameters MQ < 40.0 and QD < 2.0 for calling and SNPEff (Cingolani *et al.*, 2012) v4.3 with default parameters for annotating.

2.4.3 WGBS Quality control of reads

Basic statistics were made with FastQC (Andrews, 2010; "FastQC," 2015)in v0.11.5. Further processing was made via Trimmomatic (Bolger *et al.*, 2014) v0.36 with the parameters SLIDINGWINDOW: 4:15 ; LEADING:3, TRAILING:3 : ILLUMINACLIP: adapter.fa: 2: 30: 10 ; MINLEN:36 .

2.4.4 WGBS References genome treatment, Mapping, DMR, Functional analysis

The reference genome was transformed int a bisulfite-converted version via the software bowtie2 (Langmead & Salzberg, 2012). The Bismark software (Krueger & Andrews, 2011) v0.16.3 was then used for the aligning the reads with the reference genome with the parameters -X 700 –dovetail . DSS software (Feng *et al.*, 2014; Park & Wu, 2016; Wu *et al.*, 2015) was used to identify differentially methylated regions (DMR). Functional analysis was made in form of Gene ontology analysis using the GOSeq R package (Young *et al.*, 2010) (only GO terms with corrected p-value less than 0.05 were considered) and a KEGG analysis using KOBAS (Mao *et al.*, 2005).

3 Results

3.1 Phenotypic consequences of infecting *Arabidopsis thaliana* with Turnip Mosaic Virus

3.1.1 Confirmation of infection

A functional and reliable infection had to be established for all further experiments. In this thesis the successful virus infection was confirmed via three methods: Detection of 1) GFP, 2) viral RNA and 3) viral protein.

The TuMV virus used in this study had a co-expressed, uncoupled GFP. This allowed for tracking of the infection spread by detecting GFP fluorescence *in vivo*. Figure 6 shows an infected *A. thaliana* plant in normal light conditions (B) and under UV light (A), where the GFP signal is visible in green and the autofluorescence of the chloroplasts in red. The GFP signal spreads in the very distinct mosaic like pattern, giving the virus its name. The mosaic like pattern is visible in other hosts (f.e. *B. napus*) under normal light conditions, even without the expressed GFP. Unfortunately it is not visible in *A. thaliana* leaves. The GFP fluorescence was used for verification of successful infection before harvesting material for further experiments.

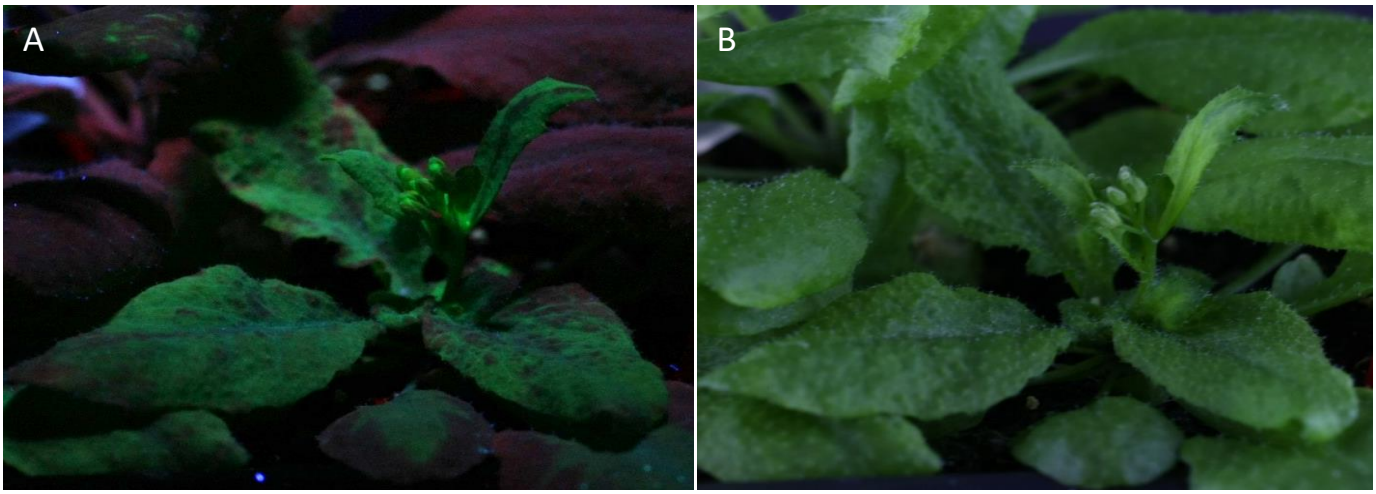


Figure 6 TuMV virus infected A. thaliana plant *The same plant was observed under different light conditions to visualize the GFP fluorescence marking TuMV infected tissue* ***A*** *UV light, GFP fluorescence visible in green, chloroplast autofluorescence in red* ***B*** *normal light conditions*

The GFP was present within the plant uncoupled from viral proteins. To verify a virus spread co-aligning with a visible GFP fluorescence, RNA and protein content of fluorescing tissues was analysed. The fluorescence was determined using a UV-lamp. Both infected and uninfected plant material, in some cases from the same leaf, was harvested. RNAs were detected via RT-PCR. Proteins with a Western Blot. For these two methods, as well as in the rest of this thesis, the house-keeping gene Actin12 was used as a control. Furthermore the presence of virus was confirmed in form of the viral NIa RNA or protein. Last but not least GFP RNA and protein presence was determined.

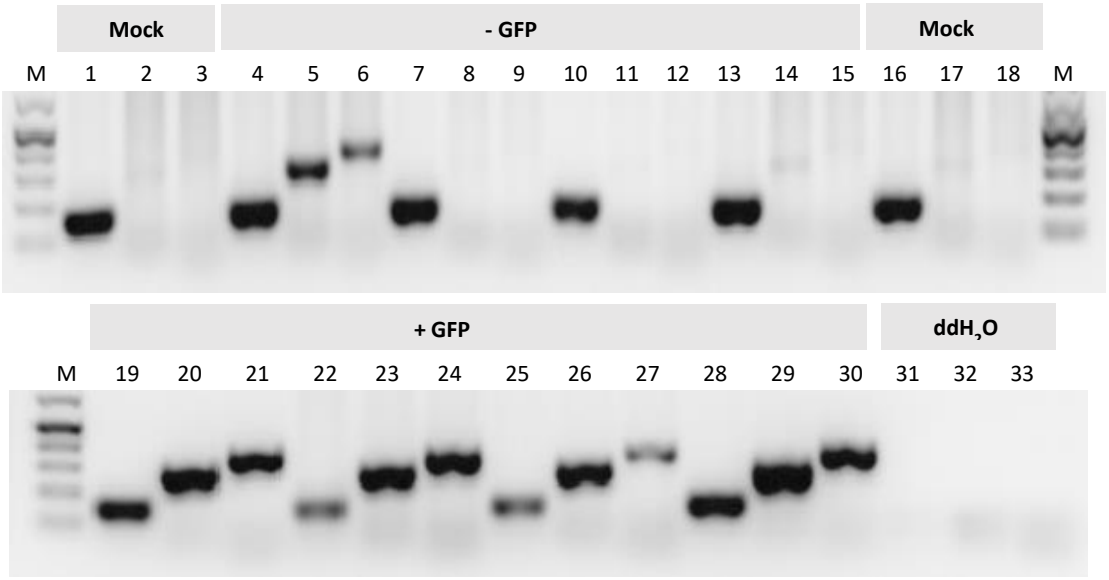


***Figure 7 Results of agarose gelelectrophoresis of RT-PCR samples**. The DNA marker represents (from top to bottom): 500, 400, 300, 200 and 75 bp. Mock, samples in which no GFP was visible (-GFP), samples in which GFP was visible (+GFP) and water controls (ddH_2O) were investigated*

RT-PCR samples were investigated via agarose gel electrophoresis (Figure 7). For each sample three primers were tested: Actin12 (control), NIa (virus) and GFP. Two mock samples are shown (1-3 and 16-17,) where only GFP was detected. Samples taken from plant material without GFP fluorescence on a virus infected plant (-GFP) are shown in 4-15. Here the first sample (4-6) shows a very good example for the sensitivity of the detection method, as here the material was not cut properly and some GFP plant material was also harvested. This resulted in a positive signal for all three categories. All other samples showed as expected neither a GFP signal nor a NIa signal. Samples from GFP positive material (+GFP) is shown in lines 19 to 30. In all of them Actin12, GFP and NIa (viral RNA) could be detected. Lastly, 3 lines (31-33) show a negative water control. To sum this up, GFP and virus exclusive coexistence during infection could be verified on RNA level.

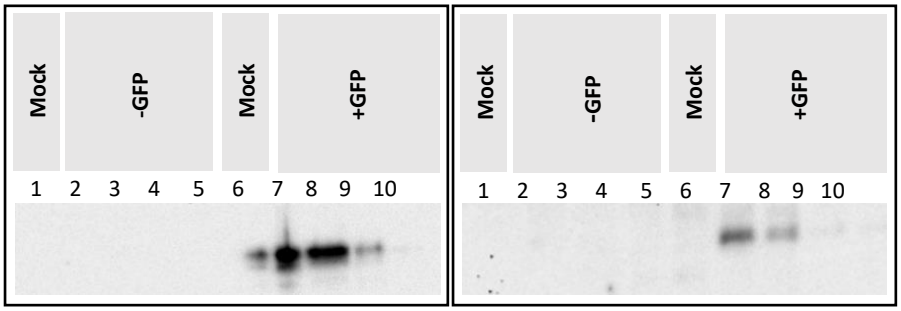


Figure 8 Results of Western Blot *Samples were previously separated by SDS-PAGE. On the left a GFP antibody was used, for the right blot a TuMV antibody*

Coexistence could also be verified on protein level (Figure 8). Again -GFP and +GFP samples are shown, resembling samples from plant material with or without visible GFP signal. The left blot was done using an anti-GFP antibody, here signals were present in +GFP samples. A spill was visible to the Mock samples, most likely from mishandling the samples in the SDS-PAGE. The -GFP samples showed no signal. The blot on the right was done using an anti-TuMV antibody. Only signals were detectable in the +GFP samples. Exclusive coexistence of GFP and viral proteins was successfully detected. Viral protein detection was not possible for all fluorescing samples. Viral RNA may be present already, causing an effect on the plant tissue, however it was not transcribed to proteins yet. At least not in the amount needed for positive results in the Western Blot method.

3.1.2 Infection phenotype

Plants have to face a variety of threats throughout their lifetime and have therefore implemented a number of mechanisms to defend themselves. However, these defence mechanisms come at a visible cost and lead to a distinct phenotype of the host. One phenotype aspect of *A. thaliana*, infected with Turnip Mosaic virus (TuMV) was investigated and results are presented in this chapter.

The phenotype manifested in an altered inflorescences growth pattern of virus infected plants. Growth differences between mock treated and virus infected plants were captured in a 2-week time window between infection and harvest of plant material for further analysis.

3-week-old *A. thaliana* plants were infected with TuMV via rub inoculation and imaged every day. The phenotype of infected plants behaved differently from mock treated plants over the observed time, with symptoms becoming more severe the stronger the infection got. Phenotypic changes ranged from a slightly reduced rosette diameter to a severely reduced inflorescence. Flowers and seeds were still produced and seeds were proven to be fertile. Only a slight delay in flowering (1-2 days) was visible. Especially plants that stayed very small showed yellowing of the leaves close to the end of the observed time frame. However yellowing was very rare and infected plants had overall a very healthy green appearance. Height measurements were made over time (Figure 9) of infected plants compared to mock treated plants.

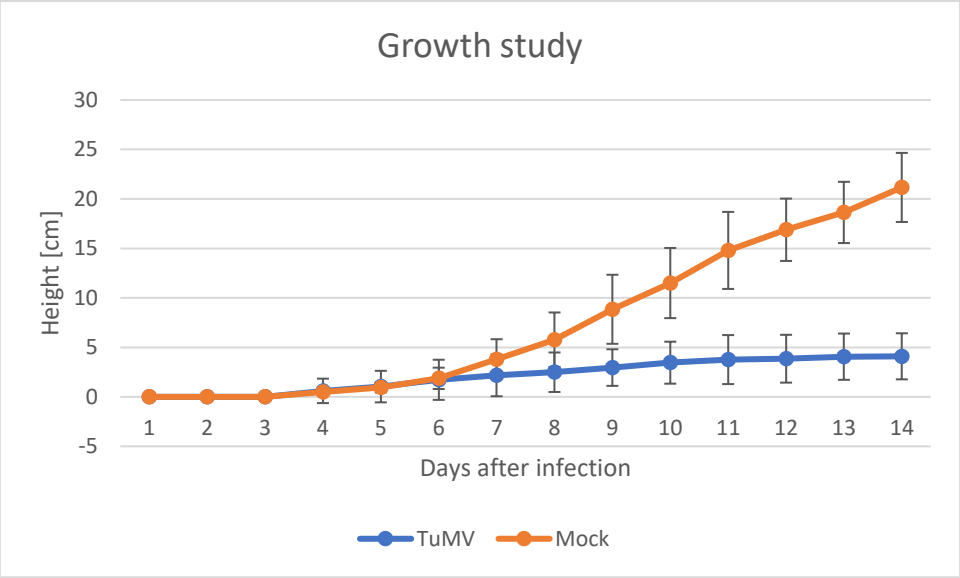


Figure 9 Growth study *Inflorescence length were measured (in cm) over a time period of 14 days of of control plants (Mock, orange) and virus infected plants (TuMV, blue)*

The growth was imaged over 14 days after infection (dai). Heights of 7 plants from each group were measured using the images, results are shown in Figure 9. A significant difference in height on the 14th dai could be determined using a one-sided students t-test with a significance interval of 5%. The enormous height difference between a virus and a mock treated plant on dai 14 can also be seen in Figure 10.

Figure 10 Visualization of growth difference *Pictures of TuMV infected (left) and Mock treated (right) plants. Pictures were taken 14 days after infection, the bar on the top left corner of each picture resembles 1 cm.*

The detected inflorescence appearance was variable and was further analysed. In the following, the determined changes in phenotype are shown and categorized.

A. thaliana plants were grown for 21 days, infected with TuMV and harvested on the 14th day of infection. Plants were imaged before harvest to analyse the phenotypic changes. Phenotypes could be separated into 4 different categories of according to their outer appearance.

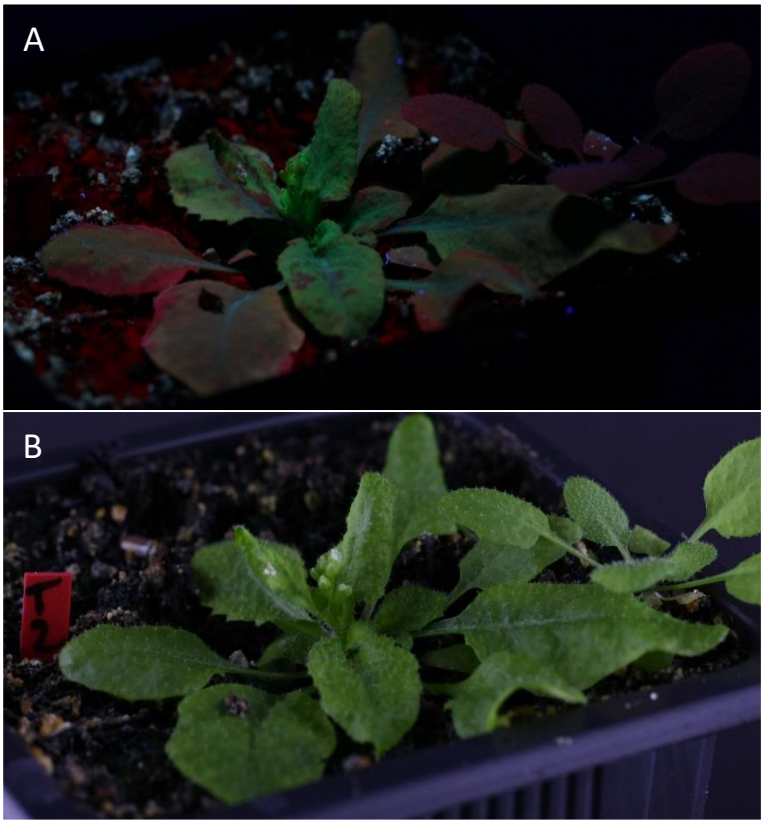
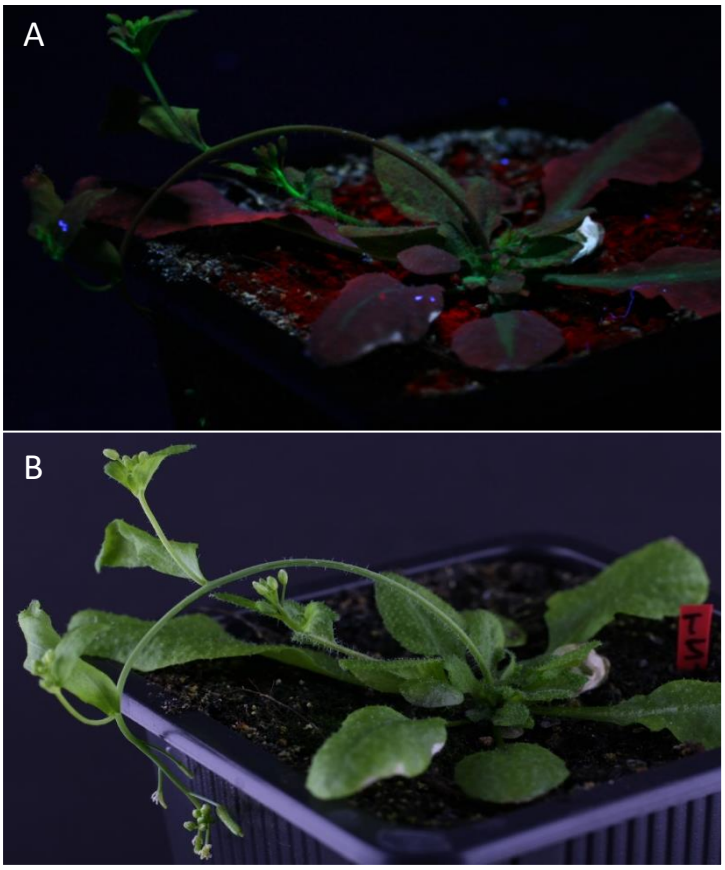


Figure 11 A. thaliana plants of category 1 A *Picture taken in UV-light, green fluorescence shows virus presence, red fluorescence is the autofluorescence of chloroplasts* ***B*** *Picture taken in normal light*

Plants of category 1 (Figure 11) had very small inflorescences with fertile seeds (seeds for 2nd generation, which was studied further, arose from plants of this category). Overall plants looked healthy, in some cases weak yellowing of the leaves was visible close to harvest.



In category 2 (Figure 12), plants showed a longer, but very crooked (nearly curled) stem. Internodes were separated along the inflorescence in comparison to category 1. The length of the inflorescences was still much smaller than those of wild type plants.

Figure 12 A. thaliana plants of category *2* ***A*** *Picture taken in UV-light, green fluorescence shows virus presence, red fluorescence is the autofluorescence of chloroplasts* ***B*** *Picture taken in normal light*

Plants of category 3 had less curled but slightly crooked inflorescences, with smaller overall length compared to category 2 plants (Figure 13).

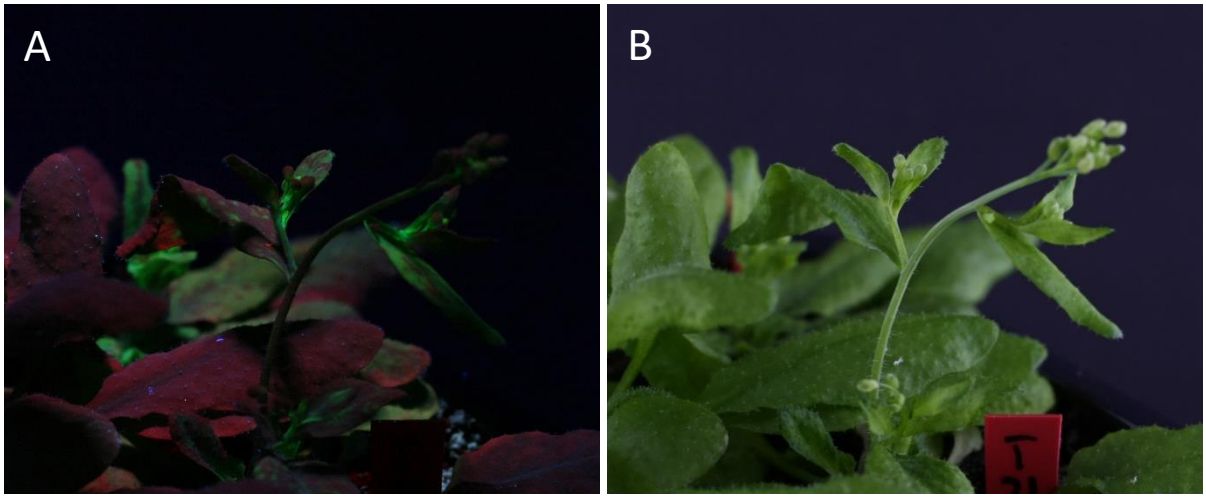


Figure 13 A. thaliana plants of category 3 A *Picture taken in UV-light, green fluorescence shows virus presence, red fluorescence is the autofluorescence of chloroplasts* ***B*** *Picture taken in normal light*

Lastly, for category 4 (Figure 14), plants showed a more straight upwards growth. Stems were still much shorter than stems of wildtype plants.

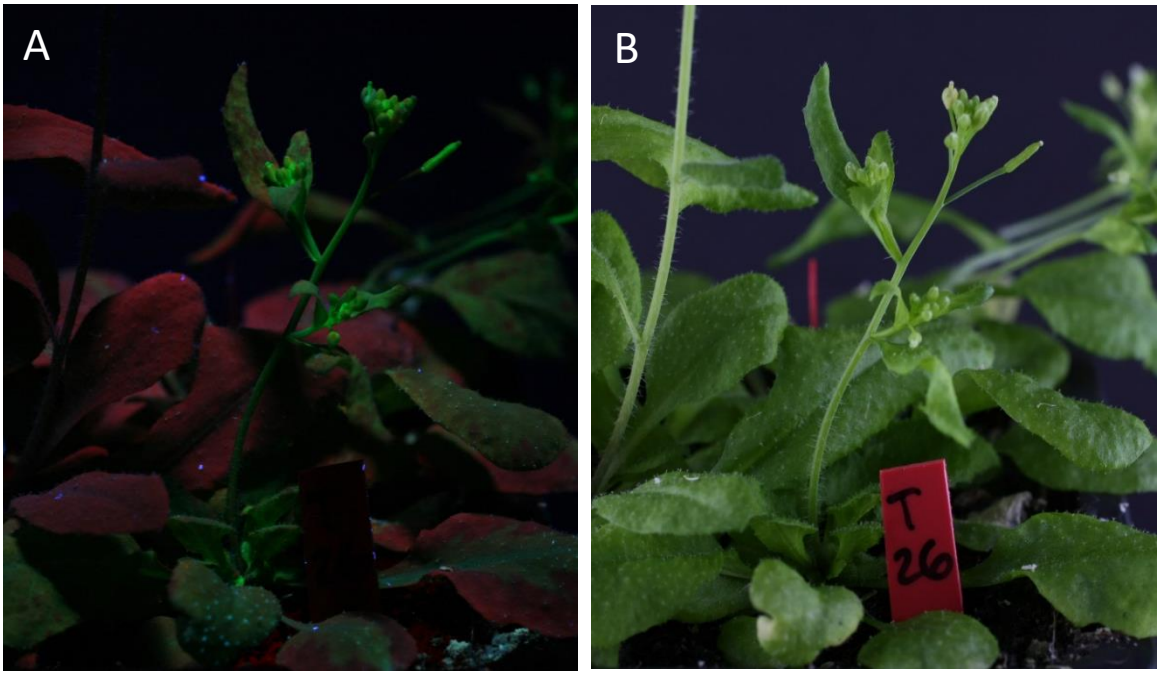


Figure 14 A. thaliana plants of category 4 A *Picture taken in UV-light, green fluorescence shows virus presence, red fluorescence is the autofluorescence of chloroplasts* ***B*** *Picture taken in normal light*

The identified categories had different frequencies (Figure 15). Category 1 was the most frequent. Plants investigated as part of the growth study fell into category 1, with one plant matching the characteristics of category 2.

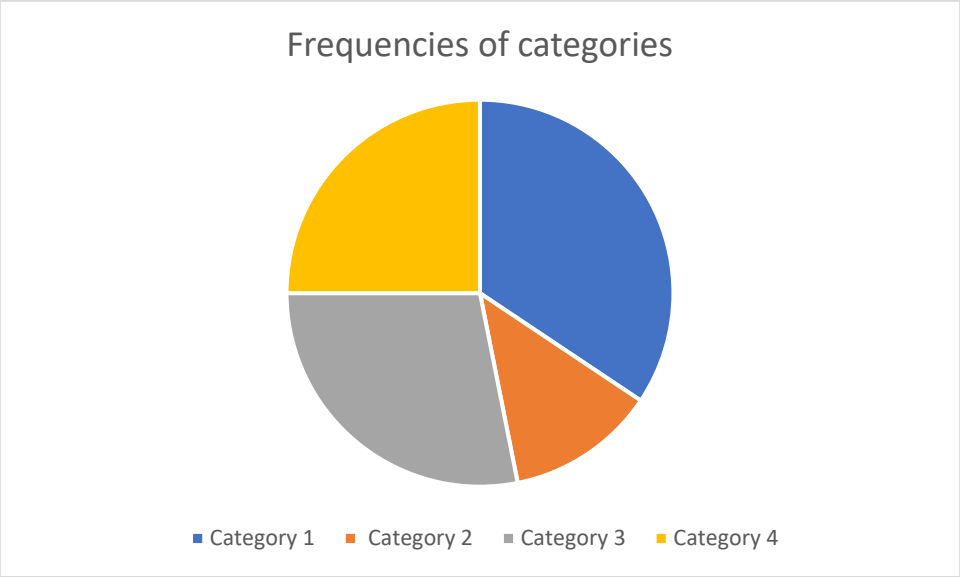


Figure 15 Frequency of the 4 different phenotype categories

3.2 Effects of TuMV virus infection on the *Arabidopsis thaliana* transcriptome

3.2.1 Effects of TuMV virus infection on the inflorescence/stem

Following up on the phenotype changes investigated in chapter 1, this chapter aims at understanding the transcriptome changes influencing the phenotypic changes after virus infection better. For this, changes were analysed in a RNA-Seq and candidate genes investigated further via RT-PCR.

Leaves and inflorescences (including flowers) were harvested separately on the 14th day of infection. To get a closer look at what mechanisms are involved, inflorescence plant material from category 1 (see chapter 1.2) was analysed by RNA-Seq. Imaged plants were used as TuMV samples and inflorescences of mock plants as the control group. Samples were send in triplicates (Mock_1-3 and TuMV_1-3).

A confirmation of virus RNA being solely present in infected samples was made. Sequencing results were mapped against the TuMV genome additionally to the RNA-Seq data set. Mapping results can be found in the supplementary. Only infected samples contained reads that mapped unto the virus genome.

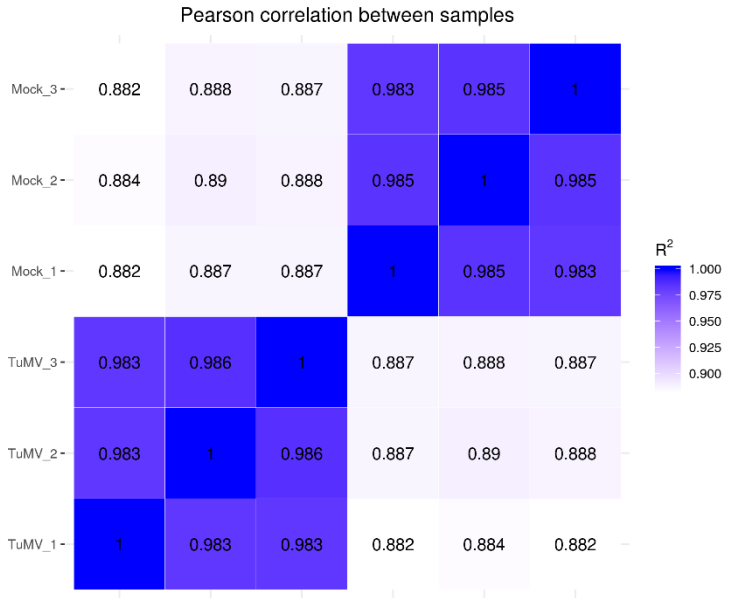


Figure 16 Pearson correlation of the phenotype RNA-Seq *This comparison shows similarity between the triplicates of mock treated and virus infected samples of the data set. The numbers range between 0 and 1, with 1 being full similarity. (Source: Novogene)*

One of the first steps in the analysis of the RNA-Seq data set after sequencing was an investigation of the data of each sample. For example the analysis via Pearson correlation (Figure 16) showed that both groups (Mock treated and TuMV infected) clustered. Differences between groups were present, however differences within the groups were still quite large.

A differential expression analysis followed in the bioinformatic analysis of the RNA-Seq data set. One of the figures created, the Volcano plot in Figure 17, showed an overview of the expression differences when comparing the transcriptome of virus infected to mock treated samples. The differentially expressed genes were distributed very equally into up- (5777 genes) and downregulation (6435 genes).

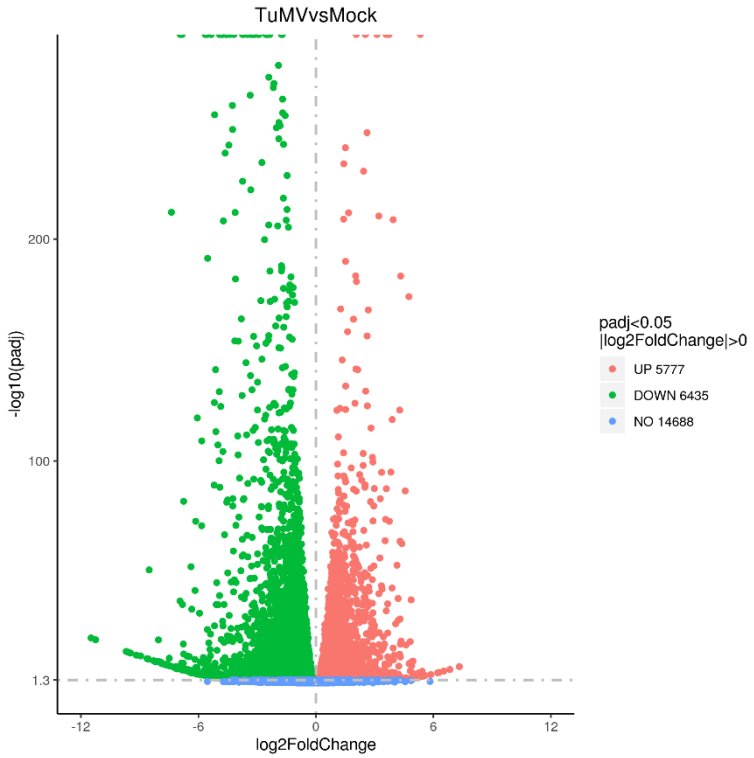


Figure 17 Volcano Plot of differentially expressed genes of phenotype experiment *The volcano plot shows genes resulting from the differential expression analysis of data sets origin from mock treated vs. TuMV infected samples; All shown genes had an adjusted p-value higher than 0.05 and are sorted into upregulated (red), downregulated (green) and not regulated (blue). DEGs ranged from log2FC of approx. 8 to -12 with a log10(padj) of up to almost 300 (Source: Novogene)*

To find possible factors influencing the change in phenotype, the most differentially expressed genes were screened. For this, the result table of the bioinformatical differential expression analysis (including details about the found genes) was used.

Only the strongest regulated genes were further on investigated for candidate genes affecting the observed change in phenotype. Fasciclin-like arabinogalactan proteins were found as potential candidates In total 19 FLA proteins are expressed in *A. thaliana*, 17 of which were differentially regulated in this data set. *FLA11*, *FLA12* and *FLA16* are known to cause short stem phenotypes (Liu et al. 2017) and were also downregulated in this data set (see Table 10) and were thus regarded as candidate genes.

Phytohormones are key players in changing the outer appearance of plants. One prominent one in determining the stem are gibberellins (Rieu *et al.*, 2008). Genes related to gibberellin metabolism were investigated in the DEG list and it was found that GA20-oxidases were downregulated. In addition to this, it was found that the Gibberellic Acid Insensitive (GAI) protein, a GA repressor, was upregulated. Lastly, a prominent GA20-Oxidase inhibitor, *ATHB12*, was found upregulated.

An overview of all candidate genes from the analysis, their changes due to infection and their expression strength can be found in Table 10.

Table 10 Candidate genes *These genes were identified from the RNA Seq data set to be involved in the visible phenotype*

Candidate	Log2FoldChange	TuMV read count	Mock read count
FLA11	-4.58	157	2775
FLA12	-5.65	84	4249
FLA16	-1.79	658	2287
GA20OX1	-1.72	36	119
GA20OX2	-2.65	13	85
GA20OX3	-5.73	1	53
GAI	0.64	1566	1000
AtHB12	1.59	480	160

The rest of this chapter will focus on investigating these candidate genes in the other described plant phenotype categories. Inflorescence and leaf material were harvested in duplicates, RNA was extracted, transcribed into cDNA and analysed by qPCR. To verify comparable results regardless of probable target RNA concentration differences, the primer pairs used were tested for primer efficiency beforehand.

Fold Changes resulting from the qPCR are shown in Figure 18 for inflorescence material and in Figure 19 for leaf material. Instead of qPCR results from inflorescence category 1 (K1I), the

results of the RNA-Seq is shown. Results of Fold Change calculations are shown for all tested candidate genes in one graph for each sample.

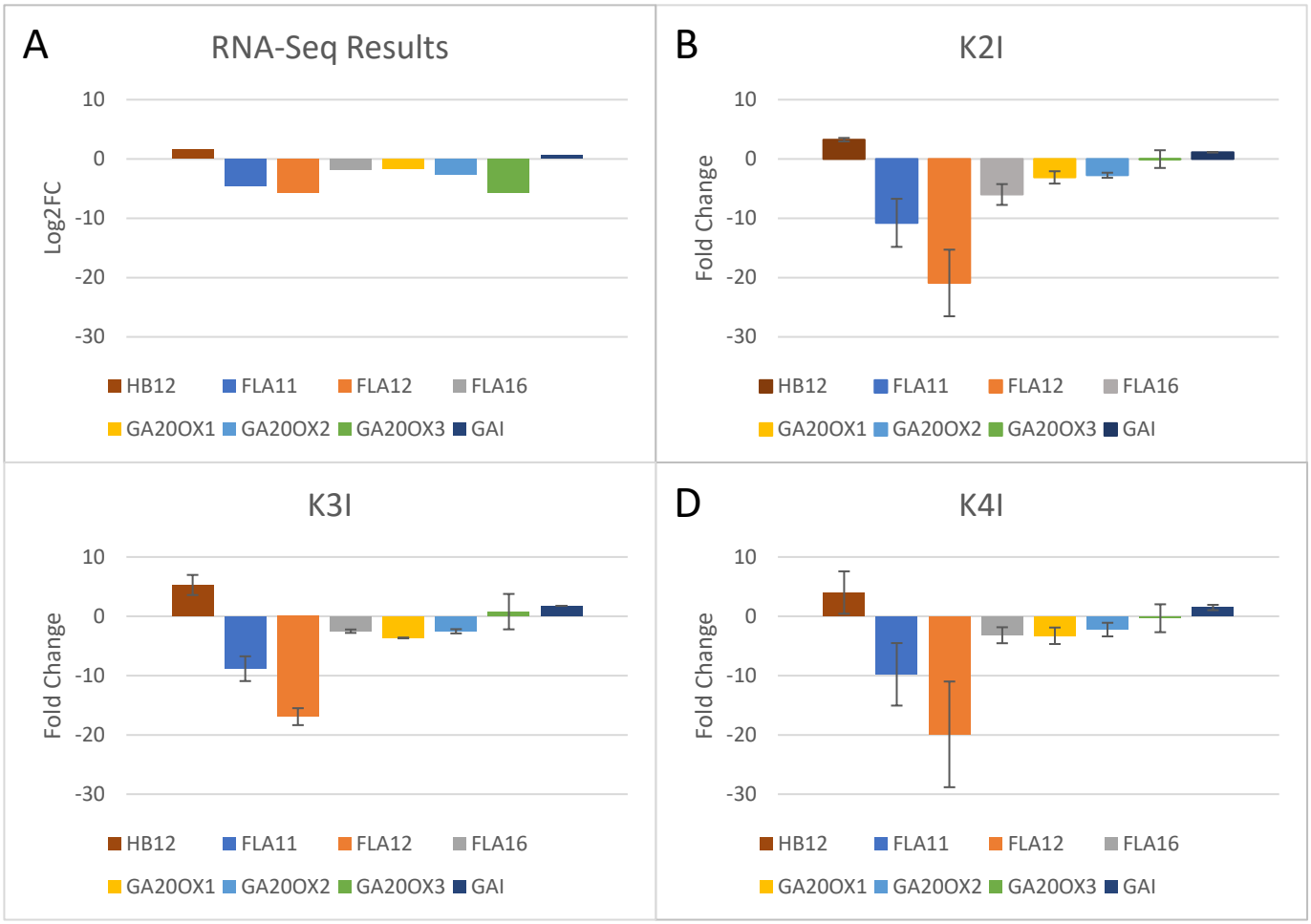


Figure 18 Results of qPCR of inflorescence *Candidate genes were tested in a qPCR for expression strength. Each bar shows the Fold Change of the respective sample and was calculated from duplicates. Error bars represent the standard deviation.*

The investigated changes of expression are very similar throughout all analysed samples. The RNA of *AtHB12* was enriched in all material after virus infection of up to 10 fold. *FLA11* and *FLA12* were downregulated after virus infection compared to mock tissue. This regulation was up to 10 times stronger in inflorescence tissue (up to -10 and -20 fold respectively) than in leaf material (up to -2 fold). Downregulation of RNA from GA20oxidases was present in both tissues. The reduction was around -5 fold, a bit less in inflorescence tissue. The RNA of GAI was found slightly upregulated in both tissue, with approximately 1-2 fold.

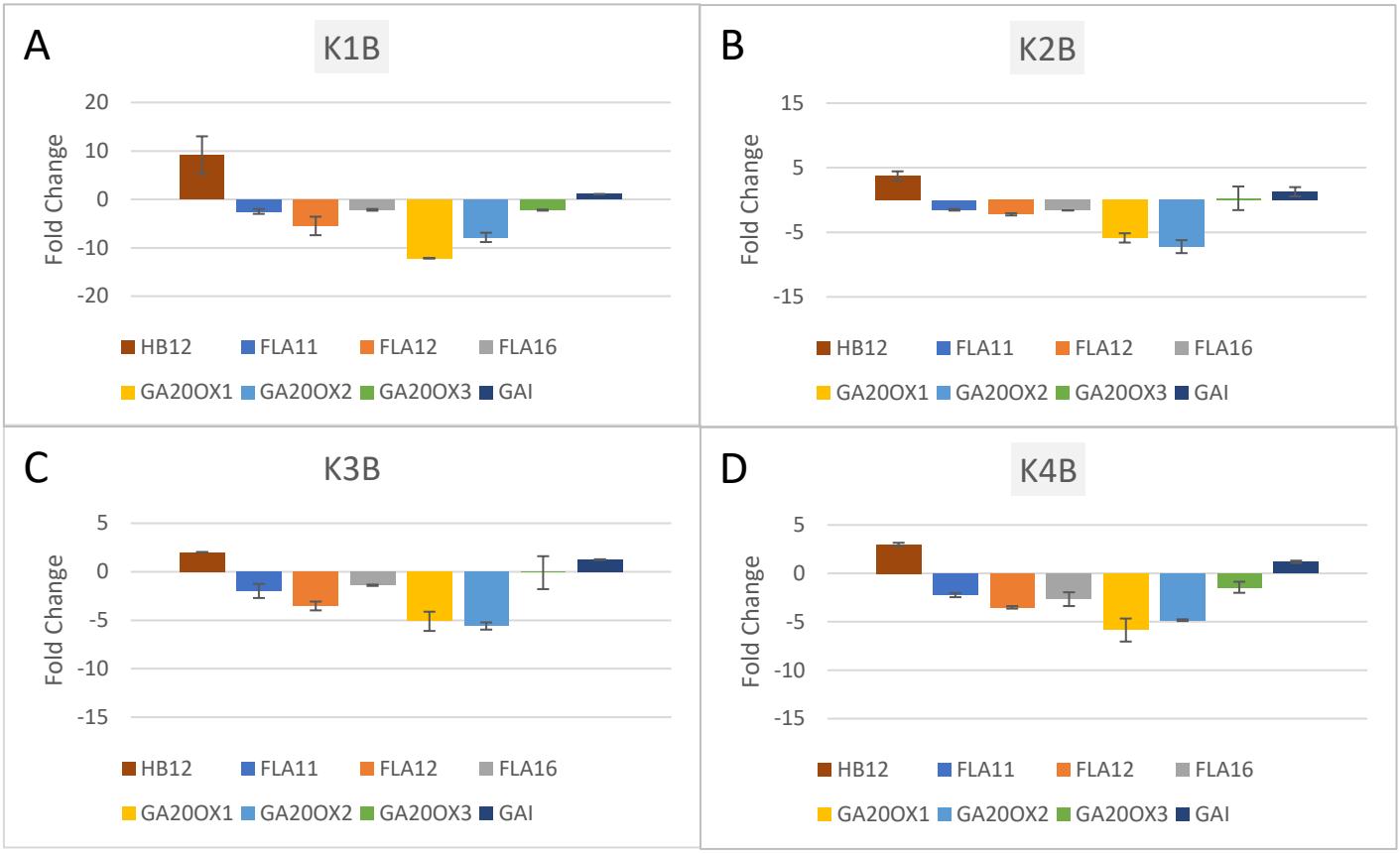


Figure 19 Results of qPCR of leaf material *Candidate genes were tested in a qPCR for expression strength. Each bar shows the Fold Change of the respective sample and was calculated from duplicates. Error bars represent the standard deviation.*

A major difference between both tissues can be seen with FLA proteins being more prominently downregulated in the inflorescence tissues and GA20oxidases being more strongly downregulated in the leaf material. Regulation ranged from approx. +5 to -21 Fold Change for the inflorescence samples and from +9 to -12 Fold Change in the leaf material. The Fold Change results of the RNA-Seq are not directly comparable to Fold Change results from qPCR, since the methods are very different, but are still shown as an indicator. The error bars from GA20OX3 samples are very large. A probable reason for this is the analysed primer efficiency outside the optimal range of 90-110 %.

3.2.2 Effects on the transcriptome of leaves

The effect of virus infection on *A. thaliana* does not stop at an altered outward appearance. Many intra- and intercellular mechanisms are involved in the battle between host and virus. One is the change of the foundation itself: the gene expression. To analyse these changes, the transcriptome of infected plants was investigated with an RNA-Seq analysis.

A. thaliana plants were grown for 3 weeks, infected via rub-inoculation with TuMV (or a mock-solution) and harvested after 14 days as biological triplicates. It was verified, that only TuMV infected leaf material was collected by using a UV light prior.

An exclusive presence of virus RNA in infected samples could be confirmed. For this sequencing results were mapped against the TuMV genome. Results can be found in the supplementary. Only infected samples contained reads mapping to the virus genome.

Samples were send to sequencing as biological triplicates (Mock_1-3 and TuMV_1-3). Sequencing and bioinformatic analysis was done with Novogene. After quality control of the produced data from each sample, several studies were made to investigate each sample. For example the consistency of the RNA-Seq data within the sample groups was analysed via Pearson correlation (Figure 21). The control group (Mock) clusters as well as the virus infected (TuMV) samples. Differences between groups are present, but small. Homogeneity of biological replicates and differences between the groups were confirmed in the gene expression distribution (not shown).

Results of the quantification analysis of expression strength were used for differential gene expression analysis. Virus-infected samples were compared to mock treated samples in this part. Again, a number of analyses were created during the bioinformatical analysis. One of them, the volcano plot (Figure 20) shows the distribution of expression strength differences. For this study, the distribution of differentially expressed genes (further on called DEGs) appeared to be even, with 4810 genes being upregulated and 4777 genes being downregulated after TuMV virus infection in *A. thaliana*. Only significant genes were used in this plot and the further analysis.

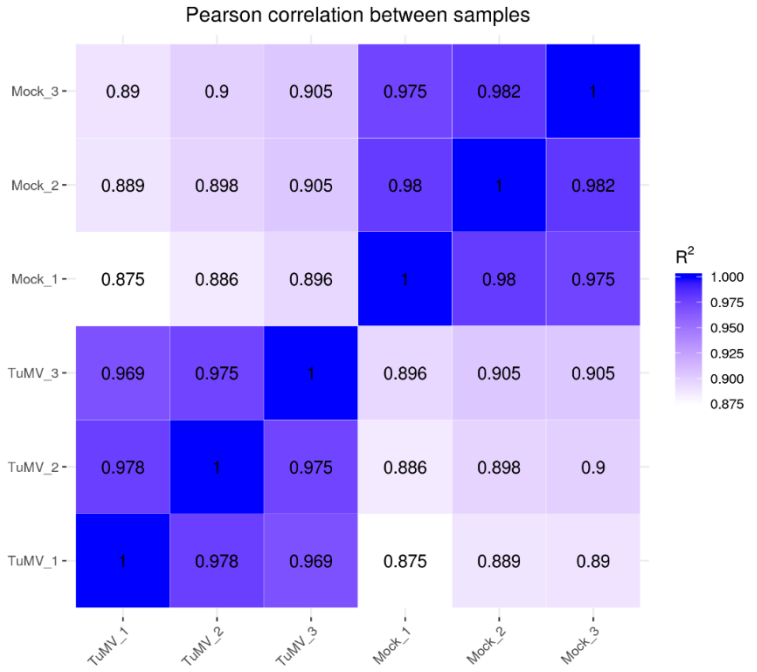


Figure 21 Pearson correlation of 1st generation *This comparison shows similarity between the triplicates of mock treated and virus infected samples of the data set. The numbers range between 0 and 1, with 1 being full similarity. (Source: Novogene)*

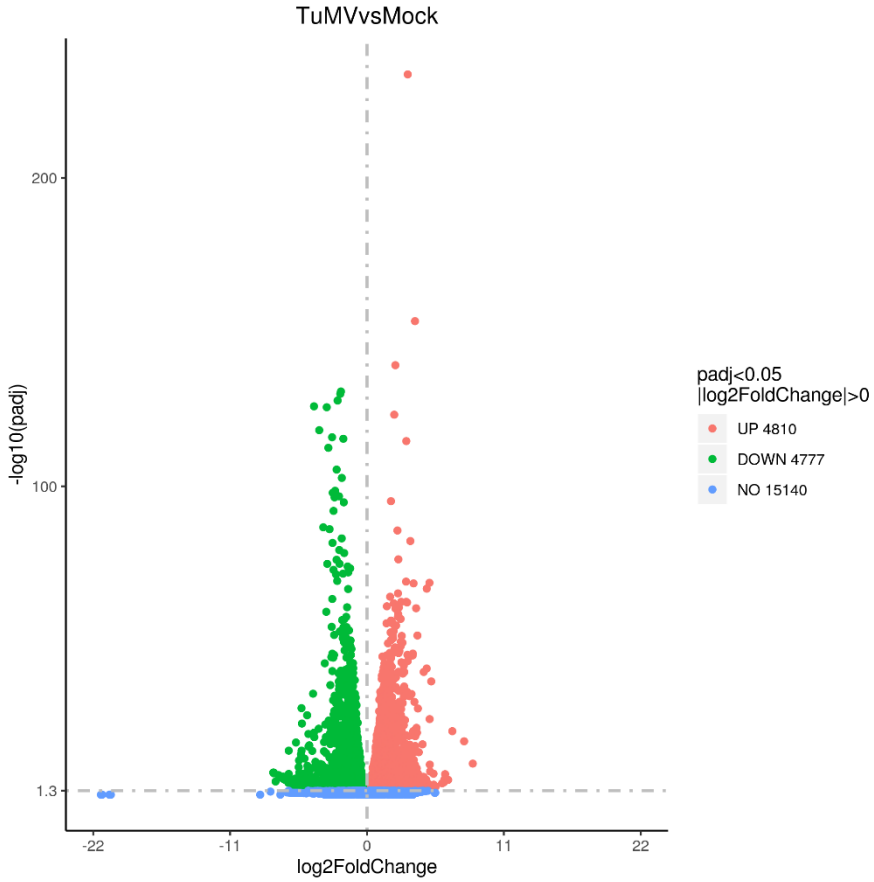


Figure 20 Volcano Plot of differentially expressed genes in 1st generation *The plot shows genes of the data set originating from mock treated vs. TuMV infected samples; All shown genes had an adjusted p-value higher than 0.05 and are sorted into upregulated(red), downregulated (green) and not regulated (blue). DEGs ranged from log2FC of approx. 10 to -10 with a log10(padj) of up to over 200. (Source: Novogene)*

Next, DEG data was investigated in a functional analysis. Two of the most interesting parts in this step are the studied enrichment of Gene-Ontology (GO) terms (not shown here) and KEGG pathways. In Figure 22, the most strongly up- and downregulated KEGG pathways are shown. Upregulation analysis showed the ribosome pathway as the most enriched in this data set. Downregulated pathways enriched were plant hormone signal transduction pathway, followed by the spliceosome pathway.

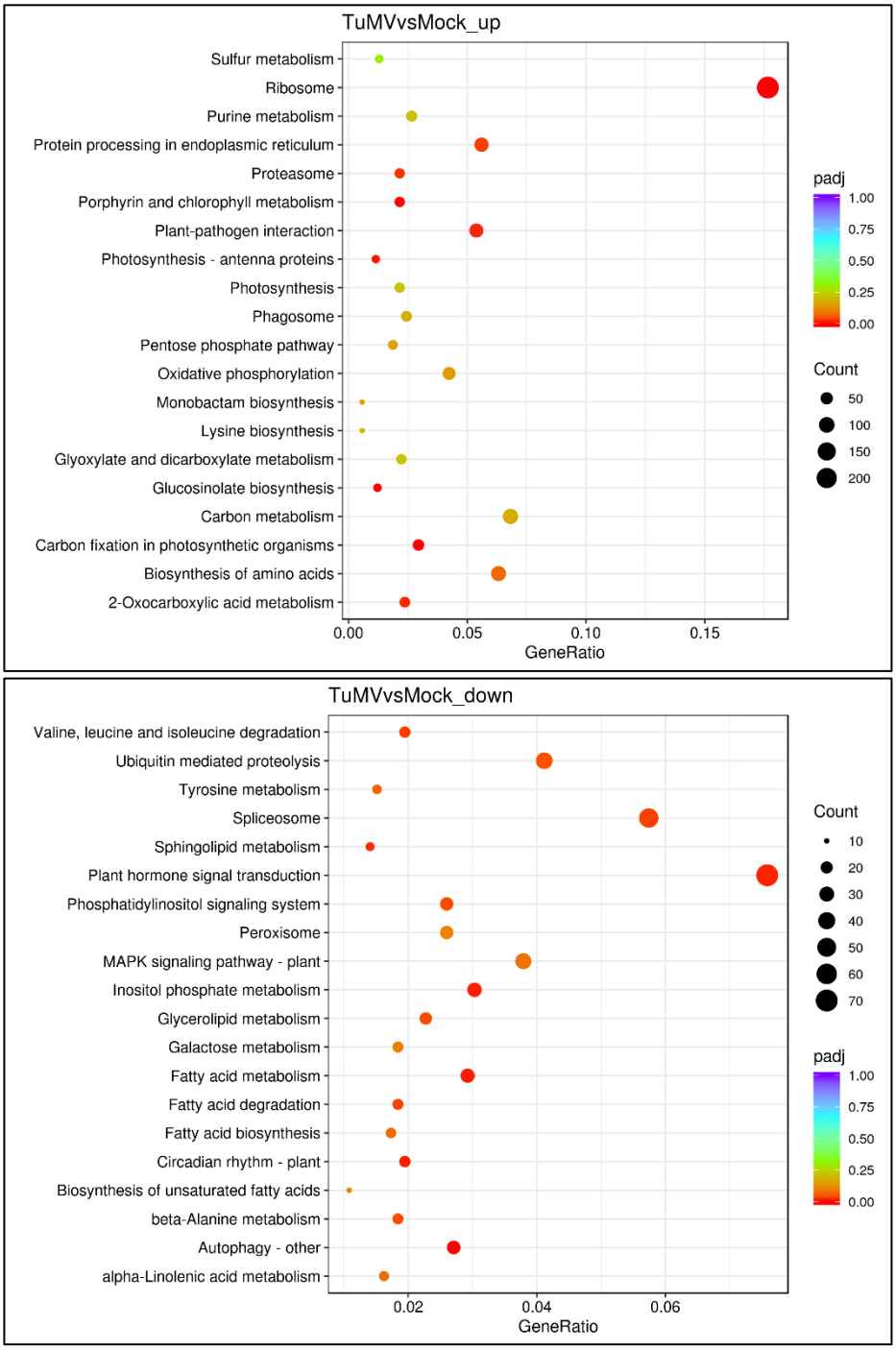


Figure 22 KEGG pathways regulated in DEG of 1st generation *The number of genes is resembled by the Count, e.g. the dot size and the significance of the padj by the color of the dots, with red being the most significant (Source: Novogene)*

The bioinformatic analysis of DEGs resulted in a list of up- and downregulated genes (further on called DEG list). A subunit of the DEG list is presented in the following, the original list contained more information. The columns in this list are the described in the following. The ATG Number (unique identification for *A. thaliana* genes) was included as well as the read counts (RC), resembling the expression strength of genes from both groups (Mock and TuMV). The Log2FC column shows the fold change of expression strength alteration between the groups. Lastly, gene description and gene name were included. All entries had a significant adjusted p-value of smaller than 0.05. Tables for all result chapters were produced similarly, if not mentioned otherwise. Table 11 shows the most strongly upregulated genes, Table 12 the most strongly downregulated genes, both sorted by Log2FC.

3.2.2.1 Differentially upregulated genes after TuMV infection

In the following, backgrounds of some of the most strongly upregulated genes (from Table 11 and the DEG list) will be described.

Table 11 20 most strongly upregulated genes in 1st generation *This list was made from the results list of differential gene analysis of the first generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene, the read counts (RC) of both samples used for DEG analysis are shown as well as the detected change (Log2FC). Further information about the gene in form of the gene description and the gene name is given.*

ATG Number	RC TuMV	RC Mock	Log2FC	Gene description	Gene name
AT3G01600	60.05	0.00	8.50	NAC domain containing protein 44 [Source:UniProtKB/TrEMBL;Acc:F4J4R5]	anac044
AT4G05540	201.13	0.87	7.81	P-loop containing nucleoside triphosphate hydrolases superfamily protein [Source:UniProtKB/TrEMBL;Acc:F4JGH5]	-
AT3G03660	173.59	1.47	6.86	WUSCHEL related homeobox 11 [Source:UniProtKB/TrEMBL;Acc:A0A1I9LND8]	WOX11
AT2G43880	15.24	0.00	6.52	Pectin lyase-like superfamily protein [Source:UniProtKB/TrEMBL;Acc:Q9SLM8]	-
AT5G39580	46.28	0.57	6.28	Peroxidase 62 [Source:UniProtKB/Swiss-Prot;Acc:Q9FKA4]	PER62
AT4G29600	24.02	0.29	6.22	Cytidine deaminase 7 [Source:UniProtKB/Swiss-Prot;Acc:Q9SU87]	CDA7

AT4G10860	36.75	0.94	5.34	F8M12.2 protein [Source:UniProtKB/TrEMBL;Acc:O81627]	-
AT1G74890	248.41	7.05	5.16	Two-component response regulator ARR15 [Source:UniProtKB/Swiss-Prot;Acc:Q7G8V2]	ARR15
AT3G45330	40.44	1.22	5.07	Putative L-type lectin-domain containing receptor kinase I.1 [Source:UniProtKB/Swiss-Prot;Acc:Q9M3E5]	LECRK11
AT5G44570	49.70	1.52	5.05	unknown protein; FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: endomembrane system; EXPRESSED IN: leaf whorl, hypocotyl, sepal, flower, leaf; EXPRESSED DURING: petal differentiation and expansion stag /.../08 eight leaves visible; Ha. [Source:TAIR;Acc:AT5G44570]	CAD1 / PROSCOOP5
AT5G62920	930.06	28.41	5.04	Response regulator 6 [Source:UniProtKB/TrEMBL;Acc:Q0WSS6]	ARR6
novel.27	437.65	13.47	5.02	-	-
AT4G09770	17.84	0.58	4.90	TRAF-like family protein [Source:TAIR;Acc:AT4G09770]	-
AT5G26170	359.52	12.88	4.80	Probable WRKY transcription factor 50 [Source:UniProtKB/Swiss-Prot;Acc:Q8VWQ5]	WRKY50
AT5G40010	224.67	8.00	4.80	AAA-ATPase ASD, mitochondrial [Source:UniProtKB/Swiss-Prot;Acc:Q9FLD5]	AATP1
AT2G21930	24.13	0.86	4.75	F-box protein At2g21930 [Source:UniProtKB/Swiss-Prot;Acc:Q9SJ06]	-
AT4G25200	21.46	0.86	4.59	HSP23.6-MITO [Source:UniProtKB/TrEMBL;Acc:A0A178USN5]	HSP23.6
AT1G13480	28.31	1.19	4.56	At1g13480 [Source:UniProtKB/TrEMBL;Acc:Q56Y29]	-
AT3G46090	511.63	21.62	4.56	ZAT7 [Source:UniProtKB/TrEMBL;Acc:A0A178VI57]	ZAT7
AT3G44350	171.75	7.58	4.49	NAC domain containing protein 61 [Source:UniProtKB/TrEMBL;Acc:B3H506]	anac061

The virus infection showed an effect on various plant mechanisms. Many of the affected groups are involved in plant defence, some directly, some via phytohormone induction. One prominent group was the NAC family: The most strongest upregulated gene (see Table 11) is *NAC044* (AT3G01600) which builds a large protein-protein network together with several WRKY factors

(Satapathy *et al.* (2018). NACs are transcription factors and very prominent regulators of plant immunity (Yuan *et al.* (2019). 31 NAC genes were found to be regulated (up or down) in the whole data set (see supplement).

Around the here upregulated *WOX11*, a whole network of around 700 regulated genes was found by researchers, these genes were related to areas like stress response and hormone signaling (Jiang *et al.*, 2017). Additionally, *WOX4* and *WOX13* were found weakly upregulated by TuMV infection in this data set.

Class III peroxidases are commonly regulated in biotic stress, as they are PR proteins (Almagro *et al.*, 2009). This study found 9 of the total 73 *A. thaliana* peroxidases upregulated, *PER62* (AT5G39580) being the most strongly. *PER62* was also found to be upregulated by PAMPs and ARRs (Arnaud *et al.*, 2017).

Genes coding for cytokinin receptors were found both enriched and reduced after virus infection. As type-B Arabidopsis response regulators (ARRs), their targets include but are not limited to genes involved in phytohormonal signaling. In this data set *ARR15* (AT1G74890) was found among the most strongly upregulated genes, it functions as a negative regulator in cytokinin-mediated signaling (T. Kiba, 2003). In total, 14 ARRs were found regulated in this data set (both up and down), with *ARR15* and *ARR6* .

WRKY transcription factors are very prominent regulators in plants, involved in various pathways. They are represented within the most strongly adjusted genes in this data set. *WRKY50* (AT5G26170) shows a strong upregulation. It has a significantly different DNA binding site from the consensus WRKY binding W-Box and it can solely induce expression of the prominent SA-induced *PR1* gene (pathogenesis-related) a marker for SAR (systemic acquired resistance) (Hussain *et al.*, 2018). Expression changes for *PR1* were detected in this data set too. Furthermore, *WRKY55* (AT2G40740) was found to be upregulated. It was shown that during bacterial infection this transcription factor induces ROS and SA accumulation, regulating thus resistance and leaf senescence (Wang *et al.*, 2020). In total, 22 WRKY genes had higher expression strength after virus infection, with the two WRKY genes described leading the list. To complete the picture, 6 WRKY genes showed a weak downregulation.

It comes as no surprise, that the prominent pathogen-related (PR) protein *PR1* is among the genes found with strong upregulations after virus infection. The 17 classes of PR genes are known to be induced upon various types of infection (van Loon *et al.*, 2006). *PR1* was shown to

be enriched after TuMV infection and new studies suggest a correlation between *PR1* accumulation and susceptibility of plants after TuMV infection (Otulak-Koziel *et al.*, 2020).

Calcium is an important signaling molecule during ETI and PTI, thus regulation of calcium channels is very important during infection, here a calcium channel was found to be within the most upregulated genes is *CNGC19* (AT3G17690) (Zhao *et al.*, 2021).

bHLH92 (AT5G43650) is part of the bHLH transcription factor group, regulating various plant mechanisms and controlling the jasmonic acid gene regulatory network and thus indirectly pathogen response (Falak *et al.*, 2021; Hickman *et al.*, 2017).

Arabinogalactan-proteins (AGPs) are involved in various developmental processes, their precise function is however still unknown. *AGP17* was highly upregulated in this data set. Researchers proposed a connection between *AGP17* on the plant surface and further intracellular changes in SA levels combined with altered expression of *PR1* (Gaspar *et al.*, 2004).

Phytohormonal changes in the plant after virus infection also cause many inducible genes to react, for example *AT5G38700* which is SA-regulated and NPR1-dependent (Singh *et al.*, 2015) or the auxin induced *SAUR7* (AT2G21200) (Goda *et al.*, 2004).

A counter-measure against the hypersensitive reaction could be the induced expression of *BON3* (AT1G08860), which together with *BON1* is thought to negatively regulate resistance-like genes (Li, 2009).

Multiple upregulated genes belong to different pathogen or stress response categories and their backgrounds are be described in the following. Firstly, zinc fingers are known to play a major role in defence response to abiotic and biotic stress; *ZAT9* plays a key role in salinity stress response (Ciftci-Yilmaz *et al.*, 2007). It is also induced by the polyamine spermine, a proposed signaling molecule for defence response and cell death in avirulent pathogen infections (Mitsuya *et al.*, 2009). Another zinc finger protein found heavily upregulated is *ZAT18*, a positive regulator upon drought stress (Yin *et al.*, 2017).

Heat shock proteins (HSPs) are induced by various stresses, here *HSP23.6* was enriched after virus infection. It is also induced by several other stresses (Sewelam *et al.*, 2019). *HSP23.6* is repressed by *IDL7*, a negative modulator of stress-related genes (Vie *et al.*, 2017).

A first wave counter measure against pathogens is the cell wall. In this data set, *XTH18* was upregulated. It was enriched too in a study investigating the effects of melatonin, supposedly

playing a role in plant defence (Weeda *et al.*, 2014). Furthermore, XTHs are involved in strengthening the cell wall and in growth modulation (Miedes *et al.*, 2013).

The mitochondria-localized *CYSTM3*, upregulated here, alters ROS homeostasis and prevents NA^+ efflux, thus regulating negatively salt stress tolerance (Xu *et al.*, 2019). *PUB54* was found upregulated and is thought to be involved in modulating defence signaling via *HMP35* (Spinti, 2021). The RLP family is involved in plant immunity, here *RLP11* was upregulated (Jamieson *et al.*, 2018). Another interesting group is the GRP family, playing a role in signaling and cellular stress response (Czolpinska & Rurek, 2018). *GRP9* (enriched in this data set) was found regulated by the multifactorial stress factor *HSFA7B*, especially in combination with a TuMV infection (Prasch, 2015). The induced cysteine-rich RLK *CRK39* is regulated by different types of stresses and hormones (Wrzaczek, 2010)

3.2.2.2 Differentially downregulated genes after TuMV infection

Following virus infection lead also to a repression of genes, serving various functions. Some of the most strongly inhibited genes (shown in Table 12) will be presented in the following.

Table 12 20 Most strongly downregulated genes from 1st generation *This list was made from the results list of differential gene analysis of the first generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene, the read counts (RC) of both samples used for DEG analysis are shown as well as the detected change (Log2FC). Further information about the gene in form of the gene description and the gene name is given.*

ATG Number	RC TuMV	RC Mock	Log2FC	Gene description	Gene name
AT5G23270	0.00	37.48	-7.54	Sugar transport protein 11 [Source:UniProtKB/Swiss-Prot;Acc:Q9FMX3]	STP11
AT1G58120	0.00	36.23	-7.49	Uncharacterized protein At1g58120/68103_m00121 [Source:UniProtKB/TrEMBL;Acc:Q9C6F6]	-
AT3G21180	0.00	36.22	-7.49	Calcium-transporting ATPase [Source:UniProtKB/TrEMBL;Acc:A0A178VER8] ;	ACA9
AT5G33370	0.35	63.94	-7.34	GDSL esterase/lipase At5g33370 [Source:UniProtKB/Swiss-Prot;Acc:Q8LB81]	CUS2
AT5G50830	0.00	27.83	-7.11	unknown protein; EXPRESSED DURING: L mature pollen stage, M germinated pollen stage, [Source:TAIR;Acc:AT5G50830]	-
AT5G28690	0.00	25.93	-7.01	At5g28690 [Source:UniProtKB/TrEMBL;Acc:Q6NQD5]	-

AT1G55570	0.00	21.84	-6.76	At1g55570/T5A14_1 [Source:UniProtKB/TrEMBL;Acc:Q9ZVV4]	sks12
AT2G26850	0.36	41.79	-6.73	F-box protein At2g26850 [Source:UniProtKB/Swiss-Prot;Acc:Q6NLB1]	-
AT1G66210	0.00	20.76	-6.69	Subtilisin-like protease SBT3.16 [Source:UniProtKB/Swiss-Prot;Acc:Q8GWX9]	SBT3.16
AT5G48140	0.38	40.10	-6.67	At5g48140 [Source:UniProtKB/TrEMBL;Acc:Q9LUB8]	-
AT5G15140	0.00	20.20	-6.65	Aldose 1-epimerase family protein [Source:UniProtKB/TrEMBL;Acc:Q9LXG7]	PEG9
AT5G58170	0.00	19.63	-6.61	Glycerophosphodiester phosphodiesterase GDPDL7 [Source:UniProtKB/Swiss-Prot;Acc:Q9LVN0]	GDPDL7
AT1G76370	0.00	19.26	-6.57	Probable serine/threonine-protein kinase PBL22 [Source:UniProtKB/Swiss-Prot;Acc:Q9SFX0]	PBL22
AT1G14420	0.38	36.27	-6.53	Probable pectate lyase 3 [Source:UniProtKB/Swiss-Prot;Acc:Q9M9S2]	AT59
AT4G03290	0.38	36.10	-6.52	Calmodulin-like protein 6 [Source:UniProtKB/Swiss-Prot;Acc:Q9ZR02]	CML6
AT1G04470	0.35	35.91	-6.51	F19P19.6 protein [Source:UniProtKB/TrEMBL;Acc:P93812]	-
AT3G28810	0.00	16.11	-6.32	Mediator of RNA polymerase II transcription subunit-like protein, putative (DUF1216) [Source:UniProtKB/TrEMBL;Acc:Q9LH93]	-
AT3G14040	0.38	31.06	-6.31	Pectin lyase-like superfamily protein [Source:UniProtKB/TrEMBL;Acc:Q9LVJ4]	-
AT4G38190	0.00	15.82	-6.29	Cellulose synthase-like protein D4 [Source:UniProtKB/Swiss-Prot;Acc:Q9SZL9]	CSLD4
AT4G31380	1.51	114.68	-6.29	Flowering-promoting factor 1-like protein 1 [Source:UniProtKB/Swiss-Prot;Acc:Q5Q0B3]	FLP1

Many downregulated genes after virus infection are flower- and pollen-specific genes, most of them involved in pollen tube growth: *STP11* (Schneidereit *et al.*, 2005), *ACA9* (Yu *et al.*, 2018), *CUS2* (Hong *et al.*, 2017), *SKS11* and *SKS12* (Zhou, 2013), *PRK4* (Chang *et al.*, 2013), *COBL10* (Li *et al.*, 2013), *CPK24* (Zhao *et al.*, 2013), *RALF4* (Mecchia, 2017), *CML6* (Nie *et al.*, 2017), *AT59* (Scarpin *et al.*, 2017), *CSDL4* (Wang *et al.*, 2011) and *SFH12* (Mo, 2007). Relations to pathogen defence are possible, as the genes code for saccharide transporters (like *STP11*) or calcium pumps (like *ACA9*), which have been shown to play a role before. However this has not been investigated for these genes yet.

Other repressed genes have various connections to pathogen defence: PBL proteins are known to be involved in PTI, however the *PBL22* downregulated here was not further investigated yet (Zhang *et al.*, 2010). Downregulation also happened to *FLA5*, a member of the FLA group which is known to respond to stress and development (Johnson *et al.*, 2003).

A probable interference with phytohormonal signaling could be caused by the downregulation of *PLC6*, a member of the phospholipase C pathway. Its activation is involved in the production of important defence signaling molecules (Canonne *et al.*, 2011).

An important player in immunity are pectin methylesterases, playing a key role in PAMP and immune response. They are especially vital in virus infection, as they are involved in virus spread (Chen, 2000). Regarding *PME67*, here downregulated, no further investigation was performed yet. Double mutant lines of *pme5* (downregulated here) and *pme31* (not regulated in this data set) were found to be more susceptible to bacteria infection (Bethke *et al.*, 2014). PMEs are also regulated after fungal infection. *PME21* downregulated by virus infection in the present study, showed an upregulation after fungi infection (Lionetti *et al.*, 2017). Lionetti et al. also investigated the importance of PME inhibitors (PMEI) in the regulation of PME. One PMEI (AT4G02250) was found downregulated in this data set, though no studies were done with this protein yet. Upregulation of PMEIs was found to improve plant resistance to various pathogens (including viruses) (Wan *et al.*, 2021).

Many of the regulated genes were found to be generally regulated by abiotic and biotic stress: *AT5G0830* is a stress responsive gene (Khan *et al.*, 2020) as well as *AT5G12000* (Coser *et al.*, 2017). Subtilases like *SBT3.16* were found induced after pathogen infection, but no studies for this specific gene were performed so far (Figueiredo *et al.*, 2014). A member of the pectin lyase-like family, *AT5G48140*, was regulated after osmotic or wound stress (Cao, 2012). Several genes involved in cell wall changes seem to be regulated. The regulation of *PEG9* for example might alter cell wall composition (Wolff *et al.*, 2015). Many of the previously mentioned genes involved in pollen growth regulation are also involved in cell wall composition.

Phytohormones influence plant defence in many ways and related genes were altered after virus infection. For example the MI oxygenase *MIOX4*, which is involved in ascorbic acid (AsA) biosynthesis (Lorence *et al.*, 2004). AsA is contributes in the plants defence to TuMV, which might be mediated by JA-dependent signaling pathways (Fujiwara *et al.*, 2016). The sulfotransferase SOT15/ST2A is involved in JA catabolism and presented itself as a molecular link between hormone signaling and photoreceptors (Fernandez-Milmanda *et al.*, 2020).

3.2.2.3 Analysis of strongest up- and downregulation genes with a read count threshold

In most cases DEG-lists sorted by Log2FoldChange revealed genes with very low read counts leading the lists as most strongly regulated. Although they might have very important functions, the differences in read count numbers between the mock treated and the infected samples were not high. The aftereffect are strong Log2FoldChanges which emerged from a possible mathematical bias and could be a "false positive" without further biological meaning. To consider this potential false picture of reality, the analysis of was slightly altered and another type of DEG lists were produced: The minimal read count difference between both groups was set to be at least 1000, which changed the most strongly regulated genes drastically. The resulting lists are not shown here. The setting of a cutoff for read count difference resulted in a decrease from 9588 to 1009 DEGs.

When the read count difference was set, the most strongly regulated genes changed. However, the overall outcome of upregulated pathways did not change dramatically: the former mentioned pathogenesis-related gene 1 (*PR1*), a key marker in pathogen defence was still leading the list.

As a new group, Arabidopsis response regulators (ARRs) came into the picture, known for their relations to cytokinin response. Here *ARR7* was found strongly regulated, acting as a transcriptional repressor for many early cytokinin-regulated genes (Lee *et al.*, 2007). Together with *ARR15*, *ARR7* also plays a role in suppressing shoot regeneration (Buechel, 2010). For many of the previously appearing groups, new members were found after shifting read count cutoff. For example the heat shock protein *HSP70-2*, that is induced by pathogens and heat shock treatment (Noel *et al.*, 2007). Also induced by pathogens is *TIR/TN10*, which pairs with TIR-NBS-LRR immune receptors to promote immunity (Chen *et al.*, 2021). TIR-NBS-LRR genes are important during defence response, as they are part of the effector triggered immunity (ETI). Here *AT5G41750* was found, which is regulated next to several other NLR genes by *HOS15* and *HDA9* via histone deacetylation (Yang *et al.*, 2020). A key player in disease resistance was detected, *ADR2*, which controls resistance via and enhanced disease susceptibility (EDS) and a SA-dependent defence signaling network (Aboul-Soud *et al.*, 2009). Another factor in immunity are SCOOP peptides, which act together with the receptor kinase MIK2 to trigger immune response. Here *SCOOP6* is regulated (Rhodes *et al.*, 2021). Regarding general stress response,

the BAM genes were found as another new group, with the here regulated *BAM5* being responsive to drought stress (Prasch, 2015).

Changes in phytohormone-related genes were already found to be regulated in many ways. Adding the read count cutoff lead to the emergence of several new candidates in this group: *CYP94C1*, which plays a role in JA-Ile hydroxylation (Caarls *et al.*, 2017). The newly appearing transcription factor *SARD1* was shown to control expression of SA synthesis genes and PTI, ETI as well as SAR genes together with *CBP60g* and both are targeted by pathogens during infection (Qin *et al.*, 2018). Next, overexpression of *PNP-A*, like observed here after virus infection, was shown to inhibit SA-mediated plant immune response (Lee *et al.*, 2020). Finally, a member of the WRKY family, strongly involved in phytohormones presented itself: *WRKY18*. It was shown to be induced by ABA and promote ABA sensitivity in plants as well as sensitivity to osmotic and salt stress, together in a network with *WRKY40* and *WRKY60* (Chen, 2010).

Regarding the downregulated genes with the read count difference threshold put into place, there were also no changes in regulated areas seen. However, also here the most strongly regulated genes changed, leading to interesting candidates:

Many genes are related to phytohormonal changes or are involved in defence. For example the strongly downregulated *BG1* gene that is induced by auxin and might regulate auxin transport (Liu *et al.*, 2015). Another example is *RD29A*, a gene induced by combined salt stress and ABA (Lee *et al.*, 2016). Regarding emerging genes related to pathogen defence, *BCAT2* was found strongly regulated. It is involved in the last step of BCAA biosynthesis (Binder, 2010) which might play a role in plant defence and SA-JA crosstalk (Maksym *et al.*, 2018). As in the original analysis approach, also here genes related to other types of stresses appeared, for example *CTPS1*, which is induced by salt stress and is involved in regulation of cell death (Alamdari *et al.*, 2021).

3.2.3 Effects of TuMV virus infection on the progeny

Virus infection, as other biotic and abiotic stresses has a huge impact on organisms. Effects can spread to next generations. For plants as sessile organisms, this can result in a more prepared and resistant offspring, which might have less struggle if faced with the similar or multiple challenges. The following chapter will focus on the analysis of transcriptional changes via RNA-Seq in plants originating from virus survivors.

To investigate changes in the offspring (from here on called 2nd generation or progeny) seeds from plants that were infected with TuMV were used. As a control group, seeds of mock treated plants were sawn. Plants were harvested after 3 weeks of growth. No additional infection took place in the 2nd generation. No relevant phenotype was visible, a slight delay in germination and a slightly smaller growth could be detected. As this could be due to several factors and number of plants grown was not big, no more detailed analysis in this direction was performed.

To provide comparability data sets were produced in the same way as for chapter 3.2.2. However, plants analysed in this chapter were 3 weeks old in contrast to plants in first generation which were 5 weeks old at the time of harvest. Additionally here all above-ground material was harvested, for chapter 3.2.2 only specific leaf material.

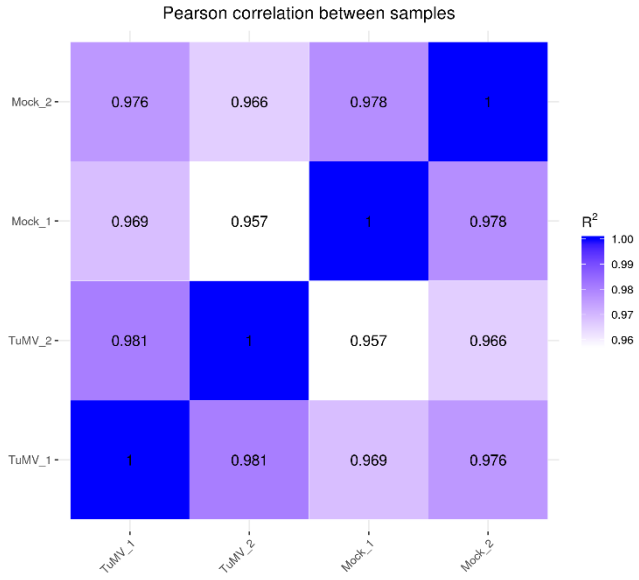


Figure 23 Pearson correlation of 2nd generation *This comparison shows similarity between the duplicates of mock treated and virus infected samples of the data set. The numbers range between 0 and 1, with 1 being full similarity. (Source: Novogene)*

The extracted RNA was used for a RNA-Seq, samples were send to sequencing as duplicates (Mock_1-2 and TuMV_1-2), where group names describe the treatment of the parent generation. Additionally to the RNA-Seq Analysis reads produced by RNA-Seq were mapped to the virus genome, with no matches (see supplementary Table 25). No viral RNA was present.

Data from each sample was investigated. One analysis created was the Pearson correlation (Figure 23). It showed that both groups (Mock and TuMV infected) clustered together resembling similarity of samples.

A differential expression analysis was made and bioinformatical tools were used to study alternating gene expression. The volcano plot (Figure 24) was one tool, it visualizes the

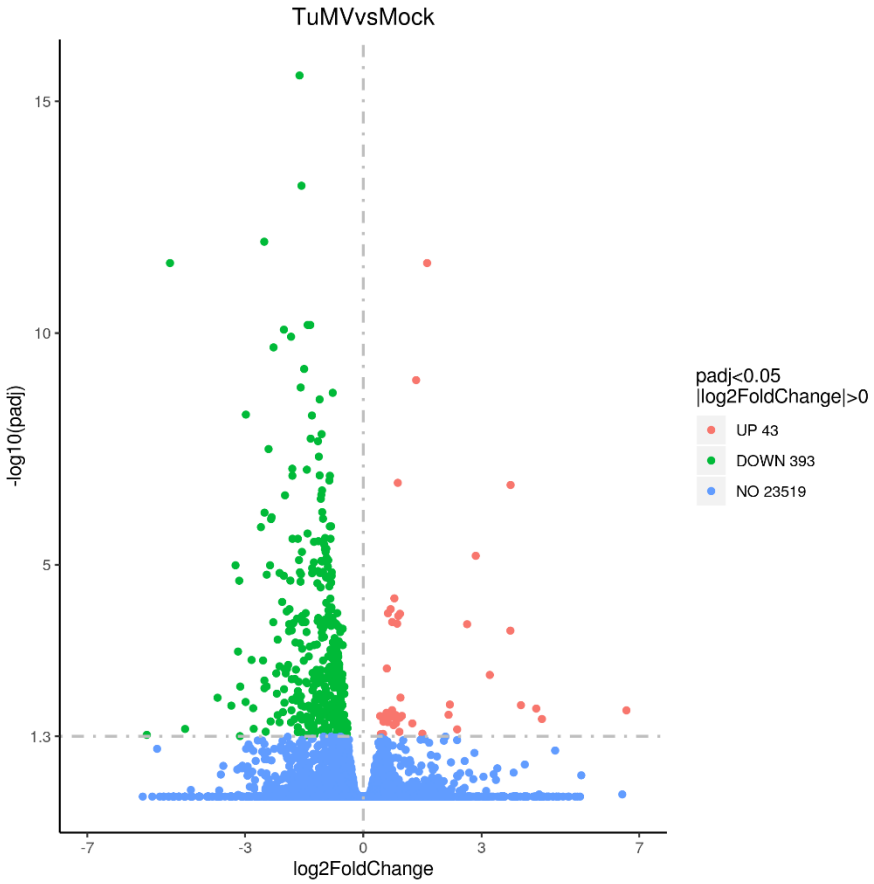


Figure 24 Volcano Plot of differentially expressed genes in the 2nd generation *The plot shows data set genes from the RNA-Seq of offspring plants of either mock treated or virus infected plants; All shown genes had an adjusted p-value higher than 0.05 and are sorted into upregulated (red), downregulated (green) and not regulated (blue). DEGs ranged from log2FC of approx. 7 to -6 with a log10(padj) of over 15 (Source: Novogene)*

distribution of differentially expressed genes. In this case the data of plant material from the offspring of virus infected and mock treated plants was compared. In the second generation only a small number of DEGs was found compared to previous generation. A large proportion of genes were downregulated (393 genes) and only 43 genes upregulated (all genes had a significance of 0.05 or lower).

The differential expression data was used for functional analysis. One of the bioinformatic tools used was to search for enriched KEGG pathways (Figure 25). Due to the small number of DEGs, the number of KEGG pathways is limited in the upregulated data. The very small count resembles a small number of genes belonging to this pathway found. The phenylpropanoid biosynthesis pathway was detected as enriched, followed by the nitrogen metabolism pathway and the cyanoamino acid metabolism pathway. All three have a small adjusted p-value, showing a strong significance. Among the downregulated genes, the plant-pathogen interaction pathway is the most enriched, followed by the protein processing in endoplasmic reticulum pathway. All pathways have a small count and only the two mentioned have a very small p-value.

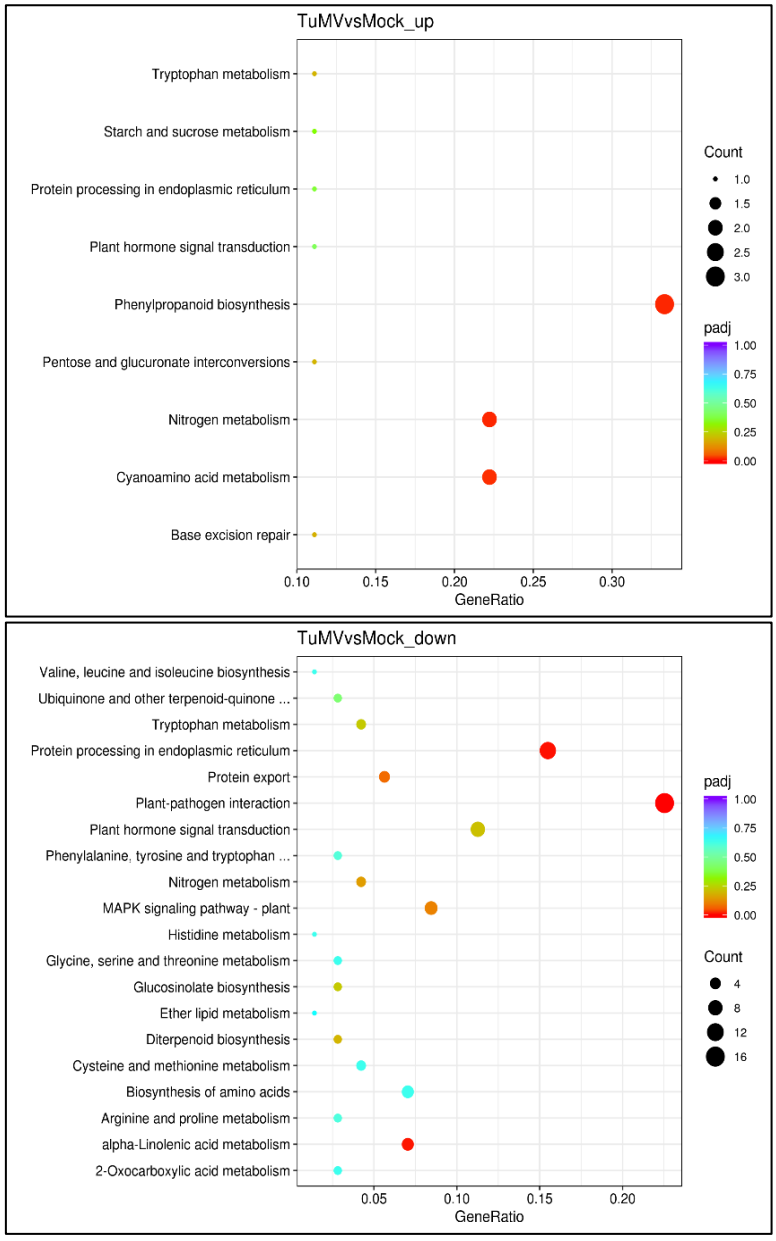


Figure 25 KEGG pathways regulated in DEG of 2nd generation *The number of genes is resembled by the Count, e.g. the dot size and the significance of the padj by the colour of the dots, with red being the most significant (Source: Novogene)*

3.2.3.1 Differentially upregulated genes in the offspring of TuMV infected plants

Investigating the differentially expressed gene (DEG) lists, the most strongly regulated DEGs were researched to gain background knowledge (Table 13). For more information on the production and outlay of the table, please see chapter 3.2.2.

Table 13 20 most strongly differentially upregulated genes in 2nd generation *This list was made from the results list of differential gene analysis of the second generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene, the read counts (RC) of both samples used for DEG analysis are shown as well as the detected change (Log2FC). Further information about the gene in form of the gene description and the gene name is given.*

ATG Number	RC TuMV	RC Mock	Log2FC	Gene description	Gene name
AT2G41850	18.13	0.00	6.67	Expressed protein [Source:UniProtKB/TrEMBL;Acc:Q8S8H2]	ADPG2
AT3G44300	279.92	12.11	4.53	ABC transporter G family member 36 [Source:UniProtKB/Swiss-Prot;Acc:Q9XIE2]	NIT2
AT3G09220	30.23	1.44	4.39	Protein ECS1 [Source:UniProtKB/Swiss-Prot;Acc:Q39066]	LAC7
AT5G39580	30.77	1.91	4.00	Uncharacterized protein At5g42530; MDH9.23 [Source:UniProtKB/TrEMBL;Acc:Q8W483]	PER62
AT1G30700	115.75	8.69	3.74	Cysteine-rich TM module stress tolerance protein [Source:UniProtKB/TrEMBL;Acc:Q8W472]	-
AT5G64100	51.25	3.84	3.73	Protein ACCELERATED CELL DEATH 6 [Source:UniProtKB/Swiss-Prot;Acc:Q8LPS2]	PER69
AT3G45060	66.78	7.22	3.21	Glutathione S-transferase F2 [Source:UniProtKB/Swiss-Prot;Acc:P46422]	NRT2.6
AT5G43580	149.92	20.79	2.85	Protein of unknown function (DUF567) [Source:TAIR;Acc:AT2G14560]	-
AT4G11650	86.83	14.00	2.63	SHV3-like 2 [Source:UniProtKB/TrEMBL;Acc:F4HQ30]	OSM34
AT5G38020	42.94	8.27	2.38	Wall-associated receptor kinase 1 [Source:UniProtKB/Swiss-Prot;Acc:Q39191]	-
AT1G09500	108.61	23.74	2.20	Calmodulin-like protein 12 [Source:UniProtKB/Swiss-Prot;Acc:P25071]	-
AT4G21680	86.65	19.40	2.16	Mediator of RNA polymerase II transcription subunit 37a [Source:UniProtKB/Swiss-Prot;Acc:Q9LKR3]	NPF7.2

AT4G37610	901.48	293.46	1.62	Aspartyl protease AED1 [Source:UniProtKB/Swiss-Prot;Acc:Q9LEW3]	BT5
AT1G73260	137.96	48.86	1.50	Lectin-like protein [Source:UniProtKB/Swiss-Prot;Acc:Q9LZF5]	KTI1
AT2G20670	801.40	316.29	1.34	Arabidopsis phospholipase-like protein (PEARLI 4) family [Source:TAIR;Acc:AT4G38550]	-
AT3G61920	169.69	71.78	1.24	Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 [Source:UniProtKB/Swiss-Prot;Acc:Q9SKB2]	-
AT4G38850	225.24	114.24	0.98	Nitrate reductase [NADH] 2 [Source:UniProtKB/Swiss-Prot;Acc:P11035]	SAUR15
AT5G56550	286.49	148.94	0.94	Heat shock protein 70 (Hsp 70) family protein [Source:TAIR;Acc:AT5G42020]	OXS3
AT5G61440	973.56	510.87	0.93	Cytochrome P450 83B1 [Source:UniProtKB/Swiss-Prot;Acc:O65782]	ACHT5
AT5G48490	233.19	122.39	0.93	Low-temperature-induced 78 kDa protein [Source:UniProtKB/Swiss-Prot;Acc:Q06738]	-

The analysis results of the progeny of virus infected plants consists only of a handful of genes with an upregulation. They are, however, quite interesting. Upregulation happens in various pathways and areas. As phytohormones are vital signaling molecules for plants, some genes related to them are altered in the offspring of virus infected plants. *NIT2* for example was found within the most strongly upregulated genes and might be involved in auxin biosynthesis, its expression is induced by bacteria and it probably affects resistance to bacteria and leaf senescence (Lehmann *et al.*, 2017). Another factor related to phytohormones is the differentially regulated *OSM34*, known as a positive regulator for ABA responses (Park & Kim, 2021). The regulated *SAUR15*, an early auxin induced gene, regulates auxin signaling (Yin *et al.*, 2020) and was shown to be downregulated by several stresses: heat, drought, virus infection and the combination of those (Prasch, 2015).

Two genes fall into the category of cell wall peroxidases: *PER62*, shown to be induced by ARRs and PAMPs (Arnaud *et al.*, 2017) and *PRX69/PER69*, which together with *PER62* mediate cell wall associated extensins and ROS homeostasis (Pacheco *et al.*, 2021). It is downregulated by NaCl treatment and is involved in cold tolerance (Jiang & Deyholos, 2006; Kim *et al.*, 2012).

Regarding direct relations to pathogen response, several genes were found upregulated. For example *BBE8*, which plays a role in stomatal response to bacteria infection (Rodrigues Oblessuc

et al., 2019). Also upregulated is the high affinity nitrate transporter *NRT2.6* which is suggested to be involved in ROS production during biotic and abiotic stress, it is induced by bacteria infection and plants show an inhibited tolerance to bacteria infection when *NRT2.6* is repressed (Dechorgnat *et al.*, 2012). The last example is *KTI1/KTI4*, which together with *KTI5* is regulating plant defence against herbivores (Arnaiz *et al.*, 2018).

Various upregulated genes are connected to different types of stresses: The oxidative stress protein 3 (*OXS3*) is involved in resistance to cadmium and could positively regulate resistance to virus (Wang, 2012).

3.2.3.2 Differentially downregulated genes in the offspring of TuMV infected plants

Decrease of expression strength was much more common in the data set from the progeny of virus infected plants than an increase. The 20 most strongly repressed genes (sorted by Log2Foldchange) are shown in Table 14 and genes will be presented in the following text.

Table 14 20 most strongly differentially downregulated genes in 2nd generation *This list was made from the results list of differential gene analysis of the second generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene, the read counts (RC) of both samples used for DEG analysis are shown as well as the detected change (Log2FC). Further information about the gene in form of the gene description and the gene name is given.*

ATG Number	RC TuMV	RC Mock	Log2FC	Gene description	Gene name
AT5G44920	0.53	23.18	-5.49	Expressed protein [Source:UniProtKB/TrEMBL;Acc:Q8S8H2]	TIK
AT5G50580	4.57	138.02	-4.90	ABC transporter G family member 36 [Source:UniProtKB/Swiss-Prot;Acc:Q9XIE2]	SAE1B-2
AT1G66600	1.02	23.58	-4.52	Protein ECS1 [Source:UniProtKB/Swiss-Prot;Acc:Q39066]	WRKY63
AT1G69930	2.59	33.43	-3.69	Uncharacterized protein At5g42530; MDH9.23 [Source:UniProtKB/TrEMBL;Acc:Q8W483]	GSTU11
AT4G10500	27.82	282.61	-3.34	Cysteine-rich TM module stress tolerance protein [Source:UniProtKB/TrEMBL;Acc:Q8W472]	DLO1
AT1G67000	9.44	88.28	-3.24	Protein ACCELERATED CELL DEATH 6 [Source:UniProtKB/Swiss-Prot;Acc:Q8LPS2]	LRK10L-2.8
AT1G65610	5.67	51.22	-3.17	Glutathione S-transferase F2 [Source:UniProtKB/Swiss-Prot;Acc:P46422]	KOR2

AT3G15536	9.44	82.73	-3.14	Protein of unknown function (DUF567) [Source:TAIR;Acc:AT2G14560]	-
AT3G48630	3.12	27.10	-3.12	SHV3-like 2 [Source:UniProtKB/TrEMBL;Acc:F4HQ30]	-
AT4G21830	35.59	309.14	-3.12	Wall-associated receptor kinase 1 [Source:UniProtKB/Swiss-Prot;Acc:Q39191]	MSRB7
AT3G24900	5.78	45.28	-2.98	Calmodulin-like protein 12 [Source:UniProtKB/Swiss-Prot;Acc:P25071]	AtRLP39
AT5G25260	20.90	163.44	-2.97	Mediator of RNA polymerase II transcription subunit 37a [Source:UniProtKB/Swiss-Prot;Acc:Q9LKR3]	FLOT2
AT1G61120	87.62	624.15	-2.83	Aspartyl protease AED1 [Source:UniProtKB/Swiss-Prot;Acc:Q9LEW3]	GES
AT1G15520	54.94	380.12	-2.79	Lectin-like protein [Source:UniProtKB/Swiss-Prot;Acc:Q9LZF5]	ABCG40
AT2G14290	5.18	35.46	-2.77	Arabidopsis phospholipase-like protein (PEARLI 4) family [Source:TAIR;Acc:AT4G38550]	-
AT4G00700	23.99	144.26	-2.59	Leucine-rich repeat receptor-like serine/threonine/tyrosine-protein kinase SOBIR1 [Source:UniProtKB/Swiss-Prot;Acc:Q9SKB2]	-
AT5G07010	11.42	66.23	-2.54	Nitrate reductase [NADH] 2 [Source:UniProtKB/Swiss-Prot;Acc:P11035]	SOT15
AT2G43570	64.94	369.13	-2.51	Heat shock protein 70 (Hsp 70) family protein [Source:TAIR;Acc:AT5G42020]	CHI
AT3G13610	10.32	58.64	-2.50	Cytochrome P450 83B1 [Source:UniProtKB/Swiss-Prot;Acc:O65782]	F6'H1
AT4G04500	10.39	58.80	-2.50	Low-temperature-induced 78 kDa protein [Source:UniProtKB/Swiss-Prot;Acc:Q06738]	CRK37

Several of the downregulated genes show a connection to defence responses. For example *SAE1B*, coding for one of the SUMO subunits, SUMO is an important key player in pathogen defence (Sharma *et al.*, 2021). Also found downregulated is *FLOT2*, which is interacting with many defence proteins (Junkova *et al.*, 2018). Next on *DLO1*, studies found this gene to be co-expressed with *DMR6*, it is induced by fungal and bacterial infection as well as SA, probably acting as a suppressor of plant immunity (Zeilmaker *et al.*, 2015). The last example from this group, *GES*, is induced by JA and catalyzes the first step in TMTT biosynthesis, a key signaling molecule in insect defence (Attaran, 2008; Herde *et al.*, 2008).

Phytohormone-related genes were also found downregulated. The ABA overly sensitive WRKY transcription factor *WRKY62/ABO3* is inhibited, this gene is involved in ABA and drought stress response (Ren *et al.*, 2010). The downregulated *KOR2/GH9A2* is a SA-induced SAR gene (Bernsdorff *et al.*, 2016). Also downregulated is the ABA transporter *ABCG40*, which is induced by TuMV (Manacorda, 2021). The last gene to be mentioned in relation with phytohormones is *SOT15/ST2A*, a sulfotransferase which is connected to JA catabolism and is a prominent linker connecting photoreceptors and hormone signaling (Fernandez-Milmanda *et al.*, 2020)

Connected to general stress are the following highly downregulated genes: *GSTU11*, regulated by WRKY factors and induced by fungi and bacterial infection (Gullner *et al.*, 2018). *MSRB7* is involved in oxidative stress and is induced by cell death activation after pathogen or UV stress (Li *et al.*, 2012; Roy & Nandi, 2017). Lastly, inhibition of *CRK37* occurred, for this gene an induction by a PTI positive regulator was shown, however a resistance to bacteria could not be detected (Yeh *et al.*, 2015).

3.2.3.3 Analysis of up- and downregulated genes with a read count threshold in the progeny

To investigate genes with a high read count difference like in the first generation, the threshold for the whole DEG list was set to 1000 and the strongest DEGs were investigated again. This cutoff reduced the number of DEGs from 437 to 55. Only 5 genes were left upregulated and the most strongly regulated genes all changed.

With this new approach, an upregulated gene comes into focus that also explains the upregulated KEGG phenylpropanoid biosynthesis pathway. *KFB50* is involved in controlling the proteolytic turnover of PAL isozymes, responsible for catalyzing the first step in phenylpropanoid biosynthesis (Zhang *et al.*, 2013).

Regarding the downregulation, some new genes appear with the new analysis approach – however they all are related to topics which emerged before. Examples for genes involved in biotic and abiotic stress response will be mentioned in the following. The Fold change of downregulation was not as strong as in lists without cutoff.

Again, many genes were found regulating defence response: The thaumatin-like *PR5* was inhibited in the offspring of virus infected plants, this gene is part of the prominent pathogenesis-related proteins and induced by SA to promote SAR during biotic stress (Ali *et al.*, 2018).. Two interesting candidates, *AED1* and *LLP1*, might be involved in a feedback

mechanisms controlling systemic immunity; they are both induced by SA and SAR with *AED1* probably being involved in a feedback loop controlling systemic immunity whereas downregulation of *LLP1* lead to a compromised SAR response with local resistance being untouched (Breitenbach *et al.*, 2014). Also downregulated was *EDA39*, this calmodulin-binding protein indirectly increases JA and promotes resistance against fungi (Lv *et al.*, 2019). *GSTF2* is induced by SA and ET signaling and was shown to reduce damage caused by pathogen infection (Lieberherr, 2003). Next on, a very important candidate *DMR6* is inhibited in this data set. This gene was found to be downregulated by JA but highly upregulated by SA treatment and is involved in inhibiting plant immunity, downregulation lead to resistance to various pathogens (Caarls *et al.*, 2017; Zeilmaker *et al.*, 2015). The cell wall associated receptor kinase *WAK1* is a PR gene and induced by pathogens, SA or INA and protects the plant from damages during defence response, it promotes for example survival during otherwise lethal SA levels in form of a feedback loop (He, 1998). The LRR-RLK *RLK7* is a receptor of *PIP1*, a PAMP-induced secreted peptide promoting immune signaling responses (Hou *et al.*, 2014). For *CRT3* an involvement in PAMP associated responses, more specifically responsiveness to elf18, has been studied (Christensen *et al.*, 2010). The senescence marker *STP13* was found upregulated after infection with CaLCuV virus, it is not involved in SA and ET pathways (Ascencio-Ibanez *et al.*, 2008). This sugar transporter is also responsible for a defence strategy against bacteria, in which uptake is enhanced to compete with bacteria for external sugar (Yamada, 2016).

Some genes repressed in this data set showed a background in induction due to phytohormones or pathogens, with no further functions studied yet. For example the resistance gene *HR4* is induced by several pathogens as well as phytohormones (Saenz-Mata & Jimenez-Bremont, 2012). *XBAT34* was shown to be repressed by ABA (Pavicic *et al.*, 2019).

Also genes acting on various stress responses appear newly within the most downregulated genes after changing the read count difference. *BCB* for example, which is known for its involvement in lignin metabolism, promotes resistance to aluminium stress as well as induction by various pathogens as a proposed early-induced cell wall-based defence mechanism (Ezaki *et al.*, 2005; Mishina & Zeier, 2007). The gene *CML12/TCH3* was shown to be regulated by touch and darkness and interacts with *PEN3*, playing a role in nonhost resistance together (Campe *et al.*, 2016; Sistrunk, 1994). *SOBIR1/EVR* is involved in RLP-mediated immunity to fungi infection (Liebrand *et al.*, 2013). The receptor kinase *MIK2* comes into play after altered cellulose biosynthesis as a cell wall damage response factor, mutant lines show furthermore changes in immune marker gene expression lignin deposition and JA biosynthesis (Van der Does *et al.*,

2017). The hypersensitive induced reaction protein *HIR2* might inhibit RPS2-mediated ETI when downregulated, as mutant lines showed stronger bacteria growth after infection, on the other hand induced *HIR2* associates with *RPS2* and contributes to ETI (Qi *et al.*, 2011); *HIR2* was also found to be upregulated after attack by virus and other pathogens (Daněk *et al.*, 2016).

3.2.4 Comparison between DEG of 1st and 2nd generation

As previously mentioned, transcriptome changes after infection are diverse and some effects can reach into the next generation. To investigate such changes, a comparison between the analysis of the first (infected) generation and the second (descendant and uninfected) generation was made.

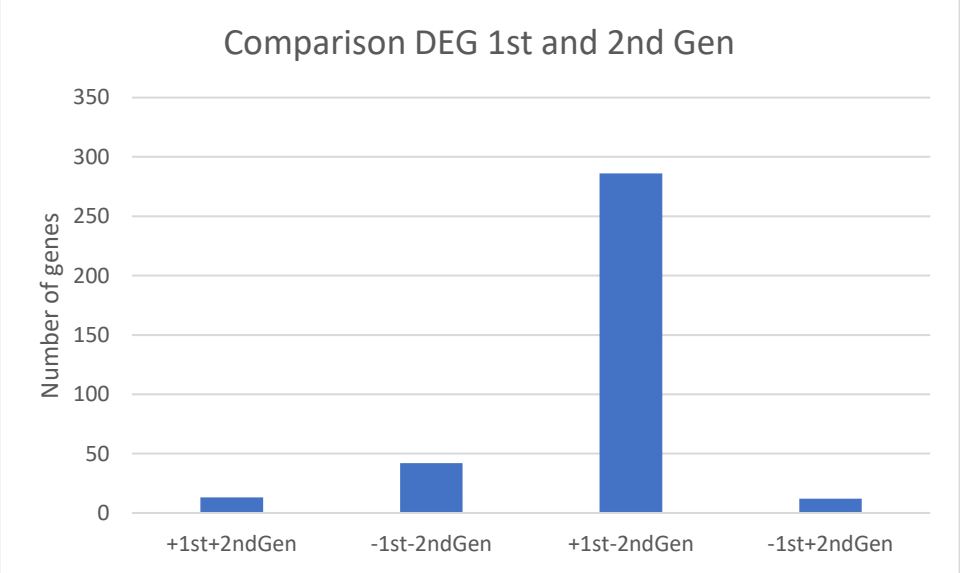


Figure 26 Comparison of DEGs between 1st and 2nd generation *Upregulation is marked with a + and downregulation with a -.*

The program Access was used to analyse genes found in two DEG lists. A comparison between 1st and 2nd generation DEG lists resulted in the following (see Figure 26) whereby upregulation is marked with a (+) and downregulation with a (-): 13 genes were found upregulated in both experiments (+1st+2ndGen), 42 genes were found downregulated in both analyses (-1st-2ndGen), 286 genes were upregulated in the 1st generation but downregulated in the 2nd (+1st-2ndGen) and finally 12 genes were found showing a vice versa regulation (-1st+2ndGen).

Particularly interesting are genes that kept their expression profile in both generations, these were further investigated. A list of the 12 genes that showed a gain in expression in both generations is shown in Table 15, no cutoffs were made.

Table 15 All genes upregulated in both 1st and 2nd generation *This list was made from the results list of differential gene analysis of the first and second generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene and the detected change (Log2FC) in both data sets is shown. Further information about the gene in form of the gene description and the gene name is given.*

ATG Number	1stGen Log2FC	2ndGen Log2FC	Gene description	Gene name
AT5G39580	6.28	4.00	Peroxidase 62 [Source:UniProtKB/Swiss-Prot;Acc:Q9FKA4]	PER62
AT5G38020	4.58	2.38	At5g38020 [Source:UniProtKB/TrEMBL;Acc:Q84MB1]	-
AT5G64100	4.45	3.73	Peroxidase [Source:UniProtKB/TrEMBL;Acc:A0A178U798]	PER69
AT2G05440	3.86	0.77	Glycine-rich protein 9 [Source:UniProtKB/TrEMBL;Acc:Q9SL23]	ATGRP9
AT3G44990	3.41	0.69	Xyloglucan endotransglucosylase/hydrolase protein 31 [Source:UniProtKB/Swiss-Prot;Acc:P93046]	XTH31
AT4G38850	3.29	0.98	Auxin-responsive protein SAUR15 [Source:UniProtKB/Swiss-Prot;Acc:Q41220]	SAUR15
AT1G13650	1.81	0.62	At1g13650 [Source:UniProtKB/TrEMBL;Acc:A2RVL9]	-
AT4G11650	1.58	2.63	OSM34 [Source:UniProtKB/TrEMBL;Acc:A0A178V0T2]	OSM34
AT3G09260	1.32	0.63	PYK10 [Source:UniProtKB/TrEMBL;Acc:A0A178VCN3]	BGLU23
AT5G44680	0.76	0.60	DNA glycosylase superfamily protein [Source:UniProtKB/TrEMBL;Acc:Q9FIZ5]	-
AT2G38870	0.73	0.59	Putative protease inhibitor [Source:UniProtKB/TrEMBL;Acc:Q9ZV18]	-
AT1G73600	0.49	0.43	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein [Source:TAIR;Acc:AT1G73600]	PMT3

DEGs found upregulated in both generations mostly had involvements with plant defence. The strongest upregulated gene was for example *PER62*, a peroxidase which is upregulated by ARRs and PAMPs during pathogen infection (Arnaud *et al.*, 2017). Another regulated peroxidase is shown, *PER69*, which was shown to be downregulated by NaCl and is involved in cold tolerance together with ROS (Jiang & Deyholos, 2006; Kim *et al.*, 2012). *GRP9* is regulated by different stresses, also regulation after TuMV infection was shown in another study (Prasch, 2015); GRPs generally are involved in stress response and signaling (Czolpinska & Rurek, 2018). *XTH31* is involved in aluminium sensitivity by modulating cell wall changes (Zhu *et al.*, 2012). Further on, *SAUR15* is induced by and regulates auxin and is downregulated by several stresses (Prasch, 2015; Yin *et al.*, 2020). *OSM34* was shown to positively regulate ABA responses (Park & Kim, 2021). The β-glucosidase *BGLU23* may be indirectly involved in abiotic and pathogen defence (Dong *et al.*, 2019).

Downregulation in both data sets was found for 42 genes, here a selection of stronger regulated genes is shown (see Table 16).

Table 16 Genes downregulated in both 1st and 2nd generation *This list was made from the results list of differential gene analysis of the first and second generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene and the detected change (Log2FC) in both data sets is shown. Further information about the gene in form of the gene description and the gene name is given. Only genes with both Log2FC greater than 1 are included with an exception if one Log2FC is greater than 2.*

ATG Number	1stGen Log2FC	2ndGen Log2FC	Gene description	Gene name
AT5G07010	-5.26	-2.54	Sulfotransferase [Source:UniProtKB/TrEMBL;Acc:A0A178UG65]	SOT15
AT5G50580	-4.68	-4.90	SUMO-activating enzyme subunit 1B-1 [Source:UniProtKB/Swiss-Prot;Acc:P0DI12]	SAE1B-2
AT1G04180	-4.33	-1.66	Flavin-containing monooxygenase [Source:UniProtKB/TrEMBL;Acc:A0A178WB22]	YUC9
AT5G61160	-4.26	-1.24	Agmatine coumaroyltransferase [Source:UniProtKB/Swiss-Prot;Acc:Q9FNP9]	ACT
AT4G32280	-3.84	-1.04	Auxin-responsive protein [Source:UniProtKB/TrEMBL;Acc:Q2VWA0]	IAA29
AT1G10070	-3.51	-2.01	Branched-chain-amino-acid aminotransferase 2, chloroplastic [Source:UniProtKB/Swiss-Prot;Acc:Q9M439]	BCAT2
AT5G12050	-3.19	-0.60	Protein BIG GRAIN 1-like D [Source:UniProtKB/Swiss-Prot;Acc:Q9LYH0]	BG1

AT4G16260	-3.10	-1.51	Probable glucan endo-1,3-beta-glucosidase At4g16260 [Source:UniProtKB/Swiss-Prot;Acc:Q8VZJ2]	-
AT5G52310	-2.84	-0.60	Low-temperature-induced 78 kDa protein [Source:UniProtKB/Swiss-Prot;Acc:Q06738]	RD29A
AT1G15125	-2.81	-0.79	F9L1.6 [Source:UniProtKB/TrEMBL;Acc:Q9XI57]	-
AT5G44920	-2.40	-5.49	TIR domain-containing protein [Source:UniProtKB/Swiss-Prot;Acc:Q0WSX8]	TIK
AT2G44910	-2.39	-1.73	Uncharacterized protein At2g44910 (Fragment) [Source:UniProtKB/TrEMBL;Acc:C0SV86]	ATHB-4
AT1G53100	-2.30	-2.12	At1g53100 [Source:UniProtKB/TrEMBL;Acc:Q6DBE8]	-
AT4G16780	-2.19	-1.13	Homeobox-leucine zipper protein HAT4 [Source:UniProtKB/Swiss-Prot;Acc:Q05466]	HAT4
AT4G21830	-1.36	-3.12	Peptide methionine sulfoxide reductase B7 [Source:UniProtKB/Swiss-Prot;Acc:Q8VY86]	MSRB7
AT5G45380	-1.34	-1.08	DUR3 [Source:UniProtKB/TrEMBL;Acc:A0A178UG75]	DUR3
AT4G02520	-1.27	-1.43	Glutathione S-transferase F2 [Source:UniProtKB/Swiss-Prot;Acc:P46422]	GSTF2
AT1G17745	-1.13	-1.07	D-3-phosphoglycerate dehydrogenase [Source:TAIR;Acc:AT1G17745]	PGDH2
AT3G45860	-1.09	-1.34	Cysteine-rich receptor-like protein kinase 4 [Source:UniProtKB/Swiss-Prot;Acc:Q9LZU4]	CRK4

Downregulation of genes in both generations was also mostly in defence related groups. Inhibited in both data sets is a SUMO subunit, *SAE1B-2* (Sharma *et al.*, 2021). Many genes are involved in phytohormonal signaling, for example *SOT15/ST2A*, a link connecting phytohormones and photoreceptors and playing a role in JA catabolism (Fernandez-Milmanda *et al.*, 2020). Also *YUC9*, which is involved in auxin biosynthesis and was also found regulated after various stresses (Cao *et al.*, 2019; Xu *et al.*, 2017). *IAA29* is a positive regulator for JA-mediated leaf senescence, the same study brought light into JA and Auxin crosstalk in leaf senescence via *WRKY57*, which was shown to interact with *IAA29* (Jiang *et al.*, 2014).

The list of most regulated genes in the context of being upregulated in 1st generation and downregulated in the 2nd generation can be found in Table 17.

Table 17 Genes upregulated in 1st and downregulated in 2nd generation *This list was made from the results list of differential gene analysis of the first and second generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene and the detected change (Log2FC) in both data sets is shown. Further information about the gene in form of the gene description and the gene name is given.*

ATG Number	1stGen Log2FC	2ndGen Log2FC	Gene description	Gene name
AT5G26170	4.80	-2.12	Probable WRKY transcription factor 50 [Source:UniProtKB/Swiss-Prot;Acc:Q8VWQ5]	WRKY50
AT1G01680	3.95	-2.19	U-box domain-containing protein 54 [Source:UniProtKB/Swiss-Prot;Acc:Q9LQ92]	PUB54
AT2G26400	3.68	-2.35	acireductone dioxygenase 3 [Source:TAIR;Acc:AT2G26400]	ARD
AT3G13610	3.59	-2.51	Feruloyl CoA ortho-hydroxylase 1 [Source:UniProtKB/Swiss-Prot;Acc:Q9LHN8]	F6'H1
AT4G03450	3.37	-2.28	Ankyrin repeat family protein [Source:UniProtKB/TrEMBL;Acc:Q9ZT73]	-
AT1G66600	3.36	-4.52	Probable WRKY transcription factor 63 [Source:UniProtKB/Swiss-Prot;Acc:Q9C6H5]	WRKY63
AT4G14370	3.30	-1.66	Disease resistance protein (TIR-NBS-LRR class) family [Source:TAIR;Acc:AT4G14370]	-
AT5G02490	3.16	-0.68	Probable mediator of RNA polymerase II transcription subunit 37c [Source:UniProtKB/Swiss-Prot;Acc:P22954]	MED37D
AT1G09080	3.15	-2.17	Probable mediator of RNA polymerase II transcription subunit 37b [Source:UniProtKB/Swiss-Prot;Acc:Q8H1B3]	MED37B
AT4G39830	3.14	-2.32	At4g39830 [Source:UniProtKB/TrEMBL;Acc:O65670]	-
AT1G56660	3.11	-0.83	F25P12.91 protein [Source:UniProtKB/TrEMBL;Acc:Q9FXB5]	-
AT3G48630	3.07	-3.13	unknown protein; BEST Arabidopsis thaliana protein match is: unknown protein (TAIR:AT3G44150.1); Ha. [Source:TAIR;Acc:AT3G48630]	-
AT5G25260	2.99	-2.98	Flotillin-like protein 2 [Source:UniProtKB/Swiss-Prot;Acc:Q4V3D6]	FLOT2
AT2G27660	2.99	-1.96	Cysteine/Histidine-rich C1 domain family protein [Source:UniProtKB/TrEMBL;Acc:Q9ZUW8]	-

AT1G01560	2.96	-1.56	Mitogen-activated protein kinase 11 [Source:UniProtKB/Swiss-Prot;Acc:Q9LMM5]	MPK11
AT5G42830	2.96	-1.82	HXXXD-type acyl-transferase family protein [Source:UniProtKB/TrEMBL;Acc:Q9FMN6]	-
AT1G72520	2.95	-1.15	Lipoxygenase 4, chloroplastic [Source:UniProtKB/Swiss-Prot;Acc:Q9FNX8]	LOX4
AT5G52760	2.89	-2.17	Heavy metal-associated isoprenylated plant protein 14 [Source:UniProtKB/Swiss-Prot;Acc:Q9LTE1]	HIPP14
AT4G18253	2.88	-1.39	Receptor Serine/Threonine kinase-like protein [Source:UniProtKB/TrEMBL;Acc:A0A1P8B6P8]	-

WRKY50 is a prominent inducer of the SAR marker PR1 (Hussain *et al.*, 2018). The other regulated WRKY gene, *WRKY63* is involved in the expression regulation of mitochondrial and chloroplastic coded stress-responsive genes (Van Aken *et al.*, 2013). *PUB54* might be involved in defence signal modulation (Spinti, 2021). *FLOT2* was fount to interact with various defence proteins (Junkova *et al.*, 2018).

In the comparison between 1st and 2nd generation DEGs, most genes were found to follow the lastly presented pattern of upregulation in 1st generation and downregulation in 2nd generation. As the number of genes is very high in this context, a GO analysis was done to analyse this group further. Analysis was done via the website http://geneontology.org/ which uses the software PANTHER (Mi *et al.*, 2019). Results of enriched biological process GO terms can be found in Table 18, with the 50 most strongly enriched GO terms shown, the full list is in Supplementary.

Table 18 Analysis results for enriched GOs *An additional GO term analysis was made for genes upregulated in 1st generation and downregulated in 2nd generation. Analysis was done using the website http://geneontology.org/ with the software PANTHER (Mi et al., 2019) .The results show the enriched GO biological proces, the number of genes in GO (# in GO) and how many of these genes were found regulated in the analysis (# regulated). Lastly, a fold enrichment describes the strength of the alteration.*

GO biological process	# in GO	# regulated	Fold Enrichment
salicylic acid biosynthetic process	8	3	37.27
regulation of salicylic acid biosynthetic process	13	4	30.58
regulation of salicylic acid metabolic process	21	6	28.4
response to singlet oxygen	14	4	28.4

nitrate assimilation	11	3	27.1
nitrate metabolic process	11	3	27.1
regulation of systemic acquired resistance	22	5	22.59
reactive nitrogen species metabolic process	14	3	21.3
nitrogen cycle metabolic process	15	3	19.88
jasmonic acid biosynthetic process	25	5	19.88
defence response by cell wall thickening	17	3	17.54
negative regulation of defence response to bacterium	17	3	17.54
oxylipin biosynthetic process	17	3	17.54
negative regulation of cell death	40	7	17.39
negative regulation of programmed cell death	29	5	17.14
phenol-containing compound biosynthetic process	18	3	16.56
pattern recognition receptor signaling pathway	24	4	16.56
cellular response to biotic stimulus	18	3	16.56
defence response by callose deposition	25	4	15.9
regulation of jasmonic acid mediated signaling pathway	42	6	14.2
endoplasmic reticulum unfolded protein response	28	4	14.2
response to molecule of bacterial origin	44	6	13.55
plant-type hypersensitive response	46	6	12.96
regulation of salicylic acid mediated signaling pathway	31	4	12.82
response to salicylic acid	156	20	12.74
programmed cell death induced by symbiont	47	6	12.69
biological process involved in interaction with symbiont	48	6	12.42
protein refolding	32	4	12.42
response to oomycetes	97	12	12.29
immune response-regulating signaling pathway	49	6	12.17
response to ozone	33	4	12.05
cellular response to unfolded protein	42	5	11.83
response to unfolded protein	42	5	11.83
cellular response to salicylic acid stimulus	55	6	10.84

salicylic acid mediated signaling pathway	46	5	10.8
response to bacterium	489	53	10.77
defence response to oomycetes	74	8	10.74
systemic acquired resistance	65	7	10.7
defence response to bacterium	372	40	10.69
recognition of pollen	48	5	10.35
regulation of cell death	97	10	10.25
jasmonic acid metabolic process	49	5	10.14
pollen-pistil interaction	69	7	10.08
cell recognition	50	5	9.94
calcium ion transmembrane transport	40	4	9.94
programmed cell death	81	8	9.82
regulation of defence response to bacterium	51	5	9.74
regulation of immune response	115	11	9.51
regulation of immune system process	115	11	9.51

First of all, the analysis shows an enrichment in GO terms involved in SA signaling as well as JA signaling. Next, an enrichment on GO terms concerning bacteria infection is visible as well as plant defence mechanisms like cell wall thickening, programmed cell death or hypersensitive response. Lastly, GO terms involved in defence were found, for example immune responses or SAR.

The list of genes downregulated after virus infection and upregulated in the offspring is very short (see Table 19).

Table 19 All genes downregulated in 1st and upregulated in 2nd generation *This list was made from the results list of differential gene analysis of the first and second generation data set. All information within the table was produced by Novogene. The ATG Number associates the entry to the specific gene and the detected change (Log2FC) in both data sets is presented. Further information about the gene in form of the gene description and the gene name is given.*

ATG Number	1stGen Log2FC	2ndGen Log2FC	Gene description	Gene name
AT5G56550	-1.50	0.94	Emb [Source:UniProtKB/TrEMBL;Acc:Q9LVB9]	OXS3
AT1G80440	-1.41	0.86	F-box/kelch-repeat protein At1g80440 [Source:UniProtKB/Swiss-Prot;Acc:Q9M8L2]	KFB20/KMD1

AT3G59940	-1.13	0.79	F-box/kelch-repeat protein SKIP20 [Source:UniProtKB/Swiss-Prot;Acc:Q9M1Y1]	KFB50/KMD4
AT5G21940	-1.12	0.88	At5g21940 [Source:UniProtKB/TrEMBL;Acc:Q9C593]	-
AT4G03510	-1.12	0.75	E3 ubiquitin-protein ligase RMA1 [Source:UniProtKB/Swiss-Prot;Acc:O64425]	RMA1
AT2G25900	-1.10	0.51	Zinc finger CCCH domain-containing protein 23 [Source:UniProtKB/Swiss-Prot;Acc:O82307]	ATCTH
AT5G17700	-1.07	0.73	Protein DETOXIFICATION [Source:UniProtKB/TrEMBL;Acc:A0A178UJQ3]	-
AT1G73260	-0.97	1.50	Kunitz trypsin inhibitor 1 [Source:UniProtKB/Swiss-Prot;Acc:Q8RXD5]	KTI1
AT5G44190	-0.84	0.49	Transcription activator GLK2 [Source:UniProtKB/Swiss-Prot;Acc:Q9FFH0]	GLK2
AT4G37610	-0.73	1.62	BTB/POZ and TAZ domain-containing protein 5 [Source:UniProtKB/Swiss-Prot;Acc:Q6EJ98]	BT5
AT5G19120	-0.72	0.56	AT5g19120/T24G5_20 [Source:UniProtKB/TrEMBL;Acc:Q93VG3]	-

Interesting candidates are for example genes like *OXS3*, involved in virus resistance promotion (Wang, 2012). *KFB20* as well as *KFB50* are involved in regulation of phenylpropanoid biosynthesis (Zhang *et al.*, 2013), these genes are also known as *KMD1* and *KMD4* and show responsiveness to cytokinin as well as a probable interaction with type-B ARR proteins (Kim *et al.*, 2013). *KTI1* was shown to play a role in defence against herbivore (Arnaiz *et al.*, 2018). Ubiquitin ligase *RMA1*, *ATCTH* and *BT5* show a regulation after bacterial and aphid infection (Barah *et al.*, 2013; Matusda, 2001). *GLK2* might be involved in JA-SA antagonism and is a defence regulator (Murmu *et al.*, 2014).

3.3 Effects of virus infection on the DNA methylome

Plants can act on stress in various fast ways, so far this study showed investigations of the transcriptome. This last chapter will focus on a rather recently found mechanism, the alteration of DNA methylation patterns. DNA methylation can influence gene expression and thus has a direct effect on plant cell behavior (Dowen *et al.*, 2012; Santos *et al.*, 2017; Zhang *et al.*, 2018). It additionally can be long lasting and even be inherited to the offspring (Boyko, 2010; Calarco *et al.*, 2012; Miryeganeh & Saze, 2019).

To be able to overview the whole picture, samples from first generation and second generation were analysed for changes in the DNA methylome. The plant material used to create the data sets was directly comparable to the material used for RNA-Seq in Chapter 3. The Whole-Genome-Bisulfite-Sequencing (WGBS) and the bioinformatic analysis was outsourced to Novogene.

Samples from the first generation were send for sequencing as triplicates (Mock_1-3 and TuMV_1-3). A confirmation of virus presence in the sequencing results, as it was done with the RNA-Seq samples, was not made. DNA samples were treated in the process of DNA extraction for RNA decontamination, so no viral RNA residue (TuMV is a RNA virus) should be left.

After sequencing the read data was bioinformatically analysed. First, an analysis focused on the produced data for each sample. All three in plants existing contexts (CG, CHG and CHH) were investigated separately. One tool to study the data is the Pearson correlation (Figure 27). It showed samples from the control group (Mock) clustering together as well as the virus infected (TuMV) samples for all three cytosine (C) contexts.

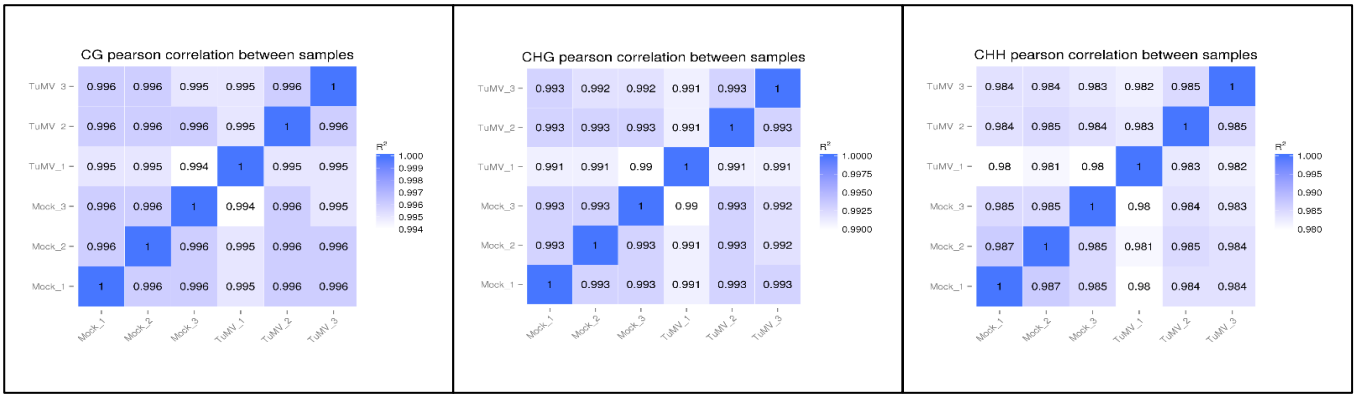


Figure 27 Pearson correlation of WGBS samples from 1st generation *This comparison shows similarity between the triplicates of mock treated and virus infected samples of the data set. The numbers range between 0 and 1, with 1 being full similarity. A correlation for each of the three cytosine contexts was made. (Source: Novogene)*

Afterwards samples were compared to each other. The aim was to find differences in methylated regions (DMRs) between samples from virus infected and mock treated plants. DMRs could be found arisen from all methylation contexts and numbers were visualized in form of Venn diagrams. (Figure 28). The CHH context was harboring the most methylation events (217) and some genes showed multiple DMRs, especially CG and CHG combined. A second Venn diagram depicts DMRs solely at promoter regions .The CHH context appeared again as the most often.

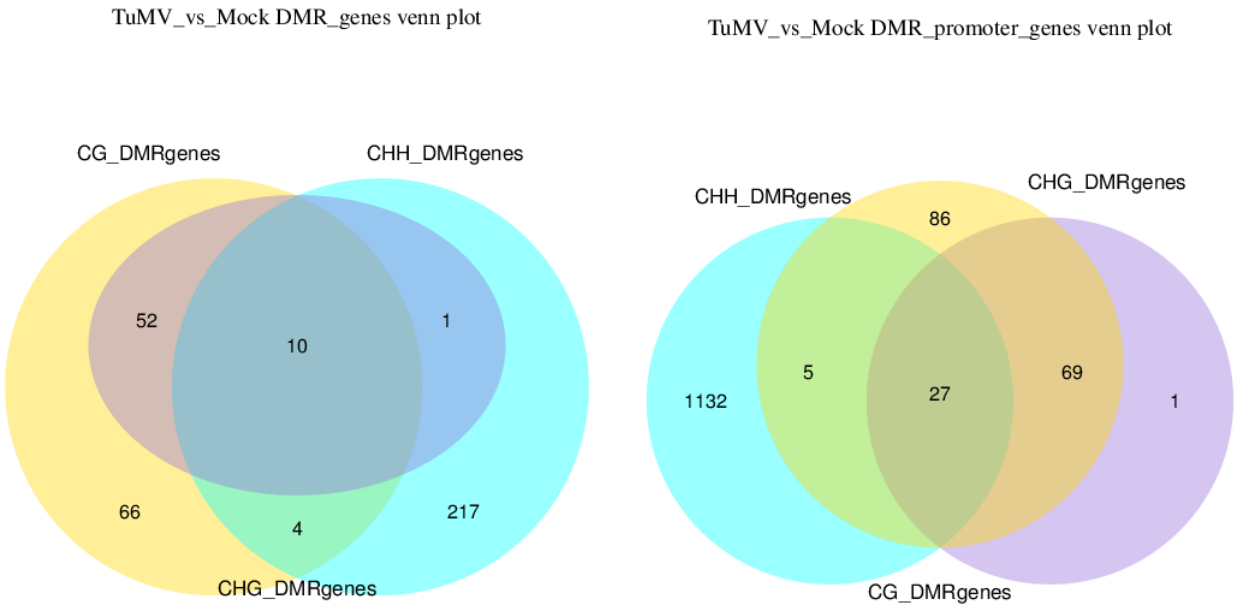


Figure 28 Venn Plots of WGBS 1st generation *In this plot differentially methylated regions (DMRs) in all three methylation contexts were compared for matching gene associations. On the left associations were made for whole genes, the right depicts DMR events only at promoters. (Source: Novogene)*

The DMRs had unique genome location patterns for all three cytosine contexts (Figure 29). DMRs were further sorted in two distinct subdivisions. A hypermethylation, where the DNA methylation of the genome loci in the treated material (e.g. the virus infected material) is enriched in comparison to the methylation level in the genome loci the control material (e.g. mock). A hypomethylation on the other hands describes a reduction of DNA methylation in a specific loci. The genome loci, the methylated region (DMR), can vary in its size. In the CG context, hypomethylation was almost only present on the mitochondrial chromosome and at areas with high transposable elements content, especially for chromosome 2. Only a few cases of hypermethylation are visible. The same was found for the CHG context. The overall

methylation was even lower but again a strong hypomethylation on the mitochondrial DNA and at the chromosome center of chromosome 2 was uphold. The last context, CHH, showed a very different pattern. A lot of hypomethylation was visible throughout all chromosomes with no specific emphasis.

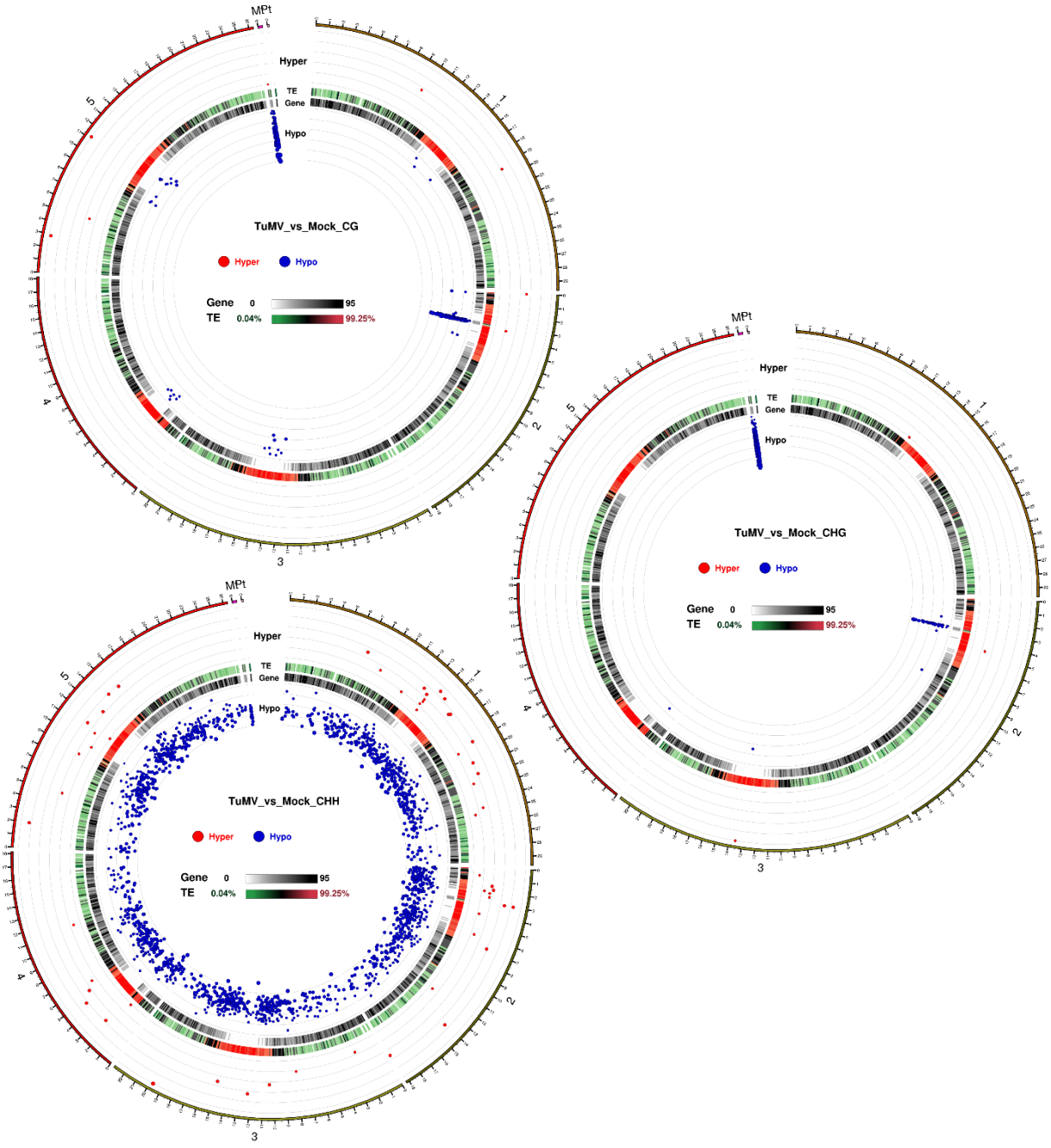


Figure 29 Circos plots of WGBS 1st generation *These plots are showing DMRs in the three cytosine contexts. From outside to inside: 1. Chromosome map 2. Hypermethylated DMR with a scale of log5 areaStat going from inside to outside 3. Heatmap of percentage of transposable elements 4. Heatmap of gene density 5. Hypomethylated DMR with a scale of log5 areaStat. (Source: Novogene)*

Further analysis gave only limited results, not many GO Terms or KEGG pathways seem to be enriched (both results not shown here). For example, the KEGG pathway analysis revealed only two differential pathways for the CG and CHG methylation context. The oxidative phosphorylation and the metabolic pathways were found, both with a small count number (marker for number of genes), but a good low q-value (marker for significance, the smaller the better). As earlier mentioned, changes in CG and CHG methylation context were small in number, whereas the CHH context revealed a lot of hypomethylation. This is why KEGG pathways analysis found more pathways regulated, with the plant hormone signal transduction pathway being the strongest. It is important to mention, that the CHH context analysis results had a small count number with not significant qvalues.

Overall, the studies showed that a variety of DNA methylation changes happened after virus infection. However methylation differences were weak as visible by the almost indistinguishable initial methylation levels of the samples. The methylated genes showed no sign of immunity related enrichment appearing in the observed functional analyses GO and KEGG.

3.3.1 Effects of virus infection on the *A. thaliana* DNA methylome in the offspring

To investigate possible inherited effects of virus infection in the offspring the DNA methylome of the next generation was analysed. Plant material was produced and harvested in the same way as the RNA-Seq samples for comparison. It has to be kept in mind that plant material differed from material harvested in the first generation (5 weeks old), as plants analysed in chapter 3.3.1 were 3 weeks old. Additionally the plant material varied between both projects: all above ground material was harvested from offspring plants (for 1st generation leaf material was used).

Samples were send to sequencing as duplicates (Mock_1-2 and TuMV_1-2). The data was processed in a bioinformatical analysis, which focused again first on the study of the data from each sample. All three in plants existing contexts (CG, CHG and CHH) were investigated separately. On type of analysis created was the Pearson correlation (Figure 30), showing that samples clustered in both the control group (Mock) as well as the virus infected (TuMV) group for each methylation context.

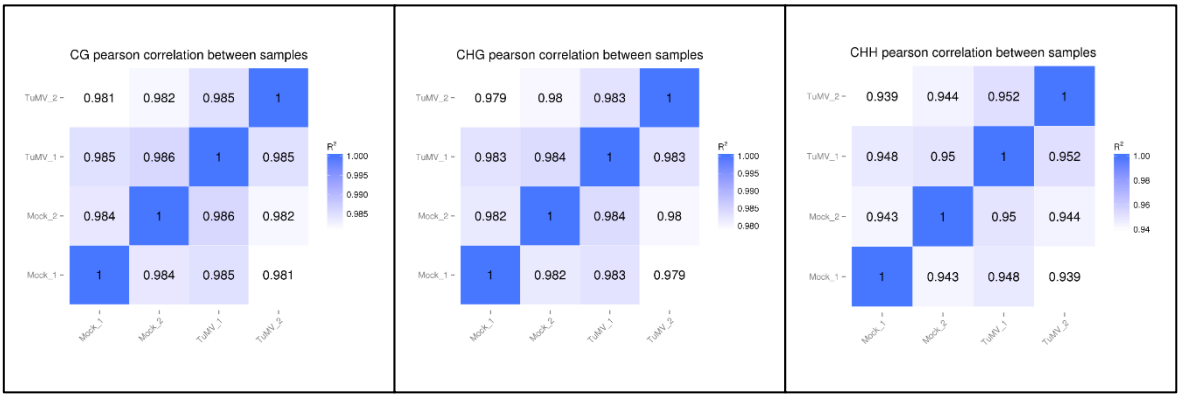


Figure 30 Pearson correlation of WGBS samples from 2nd generation *This comparison shows similarity between the duplicates of descendants originating from mock treated and virus infected samples of the data set. The numbers range between 0 and 1, with 1 being full similarity. A correlation for each of the three cytosine contexts was made. (Source: Novogene)*

After focusing on each sample, differential methylated region (DMRs) between samples were determined. The overall DNA methylation levels were very similar when progenies of TuMV infected plants and mock infected were compared (see supplementary Figure 36). The overall number of DMRs was small compared to the first generation (Figure 31). It was distributed over all three cytosine contexts, with CHH being the strongest. Regarding DMR events on the promoter (same figure right picture), the pattern stayed similar.

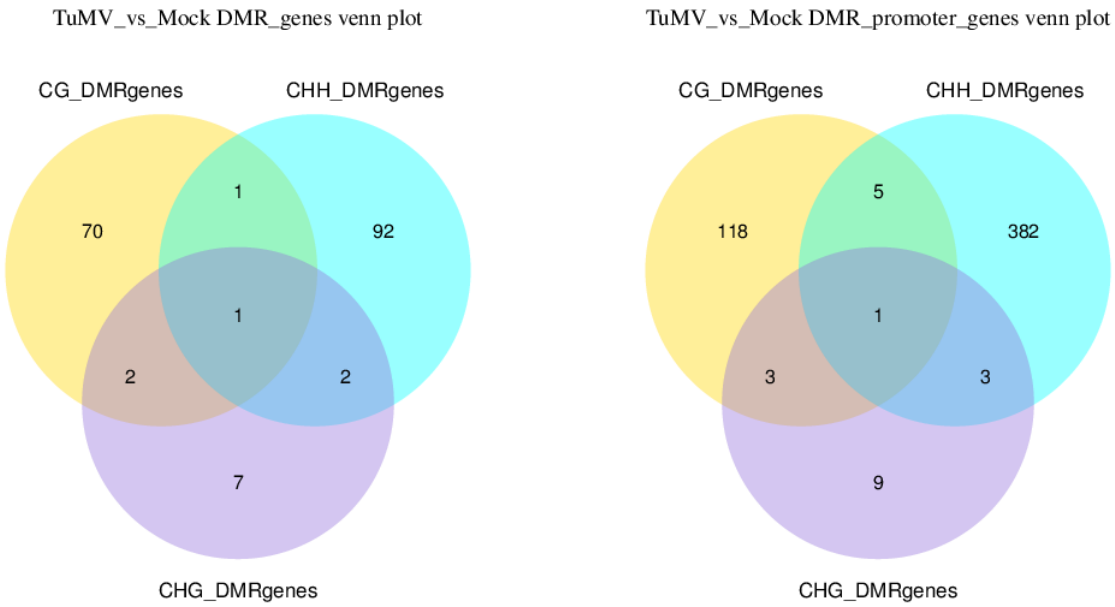


Figure 31 Venn Plots of WGBS 2nd generation *In this plot differentially methylated regions (DMRs) in all three methylation contexts were compared for matching gene associations. On the left associations were made for whole genes, the right depicts DMR events only at promoters. (Source: Novogene)*

Analysis of the location of DMRs was made visible in circos plots (Figure 32). Different patterns were visible for different cytosine contexts, altering from the analysis in the 1st generation. The CG context showed spots of hypomethylation, especially around TE rich areas, and again a bulk methylation on mitochondrial DNA and chromosome 2. However, this time spots were also visible throughout the chromosomes and a number of hypermethylation events happened, with a strong emphasis on mitochondrial DNA. The CHG context showed much less methylation, with only a handful of spots distributed into hyper- and hypomethylation over all the chromosomes. Again a bulk was visible in the mitochondria region. The context with the most methylation was the CHH methylation. Here regulation in both directions was present, with an emphasis on hypomethylation. Distribution was even over all chromosomes, with a slight tendency of hypermethylated regions to be found around transposable elements (TE)-rich areas.

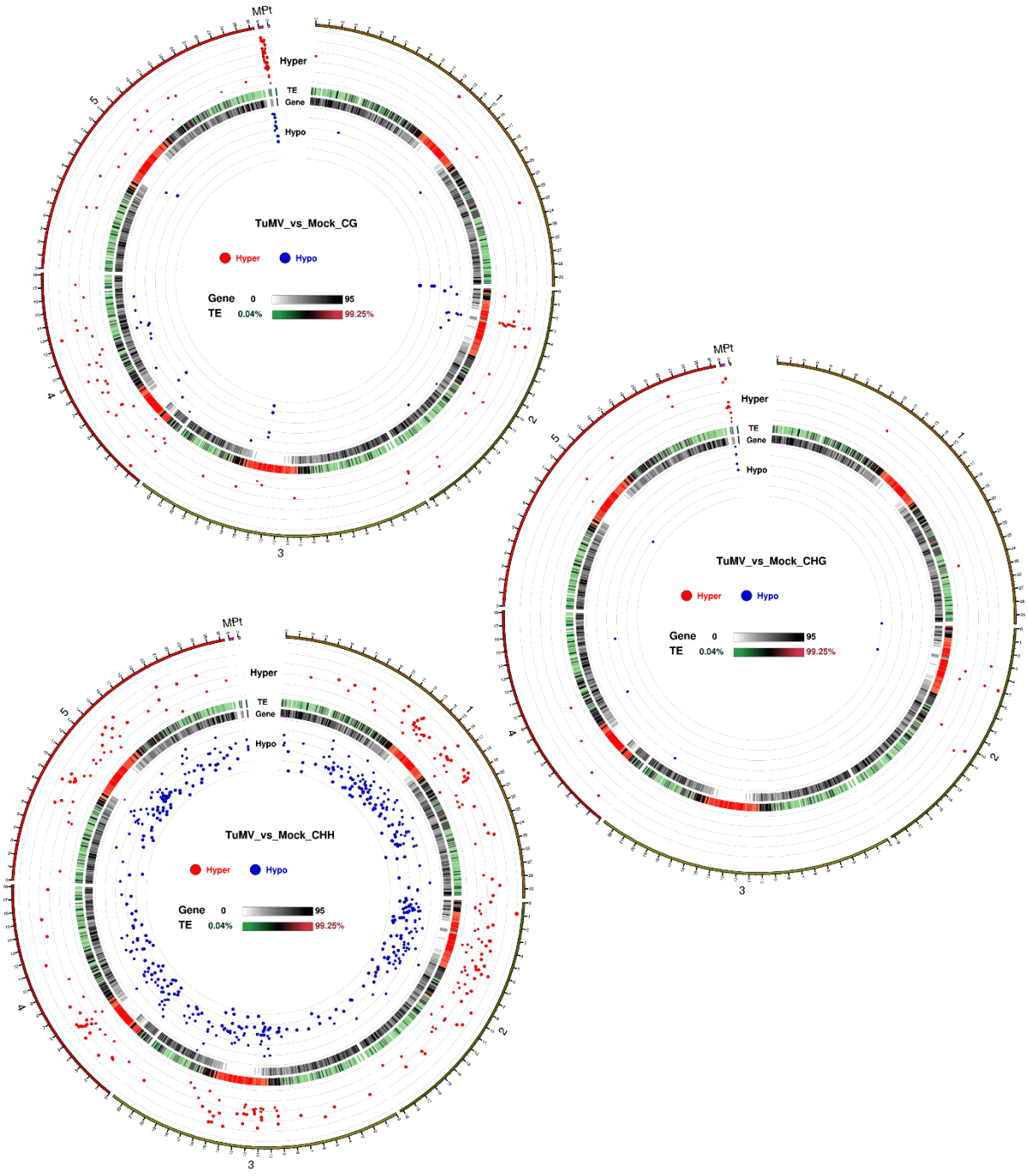


Figure 32 Circos plots of WGBS 2nd generation *These plots are showing DMRs in the three cytosine contexts. From outside to inside: 1. Chromosome map 2. Hypermethylated DMR with a scale of log5 areaStat going from inside to outside 3. Heatmap of percentage of transposable elements 4. Heatmap of gene density 5. Hypomethylated DMR with a scale of log5 areaStat. (Source: Novogene)*

The DMR data set was further investigated in a functional analysis. Two of the studies created, GO term analysis and KEGG analysis (both not shown here), resulted in almost no enrichment.

Only the CG context analysis detected pathways, differentially regulated with a low p-value (p-value resembles significance of results), the other context detected unsignificantly enriched pathways. All contexts showed pathways with a small count number (some very small, e.g. one-digit). Enriched pathways in CG context were the oxidative phosphorylation and metabolic pathways. For CHG and CHH methylation context, the gene number was even smaller and qvalues were very big.

To put it in a nutshell, the WGBS analysis showed DNA methylation changes present in the progeny of virus infected plants. Yet methylation levels are similar between the samples, changes are weak and the enrichment analyses detected only few results.

3.4 Comparison of transcriptome and DNA methylome changes of 1st and 2nd generation

Virus-host interaction in pathogen response is a very dynamic, complex machinery with many mechanisms. The transcriptome and DNA methylome changes are only two pieces inside this bigger puzzle, but they still have a lot of crosstalk. In this chapter the interplay between the DEG and DMR studies from first and second generation will be compared and similarities and differences will be brought to light. Due to the complexness of DNA methylome changes, only DMR present at the promoter will be investigated. For the correlation between DEGs and DMR, only the hypothesis of a hypermethylation leading to a reduced gene expression (and vice versa) was analysed in more detail.

There are several combinations of comparisons. Unless it is otherwise mentioned, all comparisons used only DMR in promoter regions.

3.4.1 Comparison of 1st generation DEG and DMR

Firstly, the program Microsoft Access was used to analyse genes found in two datasets. A comparison between the differentially expressed genes (further on called DEG) data and differentially methylated region (further on called DMR) data of the first generation gave the results shown in Figure 33.

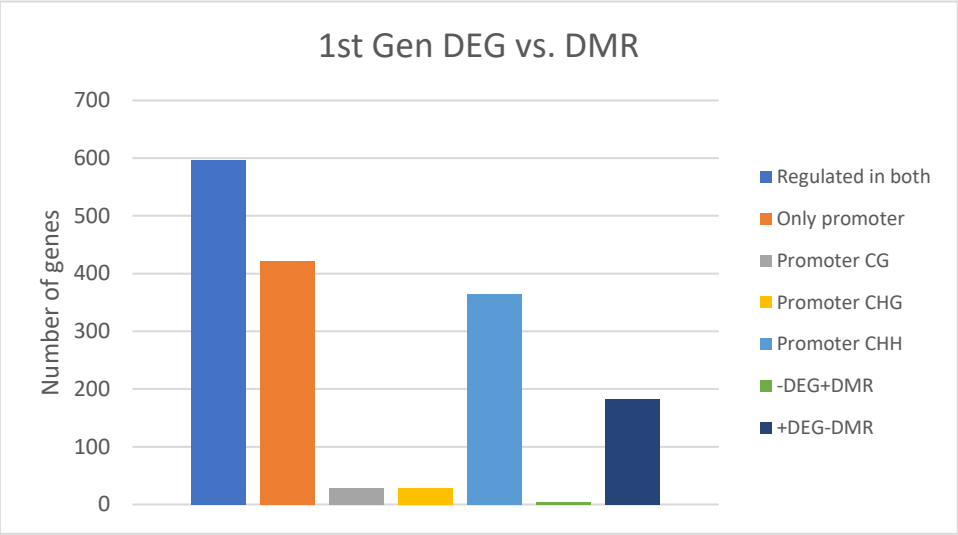


Figure 33 Comparison of DEG and DMR of first generation *The number of genes shared between the DEG and DMRs lists from the respective data sets of the first generation was determined. Different restrictions were added (see key on the right) for DMRs present at promoter (organge), on promoters only CG (grey), only CHG (yellow) and only CHH (light blue) context. Finally the number of genes showing a decreased expression combined with a hypermethylation on the promoter (green) and vice versa (dark blue) was determined.*

In total 596 matches (blue) could be found. From this, 422 methylation differences were found in a promoter region (orange) with 27 in a CG context (grey), 29 in a CHG context (yellow) and 366 in a CHH context (light blue). A correlation between DNA methylation and altered gene expression could be found with lowered expression in combination with hypermethylation for 5 genes (green), the opposite for 182 genes (dark blue). Most of these genes had a DNA methylation in a CHH context.

The 5 genes found downregulated (DEG) and hypermethylated (Table 20), all had a slight foldchange of downregulation, ranging from -0.32 to -1.34 Log2FC. All the methylation was in a CHH context.

Table 20 Genes downregulated in DEG 1st generation with hypermethylation *The list was created with information from DEG and DMR lists of 1st generation data sets produced with Novogene. The Gene ID associates the entry to a specific gene. The expression difference (Log2FC) and methylation difference (diffMethy) are shown as well as the size of the methylation change (areaStat) and the methylation context (C_context). A gene description and a gene name give further information.*

Gene ID	Log2FC	diffMethy	areaStat	C_context	Gene description	Gene name
AT3G07370	-0.32	0.104	69.61	CHH	CHIP [Source:UniProtKB/TrEMBL;Acc:A0A178VGJ7]	CHIP

AT2G48160	-0.33	0.03	183.30	CHH	Tudor/PWWP/MBT domain-containing protein [Source:TAIR;Acc:AT2G48160]	HULK2
AT5G30495	-0.37	0.08	102.71	CHH	Fcf2 pre-rRNA processing protein [Source:UniProtKB/TrEMBL;Acc:F4KCY1]	-
AT4G04221	-0.95	0.05	48.47	CHH	other RNA [Source:TAIR;Acc:AT4G04221]	-
AT1G56220	-1.34	0.09	135.28	CHH	Dormancy/auxin associated family protein [Source:TAIR;Acc:AT1G56220]	

The E3 ligase *CHIP* was shown to interact with heat shock proteins and is involved in plant immune responses (Copeland *et al.*, 2016).

Regarding genes correlated with a gain in expression and hypomethylation, 182 genes were found. The 20 most strongest DEGs are shown with their corresponding DMRs (Table 21).

Table 21 Genes upregulated in DEG 1st generation with hypomethylation *The list was created with information from DEG and DMR lists of 1st generation data sets produced with Novogene. The Gene ID associates the entry to a specific gene. The expression difference (Log2FC) and methylation difference (diffMethy) are shown as well as the size of the methylation change (areaStat) and the methylation context (C_context). A gene description and a gene name give further information.*

Gene ID	Log2FC	diffMethy	areaStat	C_context	Gene description	Gene name
AT1G52770	5.26	-0.08	-72.03	CHH	At1g52770 [Source:UniProtKB/TrEMBL;Acc:Q9C941]	-
AT1G44130	3.71	-0.12	-265.80	CHH	Eukaryotic aspartyl protease family protein [Source:UniProtKB/TrEMBL;Acc:Q9C6Y5]	-
AT1G53080	3.60	-0.14	-214.87	CHH	Lectin-like protein At1g53080 [Source:UniProtKB/Swiss-Prot;Acc:Q9LNN3]	-
AT4G08950	3.47	-0.08	-76.35	CHH	Protein EXORDIUM [Source:UniProtKB/Swiss-Prot;Acc:Q9ZPE7]	EXO

AT1G60970	3.05	-0.10	-57.04	CHH	Coatomer subunit zeta-1 [Source:UniProtKB/Swiss-Prot;Acc:Q940S5]	-
AT1G02450	2.69	-0.12	-138.83	CHH	NIMIN1 [Source:UniProtKB/TrEMBL;Acc:A0A178WC43]	NIMIN-1
AT2G04515	2.49	-0.12	-108.19	CHH	Transmembrane protein [Source:UniProtKB/TrEMBL;Acc:Q5S4Y8]	-
AT5G45960	2.41	-0.13	-160.66	CHH	GDSL esterase/lipase At5g45960 [Source:UniProtKB/Swiss-Prot;Acc:Q9FJ40]	-
AT2G35980	2.39	-0.12	-182.33	CHH	NDR1/HIN1-like protein 10 [Source:UniProtKB/Swiss-Prot;Acc:Q9SJ52]	NHL10 / NDR1
AT1G67750	2.30	-0.10	-226.79	CHH	Probable pectate lyase 5 [Source:UniProtKB/Swiss-Prot;Acc:Q9FXD8]	-
AT4G11350	2.27	-0.12	-92.99	CHH	At4g11350 [Source:UniProtKB/TrEMBL;Acc:B6IDH2]	-
AT1G21110	2.26	-0.19	-524.10	CHH	Indole glucosinolate O-methyltransferase 3 [Source:UniProtKB/Swiss-Prot;Acc:Q9LPU6]	IGMT3
AT3G23930	2.24	-0.10	-76.88	CHH	Troponin T, skeletal protein [Source:UniProtKB/TrEMBL;Acc:Q9LIR5]	-
AT5G35490	2.22	-0.07	-143.87	CHH	mto 1 responding up 1 [Source:TAIR;Acc:AT5G35490]	ATMRU1
AT5G42020	2.19	-0.05	-123.86	CHH	Heat shock protein 70 (Hsp 70) family protein [Source:TAIR;Acc:AT5G42020]	BIP

AT3G21890	2.17	-0.06	-71.55	CHH	BBX31 [Source:UniProtKB/TrEMBL;Acc:A0A178VEW7]	BBX31 / MIP1B
AT3G25882	2.02	-0.14	-272.04	CHH	NIMIN-2 [Source:UniProtKB/TrEMBL;Acc:A0A178VAB1]	NIMIN-2
AT3G25882	2.02	-0.10	-143.94	CHH	NIMIN-2 [Source:UniProtKB/TrEMBL;Acc:A0A178VAB1]	NIMIN-2
AT2G39330	2.01	-0.13	-52.90	CHH	jacalin-related lectin 23 [Source:TAIR;Acc:AT2G39330]	JAL23
AT5G01660	2.01	-0.14	-448.79	CHH	CONTAINS InterPro DOMAIN/s: Galactose oxidase/kelch, beta-propeller (InterPro:IPR011043), Kelch repeat type 1 (InterPro:IPR006652), Development/cell death domain (InterPro:IPR013989), Kelch related (InterPro:IPR013089), Kelch-type beta propeller (In /.../	-

A CHH methylation context was most common in this list. Particularly interesting is the overexpression of *NIMIN* genes coupled with a hypomethylation. NIMIN proteins are suggested to be SAR response regulators (Hermann *et al.*, 2013) and NIMIN-1 overexpression was proposed to result in SA tolerance via a limiation of NPR1 function (Mohan *et al.*, 2016). The detected gene NDR1 is involved in disease resistance and has been studied over the years in bacterial and fungal infections (Century *et al.*, 1995; Knepper *et al.*, 2011).

3.4.2 Comparison 2nd generation DEG and DMR

Another comparison was made using Access with the DEG and DMR data of the second generation. The number of DEGs in the 2nd generation were small though, contributing to a small number of genes (11) being present in both data sets (list is not shown). Three genes were found to be downregulated in gene expression coupled with a stronger DNA methylation, for the opposite context no gene was found: SERK4 is a receptor-like kinase regulating cell death *(de Oliveira et al., 2016)*, next is the flotillin-like protein FLOT2, which can interact with defence proteins *(Junkova et al., 2018)*, and finally one receptor-like protein RLP53, playing a role in sound vibration priming *(Choi et al., 2017; Tor et al., 2009)*.

3.4.3 Comparisons of DEGs shared between 1st and 2nd generation with DMR of 2nd generation

Chapter 3.2.4 investigated DEGs shared between 1st and 2nd generation. To analyse whether these genes are also marked with a methylation, the DEG lists were compared with the DMR of the second generation. Only for the comparison of upregulated gene expression in 1st generation, paired with downregulated gene expression in 2nd generation, matches with the DMR of the 2nd generation could be found (Table 22).

Table 22 Comparison DEG upregulated in 1st and downregulated in 2nd generation with DMR of 2nd generation

The list was created with information from DEG and DMR lists of 1st generation data sets produced with Novogene. The Gene ID associates the entry to a specific gene. The expression difference (Log2FC) and methylation difference (diffMethy) are shown as well as the size of the methylation change (areaStat) and the methylation context (C_context). A gene description and a gene name give further information.

Gene ID	Log2FC 1st Gen	Log2FC 2nd Gen	diffMethy	areaStat	C_context	Gene description	Gene name
AT5G25260	2.99	-2.98	0.31	68.48	CG	Flotillin-like protein 2 [Source:UniProtKB/Swiss-Prot;Acc:Q4V3D6]	FLOT2

AT5G27060	1.77	-1.83	0.14	141.57	CHH	receptor like protein 53 [Source:TAIR;Acc:AT5G27060]	AtRLP53
AT3G57460	1.15	-1.82	-0.29	-27.83	CHG	Catalytic/ metal ion binding / metalloendopeptidase/ zinc ion binding protein [Source:UniProtKB/TrEMBL;Acc:F4J3D6]	-
AT2G13790	1.06	-0.89	0.13	204.93	CHH	Somatic embryogenesis receptor kinase 4 [Source:UniProtKB/Swiss-Prot;Acc:Q9SKG5]	SERK4
AT2G23320	0.75	-0.59	-0.12	-89.58	CHH	Probable WRKY transcription factor 15 [Source:UniProtKB/Swiss-Prot;Acc:O22176]	WRKY15

The first gene appearing in the list, *FLOT2*, was shown to interact with various defence proteins (Junkova *et al.*, 2018). *RLP53* is a receptor-like protein, this family play a role in connection pathogen presence and immunity responses and this gene was found to be involved in the sound vibration priming of Arabidopsis (Choi *et al.*, 2017; Tor *et al.*, 2009). The receptor-like kinase *SERK4* is involved in regulating cell death (de Oliveira *et al.*, 2016).

3.4.4 Comparison of 1st generation DEG and 2nd generation DMR

Another approach investigated matching genes between the data set of DEG 1st generation and the DMR of the 2nd generation. Matches were found, connecting an increased expression with hypomethylation and vice versa (list is not shown). However, a GO analysis gave no results for enriched terms such as pathogen response.

4 Discussion

The tug-of-war between plants and pathogens was studied in this thesis using TuMV infected *Arabidopsis thaliana*. During infection, the host has to implement various mechanisms to reach a state of possible survival. On the other hand is the virus affecting many plant processes. Aspects of the many following changes were analysed in this thesis: the appearance (studied in chapter 3.2.1), the transcriptome of virus infected plants (studied in chapter 3.2.2) and the transcriptome in the progeny of virus infected plants (chapter 3.2.3). Another layer was added by investigating changes in DNA-methylation after virus infection and in the progeny of infected plants (chapter 3.3).

4.1 Virus infection could be confirmed in *A. thaliana*

In this thesis, verification of successful infection allowed for reliable further experiments regarding virus-host interactions as only properly infected tissue was harvested. To study effects of TuMV infection on *A. thaliana* while being able to track virus presence, a GFP-tagged TuMV strain was used. For this strain however, the GFP was not linked to any viral protein after translation. This could cause a bias as GFP is known to be cell-to-cell and even long-distance mobile (Imlau, 1999). A GFP fluorescence signal was used as a marker for harvest of sample material. Thus it had to be verified that GFP and virus protein/RNA presence coaligned. This was successfully done on RNA and protein level. Consequently, solely GFP fluoresence presence in samples could be used to verify the harvest of infected sample material.

4.2 TuMV infection lead to a short stem phenotype and cell wall alterations might contribute

The fierce fight of infection is known to result in various phenotypes and has been studied for many decades for all kinds of pathogens. The manifold changes are especially interesting when it comes to agriculturally used plants such as the family of *Brassicaceae*. Phenotypic changes caused in the host are similar for all infecting pathogen groups, like leaf lesions in fungal infection (Lebeda, 1994), bacterial infection (Katagiri *et al.*, 2002) or virus infection (Inaba *et al.*, 2011).

However, specificities of phenotypes vary between the attacking pathogen and host systems and so do the underlying mechanisms.

In this study an inflorescence phenotype (Figure 11) of *A. thaliana* following virus infection with the TuMV was investigated further. As presented in the results chapter, growth studies showed a significantly reduced heigth of TuMV infected *A. thaliana* inflorescences compared to mock treated (see chapter 3.1). Transcriptome changes behind it were investigated in chapter 3.2.1. Plants resembling the phenotype of category 1 (Figure 11) were chosen for the RNA-Seq study. Further investigation of the differential expression analysis from the bioinformatical analysis of the RNA-Seq data set resulted in eight candidate genes. They can be sorted into three groups according to their origin (Table 10) and might contribute to the observed short stem phenotype (Figure 11). Expression levels of all gene candidates were investigated via qPCR (Figure 18, Figure 19) for all observed phenotypes (Figure 11, Figure 12, Figure 13 and Figure 14) in the inflorescence plant material as well as the corresponding leaf material. The discussion will first focus on the groups potentially playing a role in this phenotype, followed by a short segment to discuss and compare the found changes with publicly available literature.

The first group of genes supposedly influencing the phenotype was found via analysis of the most strongly downregulated genes in the phenotype RNA-Seq data set. *FLA12* appeared here, which is a member of the fasciclin-like arabinogalacteran (FLA) proteins. FLAs are a subgroup of the Arabinogalactan proteins (AGPs), cell wall glycoproteins which have been studied for decades and might have a role in intercellular signaling (Seifert & Roberts, 2007). Knockout mutants of *FLA16* have shown a reduced stem length and altered stem biomechanics (Liu *et al.*, 2020), as have *FLA11* and *FLA12* to a lesser extend (Liu, 2017), a picture can be found in the supplementary (Figure 38). All three genes show a downregulation (*FLA11* -4.58; *FLA12* -5.65; *FLA16* -1.79) of their Log2FoldChange (L2FC; shown after gene inside parentheses) in the RNA-Seq results (Table 10). Downregulation was further confirmed by qPCR analysis (Figure 18) for all phenotype categories. These findings indicate a possible effect of repressed FLA genes in stem shortening during TuMV infection.

Another interesting factor modulating stem growth in plants is the phytohormone gibberelline acid (GA). This phytohormone has been studied extensively since it was first discovered to promote growth (MacMillan, 1958) and its anabolism and catabolism is very well understood nowadays (Hedden, 2020). As GA biosynthesis genes are potential candidate genes contributing to the observed short stem phenotype, they were analysed for regulation in the RNA-Seq results of the phenotype data set. It was found that 3 of the 5 known GA20-oxidases, coding for

key enzymes to catalyze the last steps of GA production (Hedden, 2020), were heavily downregulated after virus infection: *GA20OX1* (-1.72 L2FC), *GA20OX2* (-2.65 L2FC) and *GA20OX3* (-5.73). This was also confirmed for all phenotype categories in qPCR studies (Figure 18). Single, double and triple mutant of these 3 GA-oxidases were found to cause short stem and dwarf phenotypes (Plackett *et al.*, 2012; Rieu *et al.*, 2008) (Supplementary, Figure 37), similar to the phenotypes occurring after TuMV infection in this study. These findings combined might hint to a possible involvement of GA biosynthesis genes in the virus induced short stem seen in this study.

The last interesting candidate gene was *ATHB12*, a homeobox gene which was found to repress *GA20OX1* (and potentially 2 and 3) and thus lowers GA levels (Son *et al.*, 2010). Son et al. furthermore found in the same study, that ABA induces *ATHB12* expression. *ATHB12* was enhanced after TuMV infection in RNA-seq (1.59 L2FC) and qPCR results. This gene might link altered GA levels to virus infection.

Taken all of these points together, a conclusion can be drawn to state a hypothesis of a pathway potentially contributing to the observed short stem phenotype after TuMV infection. Manacorda et al. have shown, that TuMV infection increases ABA levels in *A. thaliana* (Manacorda, 2021), leading to an induction of *ATHB12* gene expression, which further represses GA20oxidases and thus resulting in a short stem due to reduced GA levels. Downregulation of *FLA11*, *12* and *16* could promote the short stem even further and lead to altered stem biomechanics. However, more experiments would be required to prove this hypothesis.

4.2.1 Severity of stem shortening cannot be exclusively explained by expression differences in the investigated candidate genes

The previously discussed stem biomechanic alterations may be the reason for the appearance of the different phenotype categories observed during virus infection. In total 4 categories could be found during investigations, all having different stem appearances considering stem length, growth direction and stem curliness. However, qPCR analysis of the inflorescence material harvested resulted in no major difference in gene expression between the categories, for any of the above mentioned candidate genes (it should be noted that the variation of expression alteration between the RNA-Seq results, presented in the same figure as the qPCR results originates from the different methods used). The difference of severity in stem shortening observed in the phenotype categories was therefore not reflected in the expression differences of the candidate genes. Nevertheless it is possible that the exact growth state of each plant at

the time of infection promotes a varying severity of altered stem biomechanics by regulation of FLA proteins. Although plants were all sawn at the same time slight delays in germination and following slight growth delays are possible, as well as different velocities of infection spread throughout the plant. After downregulation of FLA genes, altered stem biomechanics include among other things a reduced flexure strength and increased tensile stiffness (Liu *et al.*, 2020), which could explain the here observed curliness of the stem. Further investigations would be needed to gain more insight into this matter. One interesting possible hint into this direction can be already found within the results. Next to inflorescence material, the leaf material of the same plants was harvested separately and the same genes were analysed by qPCR. The results showed two main difference between the categories: 1) A stronger reduction of GA biosynthesis genes for leaf material of category 1 (most extreme short stem phenotype). 2) A stronger increase of *ATHB12* for category 1. Consequentially, additional experiments would be very interesting in this area, to potentially connect GA and AtHB12 regulation to severity of phenotype appearances after virus infection. To summarize, the observed different stem length could be a combination of GA and *ATHB12* regulation and the growth state at the time of infection, together with other yet unknown factors.

When it comes to inflorescence phenotypes in the TuMV infection of *Arabidopsis thaliana*, a very important other factor correlating with stem phenotype changes has to be named: the TuMV viral protein P3 (Lopez-Gonzalez *et al.*, 2020). Two main strains of the TuMV virus are currently used for research: the UK1 and the JPN1 strain, named after their origins, the former was used in this thesis. Lopez-Gonzalez et al. found that depending of the strain used for infection, the flower stalk elongation phenotypes vary drastically with the UK1 strain producing short stalks. They figured out that mutation of the viral protein P3 caused various alternative stalk length phenotypes in infections of both strains. They stated the TuMV P3 protein to be ER-associated and it was detected that it is a peripheral membrane protein, which movement it actomyosin-dependent. Furthermore they found, that mutations causing altered flower stalk growth also caused a differing intracellular movement and accumulation pattern of P3. However, the link between both could not be uncovered and researchers pointed in the end to possible cell wall alterations potentially being the cause for the observed phenotypes in a yet unknown way. This is where the findings of this thesis come in. It could be that GA, *ATHB12* and FLA genes might be part of the missing box connecting the viral P3 protein and the resulting virus induced short stem phenotype.

4.3 Virus-Host interaction changes the transcriptome

The visible phenotypic modification is just the tip of the ice berg when it comes to alterations during virus infection. Much more variance in gene expression can be detected when analyzing the transcriptome of infected leaves.

The most strongly regulated genes gained from two RNA-Seq data sets (Chapter 3.2.2 and 3.2.3), are presented in a mind map (Figure 34) to allow for an overview of their place in the complex machinery of virus-host interactions. This mind map includes genes regulated after virus infection in the 1st generation (marked with pink outlines) and genes regulated in the offspring of virus infected plants (marked with a purple outline). Upregulated genes are presented in green, downregulated genes are presented in red.

As visible, regulated genes were annotated to different areas and pathways. Many are related to plant immunity such as ETI and PTI, but also fields not solely playing a role in defence, like the cell wall or phytohormones, can be seen. It should be noted that the genes are not exclusively related to their annotated field but can have other functions. Plant immunity is a very complex process and the mind map therefore only hints towards influenced processes. Interesting genes will be discussed in two chapters (separating 1st and 2nd generation) loosely following the presented mind map. As virus infection is still not studied as extensive as other infection forms, research for publications showing connection to virus infection was not always successful. This could point to a relation shown in this study for the first time. Unless otherwise stated, no previous publication connecting the gene to virus infection were found.

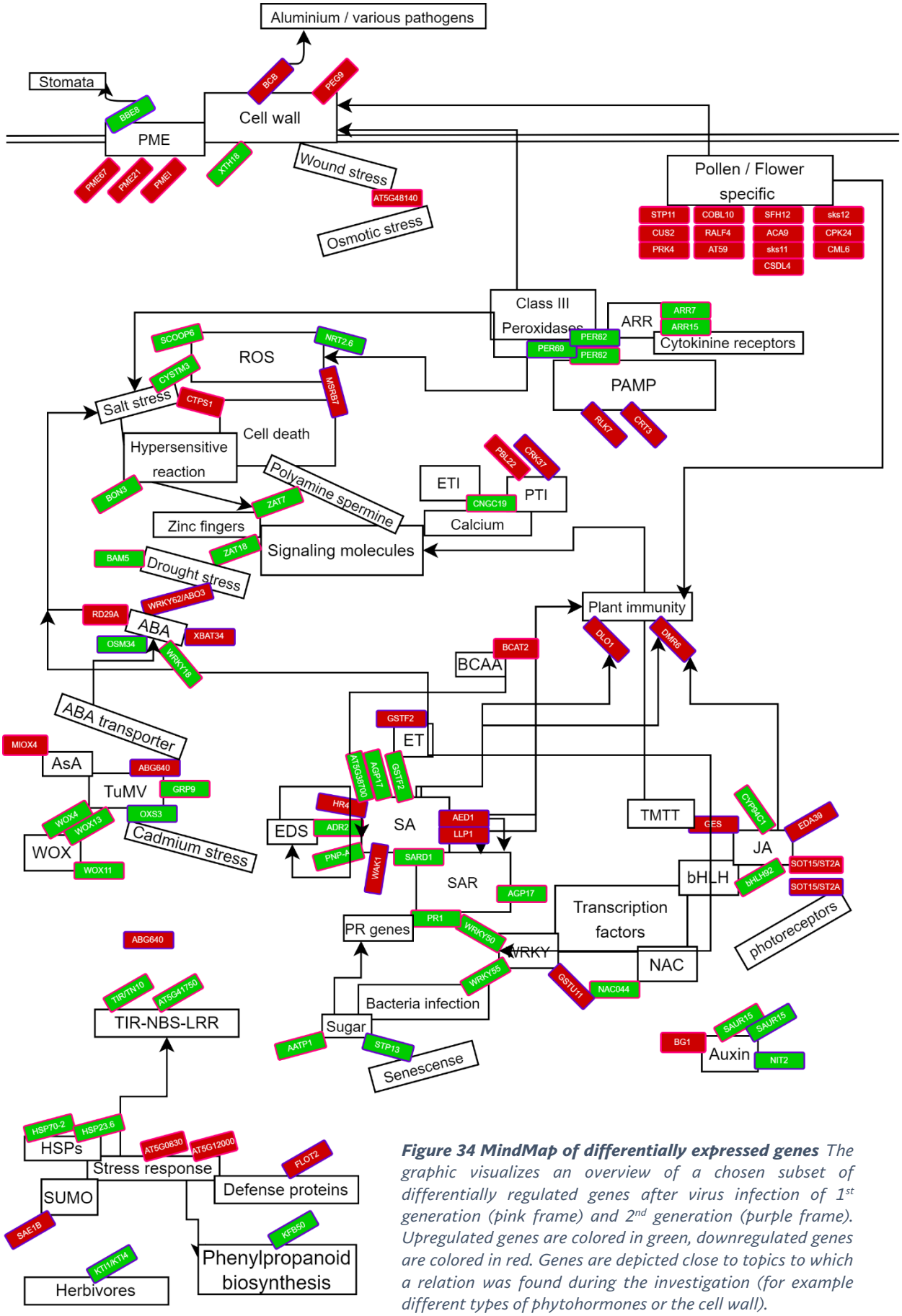


Figure 34 MindMap of differentially expressed genes *The graphic visualizes an overview of a chosen subset of differentially regulated genes after virus infection of 1st generation (pink frame) and 2nd generation (purple frame). Upregulated genes are colored in green, downregulated genes are colored in red. Genes are depicted close to topics to which a relation was found during the investigation (for example different types of phytohormones or the cell wall).*

4.3.1 TuMV infection influences transcription factor and cell wall related genes

Transcription factors (TFs) are a universal, efficient and fast way for an organism to react to stresses. In this study, mainly three groups of TFs were found regulated. Firstly, NAC TFs, which represent one of the largest group of TFs. These genes are mediated especially to serve as positive or negative regulators of plant immunity and many function during infection (Yuan *et al.*, 2019). Unfortunately, the NAC TFs found regulated after TuMV infection of *A. thaliana* in this study (Supplementary, Table 26), have not been investigated further yet. Most possibly NAC TFs play a role in virus infection, as 31 of the 96 NAC transcriptions factors annotated in *Arabidopsis* were found to be differentially regulated in this study. Including interesting genes like *NAC044*, determined to be part of a large protein-protein network together with WRKY TFs (Satapathy *et al.*, 2018). The second group of TFs regulated after virus infection are genes belonging to the WRKY family. For the WRKY family, 22 of the 72 members in *Arabidopsis* were altered in expression after virus infection in these experiments. This includes highly interesting genes such as *WRKY50* or *WRKY55* with very strong influence on plant immunity (Hussain *et al.*, 2018; Wang *et al.*, 2020). The third group are bHLH TFs, 161 were found so far in *Arabidopsis*, of which 59 showed a differential regulation in this data set, on example is *bHLH92*. bHLH proteins are involved in various areas, including stress responses and some had specific involvement in immunity, PAMP and phytohormonal controlled defence reactions (Fan *et al.*, 2014; Feller *et al.*, 2011; Song *et al.*, 2013). However, most studies were done with bacteria, not much is known about their involvement in virus infection. Not surprisingly, the plant reacts to virus infection with changes in it's transcription factors, leading downstream to many more adjustments in the transcriptome. Variation in transcription factor expression strength is a powerful tool for the plant and this study possibly revealed first hints to yet unknown roles of specific TFs in virus infection.

The cell wall as a very prominent factor during pathogen infection was heavily regulated in these studies. Cell wall alterations and defence mechanisms surrounding them have been studied extensively mostly for bacterial, fungal or oomycete infections (Molina *et al.*, 2021). During virus infection, the plant cell wall is the first barrier of defence and altered heavily (Wan *et al.*, 2021). It has a key role in resistance mechanisms and is also one of the first targets for the entered virus (Koziel *et al.*, 2021). Viral cell-to-cell mobility via plasmodesmata can be enabled or inhibited by the host for defence or by the virus for disease promotion (Koziel *et al.*, 2021). As both topics are especially interesting the number of studies in this are increased recently, to gain

more knowledge about viral spread and resistance. In this study, many of the most strongly regulated genes from the 1st generation clustered at the term cell wall. A very prominent cluster involved in cell wall modification are pectin methylesterases (PME). They are not only known for playing a large role in immunity promoted by cell wall integrity, but are especially interesting in virus infection as they bind to viral movement proteins and are required for cell-to-cell and systemic movement of viruses (Chen & Citovsky, 2003; Chen, 2000; Lionetti *et al.*, 2017; Lionetti *et al.*, 2014). This study showed many regulated PMEs, however this seems to be the first study connecting some of them specifically to virus infection. A few have previously displayed regulation during infection with other pathogens, such as *PME5* and *PME31* (Bethke *et al.*, 2014). Conclusively, pectin methylesterases play a vital role for plant immunity during virus infection and the PMEs found regulated in this study might therefore play important roles in the TuMV-*Arabidopsis* interaction and should be investigated further.

Many pollen and flower-related genes were found downregulated after virus infection – and a number of these were also involved in cell wall changes and pathogen response. Some, like *STP11* or *ACA9,* code for saccharide transporters and calcium pumps. Sugar transport is commonly altered following pathogen attack, also during virus infection (Breia *et al.*, 2021). As is calcium, it additionally plays a special role in the virus-host interaction (Cheval *et al.*, 2013; Then *et al.*, 2021).

The triangle of Arabidopsis response regulators (ARRs), PAMP and Class III peroxidases highlighted itself as peroxidases were detected strongly regulated in this study. All three categories are involved in plant immunity and studies including various pathogens have been made. For instance, Almaro and others stated class III peroxidases to be pathogenesis-related proteins having various roles in the previously discussed cell wall defence (Almagro *et al.*, 2009). The here regulated *PER62* seems to play a vital role in connecting cell wall alterations, ROS, ARRs and PAMP during bacterial infection or low-temperature stress (Arnaud *et al.*, 2017). This function might extend to viral infection, as it appears in this study. *PER62* was likewise proven by Arnaud et al. to be induced by ARRs. The type B ARRs are transcription factors involved in cytokinin signaling (Argyros *et al.*, 2008) and were suggested to play a role in plant virus resistance (Alazem & Lin, 2015). This data set showed 14 ARRs differentially regulated after virus infection, with interesting genes such as *ARR15*, a negative regulator involved in the signaling of cytokinin (T. Kiba, 2003). However, the function of *ARR15* and other ARRs is still unclear although they possibly play an important role together with the virus induced cytokinin (Alazem & Lin, 2015). Furthermore, ARRs have been proposed to build the link in the highly

interesting SA-CK crosstalk (Argueso *et al.*, 2012). To sum up, ARRs and Peroxidases are highly interesting in plant immunity and their importance also in virus infection can not be denied, although it is still missing further supporting studies.

4.3.2 Studies revealed phytohormone-related genes newly linked to virus infection

Phytohormones are used constantly to promote changes in the plant organism. Extensive studies were made to investigate effects of biotic stress on phytohormones (Burger & Chory, 2019; Denance *et al.*, 2013) although virus infection was mostly not included in pathogen studies. In this study especially SA related genes were found regulated, next to JA, ABA, ET and Auxin related genes. This is coherent with the enriched KEGG pathway *Plant hormone signal transduction* found in this RNA-Seq data set. Little is known regarding the differentially regulated SA-related genes in this study, but most genes have possible connections to plant immunity. Either they were studied in infections with other pathogens than viruses or the focus has been on other members of the same family during virus infection, making them very likely to have a similar function. For example, *GSTF2* has been shown to be regulated after fungal or bacterial infection (Gullner *et al.*, 2018), but only other members of the same family were integrated to subsequent investigation during virus infection (Sylvestre-Gonon *et al.*, 2019). The same applies to JA-related genes, here a connection to virus infection for both genes (*CYB94C1* and *SOT15/ST2A*) was not previously found. In contrast the ABA-related *RD29A* has been studied during TuMV infection (Manacorda, 2021), for *WRKY18* papers are inconclusive (Xu *et al.*, 2006). Finally, it is known that the phytohormone auxin is altered by virus infection in many ways (Mullender *et al.*, 2021). In this thesis the auxin related gene *SAUR15* was regulated. The induction by virus infection has been shown before, however, with a reversed expression regulation (Prasch, 2015). This circumstance might be explained by infection status differences between the cited paper and this study, as well as the flexibility of auxin regulation (*SAUR15* is an early auxin induced gene) during infection. Finally, the antioxidant ascorbic acid (AsA) was found to regulate resistance against TuMV (Boubraki, 2017; Fujiwara *et al.*, 2016). *MIOX4* was downregulated in this study and was previously suggested to be involved in AsA biosynthesis (Lorence *et al.*, 2004). Researchers stated that *MIOX4* furthermore increases resistance to abiotic stresses (Yactayo-Chang, 2017), showing again the complex interconnection of the plant stress response and the multifunctionality of its related genes. This paragraph highlighted genes involved in or related to phytohormone signaling. Their function in virus infection is mostly indetermined and respective experiments would be interesting.

4.3.3 TuMV infection leads to regulation of biotic and abiotic stress response genes previously not connected to virus infection

Unsurprisingly, many stress response related genes were found when studying the strongest differentially regulated genes after virus infection. Such as the indispensable immune receptors TIR-NBS-LRR, which are part of ETI. Firstly the *TIR/TN10* gene should be named, as it promotes indirectly immunity (Chen *et al.*, 2021). Secondly the NLR gene *AT5G41750*, which was found to be partly controlled by *HDA9* and *HOS15* via an epigenetic mechanisms using histone modification (Yang *et al.*, 2020), a similar role in plant defence after virus attack could be assumed. Looking further, the analysis revealed two heat-shock genes (*HSP70-2* and *HSP23.6*) influenced by TuMV infection. For *HSP70-2* regulation during TuMV infection has been shown in earlier studies, probably playing an important role in enabling infection functionality after initial virus infection (Aparicio *et al.*, 2005). *HSP23.6* has also been detected several times in studies regarding virus infection, HSPs in general seem to play a role after virus infection and are proposed to ease infection for viruses (Whitham, 2006; Wu *et al.*, 2016). However, the underlying pathways are not fully understood yet. A relatively undiscovered topic after virus infection is the effect of alterations to the BCAA biosynthesis, producing the branched-chain amino acids (BCAA) valine, leucine and isoleucine. It was discovered that alterations of their metabolism can have effects on plant defence for bacterial infection (Zeier, 2013). Appearance of a BCAA transferase *BCAT2* in this study further supports a yet unknown role in virus infection, which was proposed before (Fernandez-Calvino *et al.*, 2014). The plant stem cell regulator *WUSCHEL* was found to be heavily involved in innate immunity against viruses (Wu, 2020) and several *WUSCHEL*-related homeobox (WOX) genes were regulated after TuMV infection in this study. This study might give the first hint in their specific involvement in plant defence after virus infection.

Mechanisms behind stress responses are sometimes used universal and can have multiple outcomes. Hence genes regulated after TuMV infection have been sometimes studied previously for their function in other biotic or abiotic stresses e.g. salt, drought and wound stress. Especially multiple stress studies, including virus attack, show that genes can have multiple functions and even revealed new behaviors when the host is exposed to combinations of stresses (Prasch, 2015). Particularly interesting is the ROS-related *SCOOP6* which was found upregulated here after TuMV infection. The SCOOP family acts during defence response and is connected to the SERK family (Gully, 2019), which also shows an alteration in this dataset. Additionally connected to ROS, due to its ability to alter ROS homeostasis and inhibiting NA^+-

efflux (Xu *et al.*, 2019), is *CYSTM3*. Induced by salt stress and involved in regulating cell death is *CTPS1* (Alamdari *et al.*, 2021). One form of cell death is the hypersensitive reaction (HR), a process that can be mediated by R genes (Balint-Kurti, 2019). *BON3* plays a strong role as it represses resistance (R)-like genes causing cell death (Li, 2009). Its activation after TuMV infection, as shown here, is therefore consequential. The two remaining genes in this area are firstly *ZAT18*, a zinc finger protein connected to drought stress and ROS (Yin *et al.*, 2017). Upregulation of *ZAT18* might promote plant survival during TuMV infection in this study. This would be coherent with findings from Xu et al. that virus infection can promote drought tolerance. Researchers have various hypotheses as to why virus infection improves resistance to abiotic stress, reaching from reduced growth of the plant after initial infection to an altered phytohormonal signaling (Xu *et al.*, 2008). The second gene sorted into this context, *BAM5*, showed a responsiveness to drought stress (Prasch, 2015), but no further effects in this direction are known yet. This paragraph highlighted the various genes with induced altered gene expression after TuMV infection. Their described functions, if found, belong to various biotic and abiotic stresses, resembling the interconnectedness of plant response to virus infection.

This chapter showed that the plant immune system reacts to TuMV infection in many interesting ways. For plenty of the genes resulting from the differential transcriptome analysis literature research could not determine previous study relating them to virus infection. This could pave the way for further studies ascertaining their specific function in the plant response to viral attacks.

4.4 Effects of virus infection also proceed into the offspring

A virus infection is a very serious threat for a plant, thus the organism shows various responses to it as discussed in the previous chapter. This experience can be memorized by the plant, to enable a better survival in case of a repetition of the same or a similar threat. The strategy behind it is called priming and it is activated during abiotic or biotic stress. Induced changes range from physiological, transcriptional and metabolic to epigenetic levels (Gamir *et al.*, 2014). The goal is to react faster and more effectively to a new challenge. Priming is effective in the remaining life span of a plant and intergenerational (Gamir *et al.*, 2014). It has been shown that primed *Arabidopsis* can develop a higher resistance level to biotic stress (Slaughter *et al.*, 2012).

The offspring of virus infected plants was investigated in this thesis, the discussion will proceed in a similar way to the previous chapter. As mentioned earlier, researching whether the genes were previously shown in virus-relation was challenging. Studies concerning virus infection are smaller in numbers than studies with other pathogens. If studies could be found, it will be named specifically.

Many of the genes and topics discussed in the following are likely priming related, although no studies in this direction were found while researching the background of the genes, unless otherwise mentioned. Epigenetic mechanisms can be part of priming and will be discussed in the next chapter.

The transcriptome of the offspring of virus infected plants was investigated in an RNA-Seq. The analysis resulted in mostly downregulation of genes, when compared to the offspring of mock treated plants. The results of the strongest differentially regulated genes are part of the mind map (Figure 34), indicated by purple frames.

Likewise to the investigated virus infected plants a regulation of transcription factors was detected in the offspring. From the 72 known members of the WRKY family, 10 were found to be differentially downregulated in this study. None of the WRKY factors known to be involved in *Arabidopsis* priming (*WRKY6*, *29* and *53*) (Ando *et al.*, 2021) were regulated in this data. Interestingly, 9 of the 10 WRKY TF altered in expression in the 2nd generation had also a regulation in the 1st generation pointing to a potential function in the plant stress memory for virus infection.

The cell wall as a key player in plant defence (Molina *et al.*, 2021), was detected to be heavily regulated after TuMV infection (shown further above) and this regulation can also be found in the offspring. The cell-wall related *BBE8* was so far studied in bacterial infection for its regulation of stomatal aperture (Rodrigues Oblessuc *et al.*, 2019). For *BCB* a possible promotion of cell wall-based defence was detected (Mishina & Zeier, 2007). Both genes could have similar functions in the offspring of virus infected plants, preparing the plant for new attacks.

The triangle of ARRs, PAMP and Class III peroxidases is not only regulated after virus infection, but also the offspring shows alterations of *PER62* (and additionally *PER69*) leaving the valid question if this is part of a core memory after virus attack. Two of the strongest downregulated genes in the offspring (*CRT3* and *RLK7*) were also related to PAMP. *RLK7* has a strong role in plant defence in studies regarding bacteria infection (Hou *et al.*, 2021). Interestingly, PAMPs and the following PTI were recently associated with viruses (Mandadi & Scholthof, 2013). *CRK37*, a

gene connected to PTI is regulated in the offspring of virus infection plants (and shows also an upregulation after virus infection), here studies concerning infection with other pathogens could be found (Thatcher *et al.*, 2015; Yeh *et al.*, 2015). Although the specific function of the mentioned genes remains unclear in this context, their appearance in the offspring of virus infected plants is quite interesting and calls for further studies.

A quick glance at the mind map shows some of the strongly downregulated genes related to pathogen response or phytohormones, especially SA and JA. Four very important genes related to SA were differentially regulated (*HR4, AED1, LLP1* and *WAK1*). SA is the key phytohormone in SAR establishment and both act in plant defence to virus attacks (Zhao & Li, 2021). The transgenerational resistance by SAR has been found to be associated with the priming of SA-dependent genes (Luna *et al.*, 2012), making the here found regulated genes potentially a part of this mechanism. Especially *AED1* (participating in a feedback loop of systemic immunity) could have an important role in the offspring of virus infected plants as it was found to recognize a virus protein (Breitenbach *et al.*, 2014). It may be involved in primed transgenerational virus detection, together with the also regulated and SA-dependent *LLP1*, connecting it to SAR.

Other phytohormone-related genes were likewise targets of regulation in the offspring of virus infected plants. As in the previous generation, JA and ABA-related genes were found within the most strongest differentially regulated genes. Especially the ABA-related gene *OSM34* is very interesting in this context as it was proposed to be part of a theory of dynamical network biomarkers, indicating transition to a disease state in plants (Tarazona *et al.*, 2019). Described there as one of the top early genes it could serve in the offspring of virus infected plants as a memory marker for the disease state. Another possibly important factor could be found within the JA-related genes: *GES*, mentioned as a key signaling molecule during insect attack (Herde *et al.*, 2008). This gene might serve as a memory marker for the insect attack in the offspring of TuMV attacked plants (TuMV is naturally spread via aphids). The same theory could be proposed for the JA-related *SOT15/ST2A*. Previous studies revealed it to link phytohormone signaling and photoreceptors (Fernandez-Milmanda *et al.*, 2020). The ET-related gene *GSTF2* was detected to function in reducing pathogen infection damage (Lieberherr, 2003), this role could also be true in the offspring of TuMV infected plants.

Phytohormones and related genes are closely connected to induce plant immunity, such as the downregulated *DMR6*. In this specific case the plant might use this genes ability to enable resistance to various pathogens (Caarls *et al.*, 2017; Zeilmaker *et al.*, 2015) to further improve plant immunity in the offspring of virus infected plants. Next on, an interesting gene appears at

the link of ABA-relation to drought stress: the transcription factor *WRKY62/ABO3*. This gene was shown to interact with the histone deacetylase 19 to proposedly fine-tune plant basal defence responses (Kim *et al.*, 2008). Regulation in the offspring could point to a possible maintenance of the described function. Coherent with this thought is the detected regulation of *WRKY38*, an interaction partner of *WRKY62* in this mechanism according to Kim et al..

Although the transcriptome regulations of descendants of virus infected plants are only few in number, they are nonetheless very interesting. Changes appear in vital parts of plant immunity, like phytohormone-related fields or peroxidases. Their important functions described by other researchers sets them as attractive future study targets to reveal their function in the plant-virus interaction and particularly in priming after virus attack.

4.5 Changes of intergenerational expression strength reveal a function-related pattern

The discussion until this point focused on the altered gene expression after virus infection (1st generation) and in the offspring of virus infected plants (2nd generation) resulting from the two RNA-Seq studies. Genes were discussed mostly separate so far but connecting the knowledge won from both generations could be worthwhile. To investigate this further, differences and similarities between both RNA-Seq data sets were assessed. Why is it interesting to investigate the relations between both data sets? A pathogen attack is a very serious threat for an organism. Plants like *Arabidopsis* developed mechanisms to memorize attacks and response techniques to inherit to the next generation(s), as their survival is proof of the success of the chosen response. The plant stress memory is very complex and mechanisms like priming and epigenetic modifications (e.g. DNA methylation or histone modifications) are working hand in hand. The prime motif of all mechanisms is always a change in the expression status of certain genes.

To simplify, the comparison of both data sets in this thesis was performed in 4 different blocks i.e. Genes that kept their expression profile of up- or downregulation in both data sets (blocks 1 and 2) and genes that altered their expression profile in the opposite direction (blocks 3 and 4). The creation of more blocks would be possible, for example genes with differences in their expression strength still within the same direction of up/downregulation or also genes that were only induced in one of the two data sets. These blocks would be much more complex but nonetheless interesting, could serve important functions and should be investigated in the future. The 4 stated contexts were investigated and results are depicted in chapter 3.2.4. The

number of genes falling in each context differed significantly which will be discussed in the following as well as interesting genes or enrichment trends in each context.

Clearly, the main thread (harboring almost 300 genes) in the comparison analysis of both generations was an upregulation after virus infection and a following downregulation of the gene in the offspring (block 3). A GO term analysis resulted in enriched terms surrounding phytohormones, especially SA. This is coherent with the knowledge of SA-dependent expression induction after virus infection (Huang *et al.*, 2005) and the connected establishment of systemic acquired resistance (SAR) which was found at least for bacterial infection to act transgenerational (Luna *et al.*, 2012). According to this data, SA and SAR are the main factors in transgenerational expression alteration in *A. thaliana* after infection with the Turnip Mosaic Virus. Nonetheless, additional other mechanisms such as altered JA signaling, cell wall, programmed cell death (PCD) and hypersensitive reaction (HR) alterations were found enriched and apparently contribute to maintenance of plant defence and survival after virus infection.

Regarding the other contexts, small numbers of genes were found, which did not allow for GO enrichment analysis. Instead, trends and particular genes will be discussed in the following.

Upregulation in both data sets (block 1) was genuinely interesting, as it happened for very significant genes. For example the combination of *PER62* and *PER69*, functioning in the previously described ARR-PAMP-Peroxidase "triangle" or phytohormone-related genes like SAUR15 (Auxin) or the proposed disease state biomarker *OSM34* (ABA). The specific functions of these genes in this context remain elusive, but it is presumable that they play important roles in the plant stress memory to virus infection.

Downregulation in both data sets (block 2) happened likewise mostly for defence related genes. Mainly phytohormone related genes were found, like *SOT15/ST2A*, *IAA29* or *YUC9* (JA, Auxin). It lays the focus on phytohormonal signaling and crosstalk alterations in this expression profile change context and creates a very exciting field for further studies of their importance after virus infection in this concern.

The last block (4), containing genes downregulated after virus infection and upregulated in the offspring, showed also a unique pattern. Many genes involved in resistance to biotic stress were found promoting virus resistance like *OXS3* (Wang, 2012); *KTI1* is involved in herbivore defence (Arnaiz *et al.*, 2018) and *RMA1*, *ATCTH* and *BT5* all show regulations after infection with aphids or bacteria (Barah *et al.*, 2013; Matusda, 2001). This expression profile seems to be especially relevant for genes specific to biotic stress responses, apparently also in the context of virus

infection. Furthermore, genes in the phenylpropanoid pathway (enriched in the upregulated genes in the offspring data set) fall into this block, leaving the question if this expression pattern might be relevant for these important plant defence factors (Dixon, 2002) during virus infection.

Taking a look at changes in expression strength from one generation to the next in the virus infection context revealed interesting details. It seemed, that genes clustered depending on their related function into contexts like phytohormone regulation\relation or biotic stress. However, more studies in this direction would need to be done to confirm these preliminary hints.

4.6 DNA methylation after viral infection

Plants use various epigenetic modifications to influence gene expression quickly and/or conserve information to inherit onto further generations. One modification type is the methylation of cytosines in the genomic DNA. It has been shown before, that DNA methylation in biotic stress, for example a bacteria infection, correlates negatively with gene expression during the plants response (Zhang *et al.*, 2018). Although uniform correlation has been discussed by researchers, the common assumption of contrasting correlation was applied in this thesis. Specifically virus infection was discovered to potentially cause hypomethylation at stress-responsive genes which where found to be upregulated after infection (Wada *et al.*, 2004). Other researchers found indications suggesting that DNA methylation takes place on differentially expressed genes involved in pathogen response during virus infection (Yu *et al.*, 2019).

The data in this thesis supports this theory, although the specific effects of DNA methylation on the targeted genes remain unclear. Within this study, the DNA methylome of two types sample sets was analysed via Whole-Genome-Bisulfite-Sequencing (WGBS): Firstly, samples that were took alongside samples used for RNA-Seq from plants after 14 days of TuMV infection (e.g. mock treated). Secondly material from the progeny of virus infected or mock treated plants, harvested alongside the samples used for RNA-Seq. In the following, the results presented previously (chapter 3.3) will be discussed (each generation separately). Correlations to the associated RNA-Seq results will be included. Further on, the intergenerational discussion will be revived by adding the dimension of DNA-methylation and the most interesting genes in this context will be determined. Importantly, only differentially methylated regions (DMRs) present at the promoter were considered in this discussion. Suggested effects on the expression

implied the common conception of a negative correlation between DMR presence and expression strength alteration.

In this study TuMV infection mostly caused hypomethylation on the genome. For CG and CHG it was mainly targeted to mitochondrial DNA. Most likely this is a bias, as the amount of reads from the mitochondrial genome is enlarged due to the nature of the studied leaf samples. However, important mitochondrial encoded genes were regulated, having an influence in pathogen response. Overall methylation levels did not differ after virus infection when compared to mock treated plants, but small changes could still affect transcriptome behavior.

Effects on single genes were determined by comparing the RNA-Seq data set with the WGBS data set and searching matching genes regulated in both. For this the common assumption in the process was that DNA methylation negatively correlates with expression. Additionally the data set was resctricted to DMRs on promoters. Mostly methylation in the CHH context was discovered, 186 of the 187 matching genes had a CHH methylation. The relation between the DEGs and DMRs after virus infection was mostly an upregulation of expression combined with a reduction in methylation. None of the previously analysed genes in the transcriptome chapter showed an alternated methylation status. Still, interesting genes involved in disease response (like *NIMIN-1* and *NDR1*) were detected showing an increased expression coupled with a hypomethylation in the promoter region. This could be an indication for demethylation playing a role in the planned response to virus by enabling an increase in specific gene expression.

Progeny of virus infected and mock treated plants showed a much different pattern in their DNA methylome. As for the other data set, almost no changes in DNA methylation level between the samples were detectable (Figure 36). In contrast to their parents, only few differentially methylated regions could be found. Still this does not prove any irrelevance, as small changes can also have strong effects and the progeny was not exposed to a pathogen, which naturally does not call for many changes. The methylation patterns had similarities to the pattern seen in the 1st generation, as methylation took place in many cases on the mitochondrial genome (for CG and CHG) and CHH was against the most strongest regulated methylation context. A drastic change was that this time much more hypermethylation was found. Again, the expression changes investigated by RNA-Seq in the progeny was compared to the WGBS results and matches were analysed. The same assumptions were followed as explained earlier for the 1st generation. In total, a very small number of genes matched, most probably resulting from the small number of DMRs and DEGs in the 2nd generation data sets. Still, the genes regulated in both contexts had relations to immune response. This is in conformity with other early findings,

that a transgenerational memory of stress response, also after virus infection, exists for *A. thaliana* (Boyko, 2010). However, DNA methylation might not play a vital part in this.

Further studies into intergenerational effects were performed. The first one identified genes, which were regulated on their expression level in both generations combined with a change in their methylation status in the 2nd generation. Only one pattern was found here, genes being upregulated after virus infection, downregulated in the progeny with a hypermethylation in their promoter region. In contrast to this, researchers suggested for bacteria infection a DNA hypomethylation as a transmitter for transgenerational SAR (Luna *et al.*, 2012). Three genes are particularly interesting, as they were detected previously while comparing the DEGs and DMRs of the 2nd generation. genes all had relations to pathogen defence: *FLOT2*, which was proven to interact with several defence proteins (Junkova *et al.*, 2018). The receptor-like protein *RLP53*, whose family provides a link between pathogen detection and immunity response (Choi *et al.*, 2017; Tor *et al.*, 2009). And finally the receptor-like kinase *SERK4* regulating cell death (de Oliveira *et al.*, 2016). The second study into intergeneration effects was investigating matches for genes with an expression regulation in the 1st generation and a change in DNA methylation in the progeny. Genes for both patterns, up- and downregulation with negatively behaving methylation pattern in the progeny were found. Enrichment for genes involved in pathogen response could not be found.

FLOT2 is known to be upregulated after viral infection, and an increase has also been shown for several other pathogens (Junkova *et al.*, 2018), but a potential regulation in the next generation, influenced by the epigenetic marker DNA methylation has not been shown yet. *FLOT2* is predicted to interact with many pathogen related proteins, for example *HIR2* (Junkova *et al.*, 2018). *HIR2* could be detected too, upregulated after virus infection and downregulated in the offspring but it showed no different DNA methylation. *FLOT2*, with its complex interactome, could serve in plants inheriting the memory of virus infection as a key memory in the organisms stress memory.

RLP53 was unfortunately not studied sufficiently yet and thus its role in changing expression strength and methylation status after virus infection and in the offspring cannot be hypothesized. However, another family member, *RLP43* showed an important effect after demethylation in its promoter region, allowing PAMP-responsive WRKY factors to bind (Halter *et al.*, 2021). The two proteins do not show strong similarity (around 40% on the nucleotide level), so *RLP53* will probably not act alike, but could be part of a similar process. An involvement in intergenerational stress memory or a DNA methylation pattern has not been studied yet.

The receptor-like kinase *SERK4*, also called *BKK1*, is a prominent disease resistance protein after virus infection (and also attacks from other pathogens) and recent studies show that it might act together with *SERK3* as a co-receptor in antiviral defence (Caro *et al.*, 2022; Roux *et al.*, 2011; Yang *et al.*, 2010). The gene was not investigated in intergenerational or DNA methylome studies yet, so this data could be a first hint of a possible function of *SERK4* in retaining stress memory. The SERK family was shown to interact with important other defence response families, like SCOOP (Gully, 2019), and although it has not been shown so far, *SERK4* could play a key role in connecting plant defences after virus infection in the long term.

The presented genes were not studied yet for transgenerational effects in virus or other infection types. Throughout the last years more and more transgenerational studies considering DNA methylation changes were made in particular for bacterial infection (Hu *et al.*, 2018; Stassen *et al.*, 2018; Zheng *et al.*, 2013) and researchers work on understanding the full picture.

To sum this up, DNA methylation may not be the main epigenetic mechanism in creating a plant stress memory after virus infection. However, it still potentially regulates highly interesting disease related genes in the offspring of virus-infected plants. Further studies would be needed to ascertain the function and impact of these genes.

5 Conclusion and Outlook

The transcriptome and DNA methylome of infected plants as well as their next generation was investigated in next-generation-sequencing experiments. For the transcriptome total RNA was analysed using mRNA-Sequencing. For the DNA methylome genomic DNA was analysed via Whole-Genome-Bisulfite-Sequencing (WGBS). Sequencing data was gained for infected plants (also called the first (1st) generation) and their offspring (also called the second (2nd) generation) and bioinformatically analysed. The overall aim of these experiments was in finding differences between infected and uninfected tissues in the 1st generation. In the 2nd generation a comparison of offspring plants originating from infected and mock treated plants was made. The transcriptomal sequencing data of each context was analysed for their most strongly up- or downregulated genes, ordered by the Log2Fold-Change. The background of the genes as well as importance and relations to virus infection was investigated in this thesis. A basic analysis of the DNA methylome of all previously mentioned contexts was performed. The methylation status of the differential expressed genes was determined.

Genes altered in their expression after infection were detected manifold, the number of effected genes in the offspring was narrower. For many genes a role in stress response could be found, an overview was created in form of a mind-map. A relation to virus infection was found only for a small number of genes. Thus, detected genes in this study could function as an addition to the still small basic knowledge of virus infection. A variety of very interesting candidate genes, possibly playing important roles during virus infection, resulted from this thesis. They arose directly from or in relation to highly interesting areas such as the cell wall, phytohormones ETI, PTI and more. Further studies on them could benefit the scientific community to extend the understanding of virus-plant infections. Not only to get a clearer picture of the waves of mechanisms after infection within the same plant, but also the inheritance of information to the next generation. Especially the interconnectedness between the infected plant generation and the following led to the surprising formation of patterns. Thus happened a downregulation in the 1st generation with an upregulation in the 2nd generation mostly for genes involved in resistance to biotic stress. This distinctive partitioning is worthwhile of exploring further.

Part of this thesis was to study epigenetics effects of virus-plant infections via DNA methylation. While being heavily discussed in the human context and also for bacterial-plant infections, there is still little known regarding the virus-plant infection context. The mechanisms

behind DNA methylation playing a part in defence response (inherited also to the next generation) are very complex. Still, the results of this study show several genes with altered DNA methylation that also displayed changes in their expression after virus infection. This was detected intra- and transgenerational. Three genes are distinctively interesting (*FLOT2*, *RLP53* and *SERK4*) and are potentially linking intergenerational expression strength alteration to DNA methylation changes.

Interesting genes could be investigated in various ways in the future. Studying the transcriptome of plants in different infection stages or under differing stresses (biotic and abiotic) would be interesting. Also a combined stress study would bring forth much information. For epigenetic studies it would be highly interesting to include the analysis of histone modification changes. Apart from NGS techniques, protein-protein interaction studies could be made. Finally, mutant plants (transient or stable) showing the effect of overexpression or knock-down (-out) of the specific gene might reveal valuable information.

Furthermore drastic phenotypic changes for inflorescences after virus infection were detected in a growth study. The expression strength alterations of a handful of cell wall related genes was proposed to potentially contribute. Genes are from three areas: firstly fasciclin-like arabinogalacteran (FLA) proteins, a subgroup of cell wall glycoproteins. Secondly GA20-oxidases which are involved in the gibberellic acid biosynthesis, a phytohormone promoting growth (MacMillan, 1958). As a third part a regulated homeobox gene (*ATHB12*). It fills the gap between ABA induction after TuMV infection (Manacorda, 2021) and altered GA levels (Son *et al.*, 2010) by repressing the formerly mentioned oxidases. This leads to a hypothesized pathway: TuMV infection induces ABA, which increases *AtHB12* gene expression. This represses GA20oxidases, thus leading to low levels of GA. As this phytohormone promotes growth, inflorescences show decreased heights. These genes were detected in the RNA-Seq analysis and were further analysed in qPCR studies in additional tissue types and different stages of phenotypic changes. It appears that the detected genes can not explain the altered phenotypic appearances between the visible stages of different severity. All candidate genes showed similar expression patterns in phenotypic plants. There seem to be other important factors (f.e. exact development status at time of infection) involved. It would be interesting to study the candidate genes and the proposed additional factors further to determine if the proposed hypothesis might be the missing piece of the puzzle that results in the observed phenotype. Future studies could include comprehensive growth analyses to further determine the effect of the effect of exact development status in the infection event on the phenotype. It would be also interesting to

further investigate the hypothesis by producing mutant plants (transient or stable) of the candidate genes.

To sum it up, the results of this study are adding knowledge to the still small research about plant-virus interactions. Interconnected studies of two generations combined with the epigenetic analysis brought forward genes that were never investigated and connected in this way before. Further studies on them could bring a significant insight into defence research usable hopefully some day in the agriculture.

6 Abstract

This thesis investigated the effects of a virus infection on a plant organism. The infection was studied on the example of *Arabidopsis thaliana* plants (a prominent model organism closely related to many crop species) infected with the Turnip Mosaic virus (part of the potyvirus family which is causing much of the damage in crop species).

A virus infection was established and viral RNA and proteins were successfully and reliable detected for the type of tissue later to be harvested for further experiments.

Virus infection alters the transcriptome of infected plants. This is common knowledge in the scientific community, however through this study many genes were related to virus infection for the first time. Interesting genes related to broad topics like transcription factors, cell wall, phytohormones as well as biotic and abiotic stresses were found. Effects from virus infection also proceeds into the offspring of infected plants, as studies show. Especially the intergenerational expression strength alteration shows an interesting, function-related pattern of genes.

It was furthermore found, that DNA methylation might not be as important in virus infection as it is in other infection types. Still, the analysis of intergenerational DNA methylation on genes with expression changes resulted in three interesting genes: FLOT2, RPL53 and SERK4, all connected to disease response.

An inflorescence phenotype was observed after virus infection and growth studies were done. Alterations in the transcriptome of cell wall genes were detected which might play a role in the underlying molecular mechanisms causing the phenotype.

7 Zusammenfassung

Diese Arbeit untersuchte die Effekte einer Virusinfektion auf einen pflanzlichen Organismus. Dabei wurde am Beispiel von *Arabidopsis thaliana* Pflanzen (einem bekannten Modellorganismus verwandt mit vielen Nutzpflanzen) infiziert mit dem Turnip Mosaic Virus (Teil der Potyvirus-Familie, bekannt für ihren Befall von Nutzpflanzen) gearbeitet.

Die Virusinfektion konnte erfolgreich etabliert werden und die Detektion von viraler RNA und Proteinen konnten etabliert und genutzt werden um eine Ernte von ausschließlich infiziertem Gewebe für Experimente zu ermöglichen.

Eine Virusinfektion verändert das Transkriptom infizierter Pflanzen. Durch diese Arbeit konnten eine wertvolle Menge an pflanzlichen Genen erstmal mit einer Virusinfektion in Verbindung gebracht werden. Diese stammen aus interessanten Gebieten wie den Transkriptionsfaktoren, der Zellwand, Phytohormonen oder biotischen und abiotischen Stressantworten. Effekte der Virusinfektion wurden auch in der Nachfolgegeneration (uninfiziert) nachgewiesen. Hier waren besonders generationsübergreifende Expressionsmuster interessant.

Weiterhin wurde ermittelt das der epigenetische Mechanismus der DNA Methylierung möglicherweise in der Virusinfektion keine vergleichbar große Rolle wie in anderen Infektionstypen spielt. Dennoch erbrachte die Analyse von generationsübergreifenden Expressionsänderungen kombiniert mit entsprechenden Methylierungsmuster and den Promotern interessante Ergebnisse. Vor allem drei Gene (FLOT2, RLP53 und SERK4) zeigten dieses Muster und waren verknüpft mit der pflanzlichen Reaktion auf Krankheiten.

Letztlich konnte innerhalb der Arbeit mittels einer Wachstumsstudie ein Infloreszenz-Phänotyp ausgelöst durch die Virusinfektion ermittelt werden. Eine Studie des Transkriptoms ergab die Veränderung entscheidender Gene im Bereich der Zellwandregulation. Es wurde eine Hypothese erstellt die möglicherweise den beobachteten Phänotyp teilweise erklärt.

8 Literature

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9 Appendix

FLA11

Sequence from NCBI (https://www.ncbi.nlm.nih.gov/gene/831914):
>NM_120395.2 Arabidopsis thaliana FASCICLIN-like arabinogalactan-protein 11 (FLA11), mRNA
ACACACAACAACTACAACAATGGCTACTTCAAGAACATTCATTTTCTCTAATCTCTTCATCTTCTTC
CTCGTTATAGCCACTACTTATGGTCAGGCTCCAGCTCCAGGCCCTTCAGGTCCAACGAACATAACC
GCAATCCTAGAAAAGGCTGGTCAATTCACATTGTTCATAAGACTTCTTAAAAGCACTCAAGCCTCA
GACCAAATCAACACTCAGCTCAATTCTTCCTCGAGTAATGGCTTAACCGTGTTTGCCCCGACTGAT
AACGCCTTCAACAGCCTCAAATCCGGAACCTTAAACTCATTGTCTGACCAACAAAAAGTTCAGCTT
GTTCAGTTCCATGTCTTACCTACACTCATAACCATGCCTCAGTTTCAAACCGTTAGTAACCCTTTAC
GCACGCAAGCTGGAGATGGCCAAAACGGTAAATTCCCTCTTAACATCACTAGCTCCGGTAACCAA
GTTAACATCACCACTGGAGTTGTCAGCGCCACCGTGGCTAACTCTGTCTACAGCGATAAGCAGCTG
GCCGTTTATCAGGTTGATCAAGTTTTGCTGCCATTAGCCATGTTTGGATCAAGCGTGGCTCCTGCT
CCGGCCCCTGAGAAAGGCGGCTCTGTTTCAAAAGGCTCAGCTTCCGGTGGCGATGATGGAGGAGA
TTCTACTGATTCATCTGATGCAGAGAGGACTGGATTCGGGTTTGGGATCAGAATCACTACCGTTGC
AGCCATTGCTGCTTCTTCTTCTCTGTGGATATAACTCGAGAGAGAGCTTTTCTTGAGTCTCCACATT
TGGAATTTTAAGAATAACGAGAGCTTTAAGTTTTTGTTGTACTTTGAGATTGTTTTATAGATGTGT
TATTACGTGTGATTGTTTTGATTAAGATTGCACACAACTTTTTTTGATG

FLA12
Sequence from NCBI (https://www.ncbi.nlm.nih.gov/gene/836170):
>NM_125442.3 Arabidopsis thaliana FASCICLIN-like arabinogalactan-protein 12 (FLA12), mRNA
AAGCAATTCATGTGATAAAATCATCATTGGTTATTAACAATCAACTTAGTTGTTAAAATATGTTGA
CCTCTTAACCAATCAATTTCCCCCAGTTCAATATAACCCCTTACTCTTCATATAAACTCACACAAAC
AAAATATCAACACTCTTGAAAACAGAGCCATGGAACATTCTCTCATCATCCTCCTCTTCACCGTCC
TCCTCCTCCTCACCACCACTCCCGGAATCCTATCTCAGCCCTCTCCAGCCGTCGCTCCGGCCCCACC
AGGACCCACAAACGTTACCAAAATCCTAGAGAAAGCTGGTCAATTCACCGTCTTCATCCGACTCCT
CAAATCCACTGGAGTCGCTAACCAACTCTACGGCCAACTAAACAACTCCGACAATGGAATCACTAT
ATTCGCACCGAGTGATTCCTCGTTCACCGGTCTCAAAGCCGGGACCCTAAATTCGTTAACCGATGA
GCAACAAGTAGAGCTTATTCAGTTTCATGTCATACCAAGTTACGTCTCTTCCTCAAACTTCCAAAC
AATAAGTAACCCTCTCCGGACTCAGGCCGGTGACTCCGCCGATGGACATTTTCCTCTCAACGTTAC
CACCAGTGGCAATACCGTAAATATCACGTCCGGCGTAACCAACACCACCGTCTCCGGCAATGTCTA
CAGCGATGGACAGCTCGCTGTTTATCAGGTCGATAAGGTTTTGCTTCCACAACAAGTTTTCGATCC
TCGTCCTCCTGCTCCGGCTCCGGCTCCGTCTGTATCGAAATCAAAGAAGAAGAAGGATGACAGTGA
TAGTTCCAGTGATGATTCTCCGGCGGATGCTTCGTTTGCTTTGCGTAATGTTGGTTCTGTGTGTGAT
GCGGTGTCGTTTTGCGTCATGAGTGTAATGCTCGCATGGTTTTATTTGTGATGAAAAGCTTTTTTTT
TTTTTGTGCTGATAAATTGTTATTTTTTATTACTCATGATTTTCTGCCATGTGGGTGATTTTGGGGA
TATATTTGTGTCTTGGTTTTCATACGATTTTGTGTTAATCAATCCATTTGTTTTTTATATTTTCGTTT
ATTTTTATTAATACAAATCATATTTTCCGTTTTCGTGCGAGTGAGAATTGTGTTTTTGTTAATATCT
CGTTTATCTTTGCTTTTTCGGTTTTTCCTTTGATCCTATTTTCACATTTCTTTCAACAAGATCCAGTT
GCTTAAATAATAATGATCGATCGTATAGGTTAGCGAAACATAAAACTATATATCATCTAGATAGAT
AGCCGAATTAATTGCATAAAATGAATGTGATTTTTTATTTGAAAAATAAATAATTGGATGCTATTT
CATTAGAAACAGTGTGATATTTTATAAATGTCTTTTGCAAT

FLA16
Sequence from NCBI (https://www.ncbi.nlm.nih.gov/gene/818159):
>NM_179922.2 Arabidopsis thaliana FASCICLIN-like arabinogalactan protein 16 precursor (FLA16), mRNA
CTCTCTCCTTCTTCACAAGCAAAAAAACACCAAACCATTTTTCTTTCTTCATTGTGCCGACACTTCT
TTCCCCTCTCTCTCTCTCTCTCCACCGCCATGGATTCCTCCTATGGCGCCACAAAGTTCCTTCTCCT

CCTCTTCCTCACCACCTCCATTGCGACCGCATTACCCGATAACAAACCAGTACCGGGTCAAATAAA
CTCAAACTCA
GTCCTCGTAGCTCTCCTGGACTCTCACTACACAGAGCTAGCAGAACTAGTCGAAAAAGCTCTCCTT
CTCCAGACCCTCGAAGAAGCAGTTGGTAAACACAACATCACAATCTTCGCACCACGCAACGATGCC
TTGGAACGAAACTTAGACCCACTCTTCAAATCTTTCTTGCTCGAACCAAGAAACCTCAAATCTTTA
CAATCTTTATTAATGTTCCACATTCTTCCTAAACGAATCACTTCTCCTCAATGGCCTTCTCTTTCTC
ATCACCACCGTACTCTCTCCAACGACCATCTCCACCTCACCGTCGACGTCAACACTCTTAAAGTAG
ATTCCGCTGAGATTATCCGACCCGATGACGTCATTAGACCCGATGGTATCATTCACGGCATCGAAC
GTCTTCTCATCCCTCGCTCTGTTCAAGAAGATTTTAACCGCCGTCGTAGTCTCCGTTCAATCTCCGC
CGTTATACCAGAAGGAGCTCCTGAGGTTGACCCTAGAACTCACCGTCTCAAGAAACCATCTCCCGC
CGTTCCCGCCGGAGCTCCTCCGGTTCTTCCAATCTACGACGCTATGTCACCAGGTCCTTCCCTAGCT
CCTGCTCCAGCTCCCGGACCCGGTGGTCCACGTGGCCATTTCAACGGCGATGCTCAAGTTAAAGAT
TTCATCCACACTCTCTTGCATTACGGTGGCTACAACGAGATGGCTGATATACTCGTCAACTTAACC
TCTTTAGCCACTGAGATGGGTCGACTCGTGTCAGAAGGCTACGTTTTAACCGTTCTTGCTCCTAAC
GACGAAGCCATGGCTAAGCTCACAACGGACCAGCTTAGCGAGCCAGGTGCTCCTGAACAAATTAT
GTATTACCACATCATACCGGAGTATCAAACAGAGGAGAGTATGTACAACGCTGTTCGGAGATTCG
GAAAAGTGAAGTATGATTCATTGAGATTCCCACATAAAGTGTTGGCTCAAGAAGCTGATGGATCT
GTCAAATTCGGACACGGTGATGGTTCAGCTTACTTGTTTGATCCTGATATCTACACGGACGGTCGG
ATTTCAGTTCAGGGTATTGATGGAGTCTTGTTCCCGAAGGAGGAAACGCCGGCGACGGAGATTAA
ACCAGCTGCTCCGGTCGTTAAGAAAGTTTCTAAATCAAGAAGAGGTAAATTGATGGAGGTAGCTT
GTAGAATGATGGGGTCACGGTTTATTCCGTGTCAGTGATTTCACACGTTATCAAAAAAAAATTCCA
AGCTTCCAAATTCTTAATTCTGTAAACATGTGGAAAAAAAAAAGAAAGAGTTGAATATGTAAATG
ATGTGATTTTTGGGTTCGTTGTTATTTGCATGTTCTATTTGTAATTTTTTTATTATCATAAATATAT
ATATATATATACGTAGATTTTGATTAAGTTTGTTATTTGGGGGTGAGTAAAAAAGTTCTAGGAGTT
TAGAGAAATCAAATATGTAATACAAGTTATACAATCTGTATGGAAATAATAAAGGAAGGATCCAT
TTGTGTGCCTATTCTATTCTA

GA20OX1

Sequence from NCBI: https://www.ncbi.nlm.nih.gov/gene/828645

>NM_118674.5 Arabidopsis thaliana 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein (GA20OX1), mRNA

TTGATAACTCCATTACAGACTATAGTATTGTACTACTAGAAAACAAAAACAACAAAAAAAGAAGT
GGACAACACTATACGATCGACTTAAATGCTTGCTTATATAAAGACTAAAAGGACCATTGGTTCCCG
TATCTCCTCGCAATACTACTACTCACTTTACTATAATCTCTCAAAATGGCCGTAAGTTTCGTAACAA
CATCTCCTGAGGAAGAAGACAAACCGAAGCTAGGCCTTGGAAATATTCAAACTCCGTTAATCTTCA
ACCCTTCAATGCTTAACCTTCAAGCCAATATCCCAAACCAATTCATCTGGCCTGACGACGAAAAAC
CTTCCATCAACGTTCTCGAGCTTGATGTTCCTCTCATCGACCTTCAAAACCTTCTCTCTGATCCATC
CTCCACTTTAGATGCTTCGAGACTGATCTCTGAGGCCTGTAAGAAGCACGGTTTCTTCCTCGTGGT
CAATCACGGCATCAGCGAGGAGCTTATTTCAGACGCTCATGAATACACGAGCCGCTTCTTTGATAT
GCCTCTCTCCGAAAAACAGAGGGTTCTTAGAAAATCCGGTGAGAGTGTTGGCTACGCAAGCAGTT
TCACCGGACGCTTCTCCACCAAGCTTCCATGGAAGGAGACCCTTTCTTTCCGGTTTTGCGACGACA
TGAGCCGCTCAAAATCCGTTCAAGATTACTTCTGCGATGCGTTGGGACATGGGTTTCAGCCATTTG
GGAAGGTGTATCAAGAGTATTGTGAAGCAATGAGTTCTCTATCACTGAAGATCATGGAGCTTCTG
GGGCTAAGTTTAGGCGTAAAACGGGACTACTTTAGAGAGTTTTTCGAAGAAAACGATTCAATAAT
GAGACTGAATTACTACCCTCCATGTATAAAACCAGATCTCACACTAGGAACAGGACCTCATTGTGA
TCCAACATCTCTTACCATCCTTCACCAAGACCATGTTAATGGCCTTCAAGTCTTTGTGGAAAATCA
ATGGCGCTCCATTCGTCCCAACCCCAAGGCCTTTGTGGTCAATATCGGCGATACTTTCATGGCTCT
ATCGAACGATAGATACAAGAGCTGCTTGCACCGGGCGGTGGTGAACAGCGAGAGCGAGAGGAAA
TCACTTGCATTCTTCTTGTGTCCGAAAAAAGACAGAGTAGTGACGCCACCGAGAGAGCTTTTGGAC
AGCATCACATCAAGAAGATACCCTGACTTCACATGGTCTATGTTCCTTGAGTTCACTCAGAAACAT
TATAGAGCAGACATGAACACTCTCCAAGCCTTTTCAGATTGGCTCACCAAACCCATCTAAGAAATA
AAATATTCATGTCTTGTCTTGTTAGTTACTAGTATCTTCTTTATATTTCATGTATGTATATGGTAAT
AGGCAATAACACCTTTTAGCATCTCATTTATAAAATCTATCCGTTAAACTAAAATACT

GA20OX2

Sequence from NCBI: https://www.ncbi.nlm.nih.gov/gene/835256
>NM_124560.4 Arabidopsis thaliana gibberellin 20 oxidase 2 (GA20OX2), mRNA
TACACTAACATGACTTGAAGCTTGCTTATATAAAGACTTAAAGGACCCTTTGTTCCCCCATCTCCTC
AACAACACTCACTCAGAACAAGACAAAACAAAAACCCCAAAACTCTCAAGAAAAAAAAAAGAAAA
GAAATGGCGATACTATGCACAACAACATCTCCGGCAGAGAAAGAACACGAACCAAAACAAGATCT
TGAAAAAGACCAAACTTCTCCACTAATCTTTAACCCTTCTCTTCTTAACCTCCAATCCCAAATCCCA
AACCAATTCATTTGGCCAGACGAAGAGAAACCTTCCATTGACATTCCAGAGCTCAACGTCCCGTTC
ATCGATCTCTCAAGCCAAGACTCGACTCTTGAAGCTCCTAGAGTCATCGCAGAAGCTTGCACCAAA
CACGGCTTCTTCCTCGTCGTCAATCATGGCGTCAGCGAGTCACTAATAGCGGATGCTCACCGTTTG
ATGGAAAGTTTCTTCGACATGCCTCTCGCCGGCAAACAGAAAGCTCAGAGAAAACCCGGTGAGAG
TTGTGGCTATGCAAGTAGCTTCACCGGCAGATTCTCCACTAAGCTCCCATGGAAGGAGACTCTCTC
TTTTCAGTTTTCCAACGATAATAGTGGCTCGAGAACCGTTCAAGATTACTTTTCCGATACATTAGG
ACAAGAGTTCGAGCAGTTTGGGAAGGTGTATCAAGACTATTGTGAAGCAATGAGTTCTCTATCACT
CAAGATCATGGAGCTTCTGGGCTTAAGTTTAGGCGTAAACCGAGACTATTTCCGAGGATTTTTCGA
AGAGAACGATTCGATAATGAGGCTCAATCATTATCCTCCATGCCAAACACCAGATCTCACGTTAGG
TACAGGACCTCATTGTGATCCAAGTTCTTTGACCATCCTTCATCAAGACCATGTCAATGGCCTTCA
AGTCTTTGTCGACAATCAATGGCAATCCATTCGTCCCAATCCCAAGGCTTTCGTTGTCAATATTGG
TGACACTTTCATGGCTCTATCGAACGGGATATTCAAGAGCTGTTTGCATAGAGCGGTTGTGAATAG
AGAGAGCGCGAGAAAATCGATGGCGTTTTTCTTGTGTCCGAAGAAAGACAAAGTGGTGAAACCAC
CAAGTGATATTTTGGAGAAGATGAAAACAAGAAAATACCCTGACTTCACTTGGTCTATGTTCCTTG
AGTTCACTCAAAAACATTACCGAGCAGATGTGAATACTCTCGATTCCTTTTCGAATTGGGTTATTA
CCAACAACAATCCCATCTAAGAAACAAAATTATTTACTATCTCAATCTTTTGTTTTTCTTTGGTTAC
TTTGTGTCCTTTGTTCTCATGGTGAAATGCATTAAATTGCATTTCAAAGTTTTAAACGTTTGTATAT
TGATTGTTCCAAGCTTTAGACCAATCCCTACCGTATGAGCTCGTTCAATGAATAATTTGAATGAAA
AATTCAAAGAAATTTTTCTTCATCTTTGTT

GA20OX3
Sequence from NCBI: https://www.ncbi.nlm.nih.gov/gene/830611
>NM_120802.2 Arabidopsis thaliana gibberellin 20-oxidase 3 (GA20OX3), mRNA
CCCAAATGCCGCTTACGTACTATTCCCTGCACCTAAGTTTCCCTCTCGCACCTATATATACCACTCC
TTTCCTCTCCCACTTACCGACCACTGTACTGAATCTTTAAGCCTCTCAACGTGTTTTTTATATATAT
TTTGAAAATCTTTTACGCCTTAAAAGGATCTACGATAATTAAAAAAAAAATGGCAACGGAATGCATT
GCAACGGTCCCTCAAATATTCAGTGAAAACAAAACCAAAGAGGATTCTTCGATCTTCGATGCAAA
GCTCCTTAATCAGCACTCGCACCACATACCTCAACAGTTCGTATGGCCCGACCACGAGAAACCTTC
TACGGATGTTCAACCTCTCCAAGTCCCACTCATAGACCTAGCCGGTTTCCTCTCCGGCGACTCGTG
CTTGGCATCGGAGGCTACTAGACTCGTCTCAAAGGCTGCAACGAAACATGGCTTCTTCCTAATCAC
TAACCATGGTGTCGATGAGAGCCTCTTGTCTCGTGCCTATCTGCATATGGACTCTTTCTTTAAGGC
CCCGGCTTGTGAGAAGCAGAAGGCTCAGAGGAAGTGGGGTGAGAGCTCCGGTTACGCTAGTAGTT
TCGTCGGGAGATTCTCCTCAAAGCTCCCGTGGAAGGAGACTCTGTCGTTTAAGTTCTCTCCCGAGG
AGAAGATCCATTCCCAAACCGTTAAAGACTTTGTTTCTAAGAAAATGGGCGATGGATACGAAGAT
TTCGGGAAGGTTTATCAAGAATACGCGGAGGCCATGAACACTCTCTCACTAAAGATCATGGAGCTT
CTTGGAATGAGTCTTGGGGTCGAGAGGAGATATTTTAAAGAGTTTTTCGAAGACAGCGATTCAAT
ATTCCGGTTGAATTACTACCCGCAGTGCAAGCAACCGGAGCTTGCACTAGGGACAGGACCCCACT
GCGACCCAACATCTCTAACCATACTTCATCAAGACCAAGTTGGCGGTCTGCAAGTTTTCGTGGACA
ACAAATGGCAATCCATTCCTCCTAACCCTCACGCTTTCGTGGTGAACATAGGCGACACCTTCATGG
CTCTAACGAATGGAAGATACAAGAGTTGTTTGCATCGGGCGGTGGTGAACAGCGAGAGAGAAAGG
AAGACGTTTGCATTCTTCCTATGTCCGAAAGGGGAAAAAGTGGTGAAGCCACCAGAAGAACTAGT
AAACGGAGTGAAGTCTGGTGAAAGAAAGTATCCTGATTTTACGTGGTCTATGTTTCTTGAGTTCAC
ACAGAAGCATTATAGGGCAGACATGAACACTCTTGACGAGTTCTCAATTTGGCTTAAGAACAGAA
GAAGTTTCTAAAATTGAGGAGACAATGTTGTGGTCCAGAAGGTCACTTGTAGTTATGTATAGAGCT
TTCACTGTTTTTCTTAAGATGTTCAAGACTTGTTAAGGATCGGAGACTTTTTTGTTCTTTTATTATC
TTGCTCTAGGTTGTCTGGTGTTAAATAAAAGTAGCAAAAAAATAAAAATATATGTACTCCATTGGG
TCTACTTATGATTTGTTTCGAAATGGTTTTTTTATAGTCTCTAATGTTAAATTTATGTCACTATTAC
TATTATTAAATGAAGTTTATCAAGTAC

GAI

Sequence from NCBI: https://www.ncbi.nlm.nih.gov/gene/838057

>NM_101361.3 Arabidopsis thaliana GRAS family transcription factor family protein (GAI), mRNA

CTAAACTCTAAAGTAATACTAACTTTAGAAAATATTAATCTGTAAAAAGCTATATATTCCCTAATA
TATTAGTTTATTAATTTCAATATATTCCCTAATTACAAATTTTGGTGTGTGTGTGATTTTCAGCCTT
ATCTCTTTGAGCTGTAGATGTTGCTGTTAGCCCTCTGATCCATGATTCATCGGACATCGAGCCCCA
TCACGAGCCTTTTTATTCTCAACAATAATAATCATTTTTTTTCTTATAACCTTCCTCTCTATTTTTAC
AATTTATTTTGTTATTAGAAGTGGTAGTGGAGTGAAAAAACAAATCCTAAGCAGTCCTAACCGATC
CCCGAAGCTAAAGATTCTTCACCTTCCCAAATAAAGCAAAACCTAGATCCGACATTGAAGGAAAA
ACCTTTTAGATCCATCTCTGAAAAAAAACCAACCATGAAGAGAGATCATCATCATCATCATCATCA
AGATAAGAAGACTATGATGATGAATGAAGAAGACGACGGTAACGGCATGGATGAGCTTCTAGCTG
TTCTTGGTTACAAGGTTAGGTCATCCGAAATGGCTGATGTTGCTCAGAAACTCGAGCAGCTTGAAG
TTATGATGTCTAATGTTCAAGAAGACGATCTTTCTCAACTCGCTACTGAGACTGTTCACTATAATC
CGGCGGAGCTTTACACGTGGCTTGATTCTATGCTCACCGACCTTAATCCTCCGTCGTCTAACGCCG
AGTACGATCTTAAAGCTATTCCCGGTGACGCGATTCTCAATCAGTTCGCTATCGATTCGGCTTCTT
CGTCTAACCAAGGCGGCGGAGGAGATACGTATACTACAAACAAGCGGTTGAAATGCTCAAACGGC
GTCGTGGAAACCACTACAGCGACGGCTGAGTCAACTCGGCATGTTGTCCTGGTTGACTCGCAGGA
GAACGGTGTGCGTCTCGTTCACGCGCTTTTGGCTTGCGCTGAAGCTGTTCAGAAAGAGAATCTGAC
TGTAGCGGAAGCTCTGGTGAAGCAAATCGGATTCTTAGCCGTTTCTCAAATCGGAGCGATGAGAA
AAGTCGCTACTTACTTCGCCGAAGCTCTCGCGCGGCGGATTTACCGTCTCTCTCCGTCGCAGAGTC
CAATCGACCACTCTCTCTCCGATACTCTTCAGATGCACTTCTACGAGACTTGTCCTTATCTCAAGTT
CGCTCACTTCACGGCGAATCAAGCGATTCTCGAAGCTTTTCAAGGGAAGAAAAGAGTTCATGTCAT
TGATTTCTCTATGAGTCAAGGTCTTCAATGGCCGGCGCTTATGCAGGCTCTTGCGCTTCGACCTGG
TGGTCCTCCTGTTTTCCGGTTAACCGGAATTGGTCCACCGGCACCGGATAATTTCGATTATCTTCAT
GAAGTTGGGTGTAAGCTGGCTCATTTAGCTGAGGCGATTCACGTTGAGTTTGAGTACAGAGGATTT
GTGGCTAACACTTTAGCTGATCTTGATGCTTCGATGCTTGAGCTTAGACCAAGTGAGATTGAATCT
GTTGCGGTTAACTCTGTTTTCGAGCTTCACAAGCTCTTGGGACGACCTGGTGCGATCGATAAGGTT
CTTGGTGTGGTGAATCAGATTAAACCGGAGATTTTCACTGTGGTTGAGCAGGAATCGAACCATAAT
AGTCCGATTTTCTTAGATCGGTTTACTGAGTCGTTGCATTATTACTCGACGTTGTTTGACTCGTTGG
AAGGTGTACCGAGTGGTCAAGACAAGGTCATGTCGGAGGTTTACTTGGGTAAACAGATCTGCAAC
GTTGTGGCTTGTGATGGACCTGACCGAGTTGAGCGTCATGAAACGTTGAGTCAGTGGAGGAACCG
GTTCGGGTCTGCTGGGTTTGCGGCTGCACATATTGGTTCGAATGCGTTTAAGCAAGCGAGTATGCT
TTTGGCTCTGTTCAACGGCGGTGAGGGTTATCGGGTGGAGGAGAGTGACGGCTGTCTCATGTTGG
GTTGGCACACACGACCGCTCATAGCCACCTCGGCTTGGAAACTCTCCACCAATTAGATGGTGGCTC
AATGAATTGATCTGTTGAACCGGTTATGATGATAGATTTCCGACCGAAGCCAAACTAAATCCTACT
GTTTTTCCCTTTGTCACTTGTTAAGATCTTATCTTTCATTATATTAGGTAATTGAAAAATTTTAATC
TCGCTTTGGAGAGTTTTTTTTTTTTGCATGTGACATTGGAGGGTAAATTGGATAGGCAGAAATAGA
AGTATGTGTTACCAAGTATGTGCAATTGGTTGAAATAAAATCATCTTGAGTGTCACCATCTATAAA
ATTCATTGTAATGACTAATGAGCCTGATTAAACTGTCTCTTATGATAATGTGCTGATTCTCATGAA
TATGCTCTTTTAATGTGCATGGTATTATAGGTGGACCAGATTATTTTAACAATGCTAAG

ATHB12

Sequence from NCBI: https://www.ncbi.nlm.nih.gov/gene/825362

>NM_116054.3 Arabidopsis thaliana homeobox 12 (HB-12), mRNA

CTACACAAGCACACATAGTAACTCCCACATTATATATAAGCGGCCAATATCAGCAACTCAGAGATT
CCAGAAAGAAAGAAAAAAAAGAAACAAATAATTCCAAAACCTTCTCTCTTAATCAAAATCAAGAA
ACTTACAAGATCTGGTGAAAACCATGGAAGAAGGAGATTTTTTCAACTGCTGTTTCAGCGAGATTA
GTAGTGGCATGACCATGAATAAGAAGAAGATGAAGAAGAGCAATAACCAAAAGAGGTTTAGCGA
GGAACAGATCAAGTCACTTGAGCTTATATTTGAGTCTGAGACGAGGCTTGAGCCGAGGAAGAAGG
TTCAGGTAGCTAGAGAGCTAGGGCTGCAACCAAGACAAGTGGCTATATGGTTTCAAAACAAGAGG
GCTCGATGGAAAACTAAGCAACTTGAGAAAGAGTATAACACTCTTAGAGCCAATTACAACAATTT
GGCTTCACAATTTGAAATCATGAAGAAAGAAAAGCAATCTCTGGTCTCTGAGCTGCAGAGACTAA
ACGAAGAGATGCAAAGGCCTAAAGAAGAAAAGCATCATGAGTGTTGTGGTGATCAAGGACTGGCT

CTAAGCAGCAGCACAGAGTCGCATAATGGAAAGAGTGAGCCAGAAGGGAGGTTAGACCAAGGGA
GTGTTCTATGTAATGATGGTGATTACAACAACAACATTAAAACAGAGTATTTTGGGTTCGAGGAA
GAGACTGATCATGAGCTGATGAACATTGTGGAGAAAGCTGATGATAGTTGCTTGACATCTTCTGA
GAATTGGGGAGGTTTCAATTCTGATTCTCTCTTAGACCAATCTAGCAGCAATTACCCTAACTGGTG
GGAGTTTTGGTCATAAAAGCATATAAGAAAAAAACAGAACATAAGCGAAGAGAAAGAGTGTGAA
TAGTTTGTAAATTATGTGTTAAGAAAAATAAATTTAGTTTAGTTTAAATCTTGTTTCGATCTATGT
ATCTACTATGTTCAATACTCTTTGTAGCTAATTAGTAGCTTATAATGAGACTAGAAAAGTTTTGAA
GTCACCAAGGTT

ACT8

Sequence from NCBI: https://www.ncbi.nlm.nih.gov/gene/841347

>NM_103814.4 Arabidopsis thaliana actin 8 (ACT8), mRNA

TCTTTGTCGAATTTAATTATTTCCAAAATTGATGACTCTAAAGAAAAAAAAAATAGTTTTTCAGATA
AACCCGCCTATATAAATAGTTCAACACTCGGTTTATTTCTTCTCCCCTCTTTGAATTGCCTCGTCGT
CTTCAGCTTCATCGGCCGTTGCATTTCCCGGCGATAAGAGAGAGAAAGAGGAGAAAGAGTGAGCC
AGATCTTCATCGTCGTGGTTCTTGTTTCTTCCTCGATCTCTCGATCTTCTGCTTTTGCTTTTCCGATT
AAGATCGTAGACCATGGCCGATGCTGATGACATTCAACCTATTGTCTGTGACAATGGTACTGGAAT
GGTTAAGGCTGGATTCGCTGGAGATGATGCTCCCAGAGCGGTTTTCCCCAGTGTTGTTGGTCGACC
TAGACATCATGGTGTCATGGTTGGGATGAATCAGAAAGATGCGTATGTTGGTGATGAAGCACAAT
CCAAAAGAGGTATCCTCACATTGAAATACCCTATTGAGCATGGTGTTGTTAGCAACTGGGATGACA
TGGAGAAGATTTGGCATCACACTTTCTACAATGAGCTCCGTATTGCTCCTGAAGAGCACCCGGTTC
TACTTACCGAGGCTCCTCTTAACCCAAAAGCCAACAGAGAGAAGATGACTCAGATCATGTTTGAGA
CCTTTAATTCTCCAGCTATGTATGTTGCCATTCAAGCTGTTCTATCACTTTACGCCAGTGGTCGTAC
AACCGGTATTGTGTTGGACTCTGGTGATGGTGTGTCTCACACTGTGCCTATCTACGAGGGTTTCTC
ACTTCCACATGCTATCCTCCGTCTCGACCTTGCTGGTCGTGACCTTACTGATTACCTCATGAAGATC
CTTACCGAGAGAGGTTACATGTTCACCACAACAGCAGAACGGGAAATTGTGAGAGACATCAAGGA
GAAGCTTTCCTTTGTCGCTGTCGACTACGAGCAAGAGATGGAGACCTCGAAAACCAGCTCCTCCAT
CGAAAAGAACTATGAATTACCCGACGGACAAGTGATCACGATCGGTGCTGAGAGATTCAGGTGCC
CAGAAGTCCTTTTCCAGCCATCATTTGTTGGAATGGAAGCTGCAGGGATCCACGAGACAACTTACA
ACTCGATCATGAAGTGTGATGTTGATATCAGGAAGGACCTTTACGGTAACATTGTGCTCAGTGGTG
GTACAACTATGTTCTCAGGTATTGCAGACCGTATGAGCAAAGAGATCACAGCTCTTGCCCCGAGCA
GCATGAAGATTAAGGTCGTGGCACCACCCGAGAGGAAGTACAGTGTCTGGATTGGTGGTTCTATC
CTTGCTTCCCTCAGCACTTTCCAGCAGATGTGGATCTCTAAGGCAGAGTATGATGAAGCAGGTCCA
GGCATTGTCCACAGAAAATGCTTCTAAACTAAAGAGACATCGTTTCCATGACGGGATCACATTTCT
TTCTATTTCTCCAATTTGTTTGTTTCAAATTTTTTTCCCCTTTGTCATTTGTGCACTATGTGAGAAAC
TTTCCGGTTACAGCGTTTGGAGAGATGTCTAAGGAGGAGCAGGTTTGAAAACCCGCTCTCGCTCTT
ACCTGAGGCACTAATCCGCGTTTCAAACTCAGCTTCATTCTCTATTCTTGTCCATTTGTTTGTTTGT
TTGTAGCCTCTTCAAACTCGGATAAAAACAAAAGTTTTTGGACTATTGATATTTGTACTTTATTTG
ACAGAATTTCTGTGTTAGGAAGTATCACAACAAATTCGAAAAATTACCTATTGTTAGCCTAACATT
CGAGTTTTAGAACAAATCAG

Table 23 *Summary of mapping statistics after sequencing*

Samples Name	Total reads	Total mapped reads	Uniquely mapped reads	Multiple mapped reads	Total mapping rate	Uniquely mapping rate	Multiple mapping rate
Mock1 1. Gen	41076504	40378782	39661742	717040	98.30%	96.56%	1.75%
Mock2 1. Gen	44984044	44313784	43491970	821814	98.51%	96.68%	1.83%

Mock3 1. Gen	44923030	44209658	43375891	833767	98.41%	96.56%	1.86%
TuMV1 1. Gen	43769406	36797332	36109549	687783	84.07%	82.50%	1.57%
TuMV2 1. Gen	42548268	33857439	33190616	666823	79.57%	78.01%	1.57%
TuMV3 1. Gen	38993250	32559401	31972413	586988	83.50%	81.99%	1.51%
Mock1 2. Gen	43413058	42913731	41937455	976276	98.85%	96.60%	2.25%
Mock2 2. Gen	42797876	42201685	41227261	974424	98.61%	96.33%	2.28%
TuMV1 2. Gen	40287380	39711002	38784145	926857	98.57%	96.27%	2.30%
TuMV2 2. Gen	42410886	41881367	40894280	987087	98.75%	96.42%	2.33%
Mock1 phenotype	39096138	38466481	37587016	879465	98.39%	96.14%	2.25%
Mock2 phenotype	44955552	44324828	43316463	1008365	98.60%	96.35%	2.24%
Mock3 phenotype	39006916	38436049	37553634	882415	98.54%	96.27%	2.26%
TuMV1 phenotype	42040948	36716113	35895831	820282	87.33%	85.38%	1.95%
TuMV2 phenotype	43490198	36678456	35862638	815818	84.34%	82.46%	1.88%
TuMV3 phenotype	42941798	36544259	35747210	797049	85.10%	83.25%	1.86%

Table 24 *Summary of quality control after sequencing*

Sample name	Raw reads	Clean reads	Raw bases	Clean bases	Error rate(%)	Q20(%)	Q30(%)	GC content(%)
Mock1 1. Gen	20691267	20538252	6.2G	6.2G	0.03	97.86	93.61	45.59
Mock2 1. Gen	22658928	22492022	6.8G	6.7G	0.02	98.18	94.44	45.56
Mock3 1. Gen	22636331	22461515	6.8G	6.7G	0.02	98.19	94.48	45.54

TuMV1 1. Gen	22093805	21884703	6.6G	6.6G	0.03	98.02	94.13	45.8
TuMV2 1. Gen	21451700	21274134	6.4G	6.4G	0.02	98.15	94.33	45.59
TuMV3 1. Gen	19695227	19496625	5.9G	5.8G	0.02	98.04	94.17	45.73
Mock1 2. Gen	22341828	21706529	6.7G	6.5G	0.03	97.98	93.96	45.74
Mock2 2. Gen	21951560	21398938	6.6G	6.4G	0.03	97.9	93.88	45.89
TuMV1 2. Gen	20595476	20143690	6.2G	6.0G	0.02	98.06	94.39	46.08
TuMV2 2. Gen	21763242	21205443	6.5G	6.4G	0.02	98.2	94.69	46.05
Mock1 phenotype	21331287	21020474	6.4G	6.3G	0.02	98.05	94.3	45.23
Mock2 phenotype	22143853	21745099	6.6G	6.5G	0.02	98.26	94.77	45.27
Mock3 phenotype	21894448	21470899	6.6G	6.4G	0.02	98.2	94.61	45.21
TuMV1 phenotype	19859770	19548069	6.0G	5.9G	0.03	97.81	93.82	45.36
TuMV2 phenotype	22905520	22477776	6.9G	6.7G	0.02	98	94.2	45.49
TuMV3 phenotype	19894155	19503458	6.0G	5.9G	0.03	97.87	93.91	45.53

Table 25 *TuMV presence in sequencing samples*

Origin	Overall alignment rate
Mock1 1. Gen	0.00%
Mock2 1. Gen	0.00%
Mock3 1. Gen	0.00%
TuMV1 1. Gen	14.41%
TuMV2 1. Gen	19.02%
TuMV3 1. Gen	14.99%
Mock1 2. Gen	0.00%
Mock2 2. Gen	0.00%

TuMV1 2. Gen	0.00%
TuMV2 2. Gen	0.00%
TuMV1 phenotype	11.36%
TuMV2 phenotype	14.56%
TuMV3 phenotype	13.73%
Mock1 phenotype	0.00%
Mock2 phenotype	0.00%
Mock3 phenotype	0.00%

Table 26 *NAC genes regulated in first generation data set*

ATG Number	Gene name	Log2FC
AT3G01600	NAC044	8.5
AT3G44350	ANAC061	4.49
AT5G18270	NAC087	2.86
AT1G02230	NAC004	2.25
AT5G22380	NAC090	1.98
AT2G17040	ANAC036	1.45
AT1G34180	NAC016	0.935
AT1G02220	NAC003	0.846
AT5G13180	NAC083	0.788
AT5G24590	NAC091	0.74
AT1G01720	NAC002	0.56
AT3G10500	NAC053	0.41
AT5G14000	ANAC084	-2.9
AT3G29035	NAC59	-2.6
AT1G69490	NAC029	-2.18
AT5G39610	NAC92	-2.1
AT5G22290	NAC089	-1.42
AT1G56010	NAC021	-1.42
AT5G66300	NAC015	-1.38
AT3G15500	NAC055	-1.04
AT3G10490	ANAC052	-0.9

AT5G07680	NAC079	-0.7
AT3G15510	NAC056	-0.7
AT1G52890	NAC019	-0.7
AT3G10480	NAC050	-0.7
AT1G52880	NAC018	-0.5
AT1G33060	NAC014	-0.5
AT2G33480	NAC041	-0.5
AT4G01550	NAC69	-0.4
AT5G63790	ANAC102	-0.2

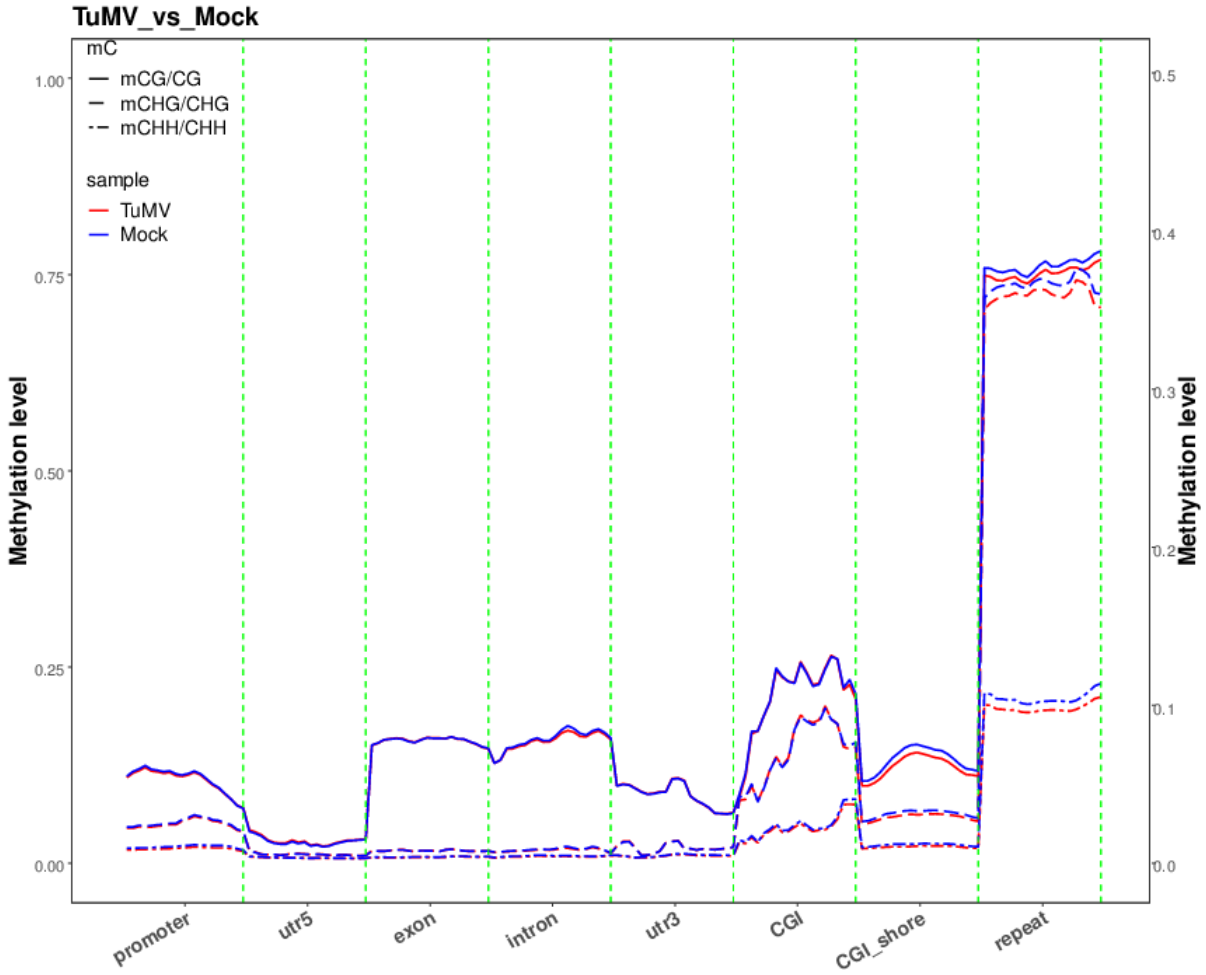

Figure 35 Methylation level distribution at functional genetic elements for all three methylation contexts of WGBS data set of 1st generation, TuMV infected samples are shown in red, Mock infected samples in blue (Source: Novogene)

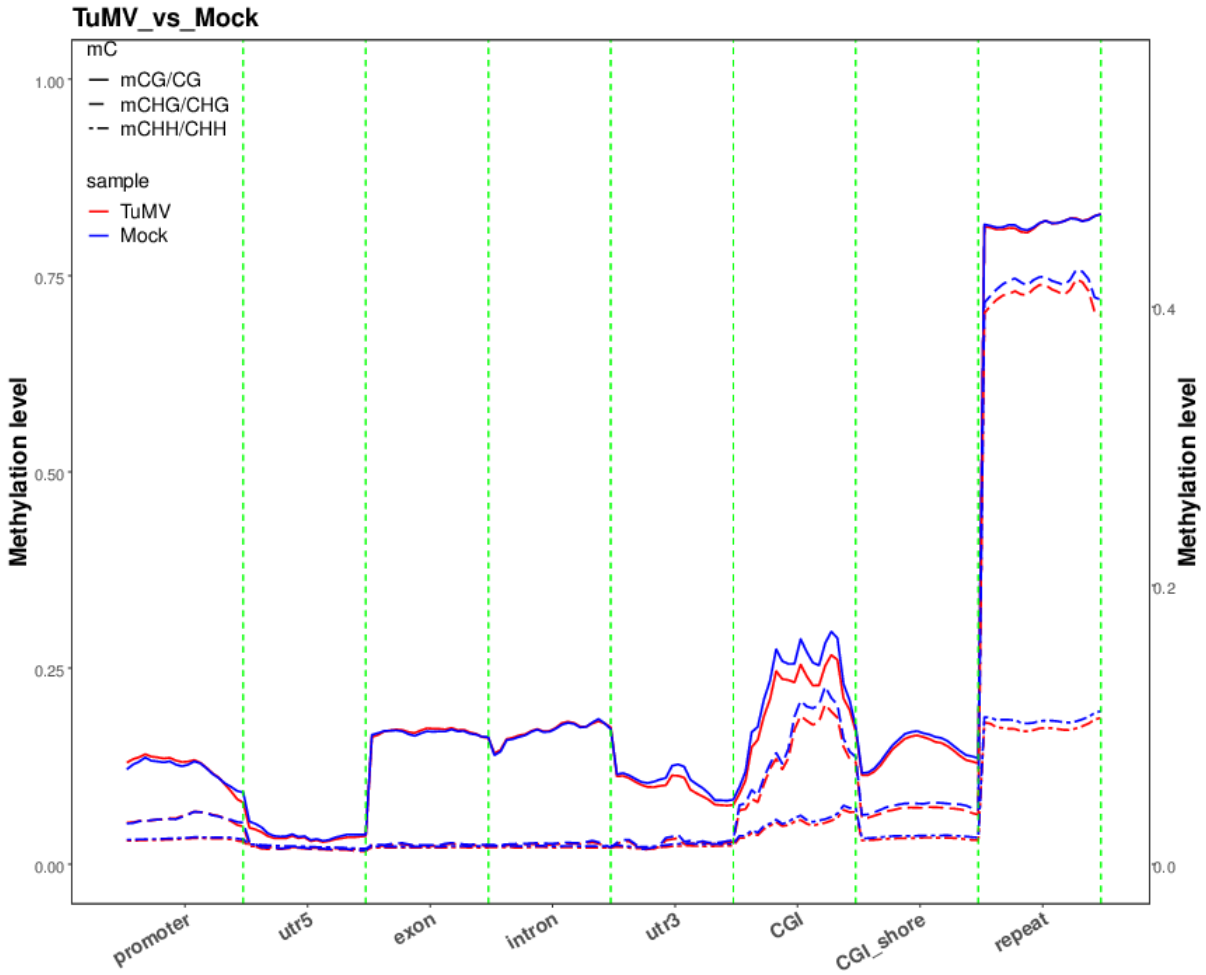

Figure 36 Methylation level distribution at functional genetic elements for all three methylation contexts of WGBS data set of 2nd generation, TuMV infected samples are shown in red, Mock infected samples in blue (Source: Novogene)

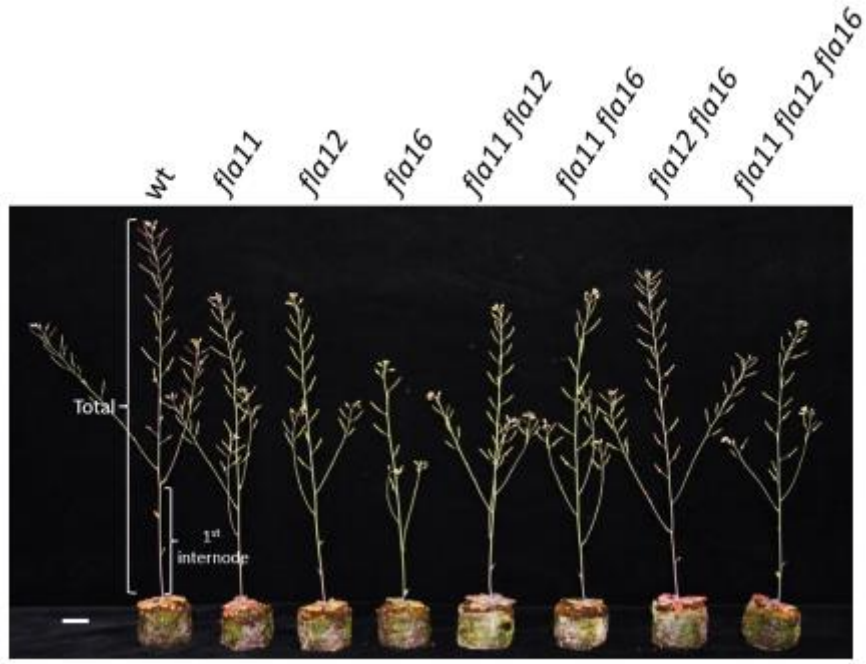

Figure 38 Figure from (Liu, 2017) showing the stem length phenotypes of fla11, fla12 and fla16 as single, double and triple mutant plants with a wildtype plant for comparisons on the far left.

Figure 37 Short stem phenotypes of GA20oxidase mutant lines (Rieu et al., 2008)

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Zuletzt möchte ich mich bei all denjenigen bedanken die stets an mich geglaubt haben – ohne euch wäre diese Arbeit nicht entstanden!

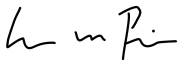
Eidesstaatliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare upon oath that I have written the present dissertation independently and have not used further resources and aids than those stated in the dissertation.

Hamburg, 19.10.2022

Ort, Datum | City, Date



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