Arabidopsis thaliana class II TGA transcription factors provide a molecular link between salicylic acid and ethylene defense signalling

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Betreuungskommission	**Prof. Dr. Christiane Gatz**
	Prof. Dr. Volker Lipka
Referentin	**Prof. Dr. Christiane Gatz**
Koreferent	**Prof. Dr. Volker Lipka**
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Chapter I

Summary

Since plants are exposed to a multitude of different attackers, a complex innate immune system has evolved to protect them from devastating diseases. Depending on the type of pathogen or pest, at least one the three major stress signalling hormones which coordinate further defense responses is synthesized (Glazebrook, 2005): Salicylic acid (SA) requires the redox-regulated co-activator NPR1 and TGA bZIP transcription factors to induce defense genes, is required to combat biotrophic pathogens; jasmonic acid (JA) leads to the degradation of JAZ repressor proteins to release MYC transcription factors; ethylene (ET) leads to the stabilization of transcription factor EIN3. Both pathways merge at the promoter of transcription factor ORA59 which triggers defense responses against necrotrophic pathogens (Pre et al., 2008). In the absence of ET, JA-activated MYC2 coordinates the response against insects. The three defense pathways act mutually antagonistic indicating that their simultaneous activation is evolutionary disadvantageous. This thesis has identified subclass II TGA transcription factors and their interacting CC-type glutaredoxins as the key regulatory module that mediates the antagonistic action of SA and JA on the JA/ET-pathway.

In Chapter II we demonstrate that the *Arabidopsis thaliana* subclass II TGA transcription factors, which had been previously described as essential activators of the SA pathway, are positive regulators the JA/ET pathway. Evidence for this was provided by the increased susceptibility of *tga256* triple mutant plants against the necrotrophic fungus *Botrytis cinerea* and decreased expression of the marker gene of the JA/ET response, *PDF1.2*. In contrast, mutations in AtMYC2, the key positive regulator of the JA pathway, led to hyper-induction of the pathway. JA/ET-induced expression of *PDF1.2* was restored in the *tga256 myc2* quadruple mutant, indicating that TGA factors and MYC2 act as mutual suppressors on the JA/ET pathway. Interestingly, this *tga256 myc2* mutant is insensitive to the antagonistic effect of SA establishing the concept that the positive function of TGA factors in the JA/ET-pathway serves to install the SA sensitivity.

In Chapter III we show that the compromised defense gene expression in *tga256* mutant plants after ET treatment is a direct consequence of the reduced expression of *ORA59*, the master integrator of the JA/ET pathway (Pre et al., 2008). Chromatin immunoprecipitation analyses demonstrated ET-induced direct binding of TGA factors to the TGACG motif at the *ORA59* promoter. The functional importance of the TGA binding was further supported by analyses of transgenic $ORA59_{Pro}$*:GUS* plants which

indicated a crucial function of the TGACG motif for promoter activity. Moreover, SA-induced susceptibility of *Arabidopsis* plants towards infection with *Botrytis cinerea* was abolished in the *tga256* mutant. Microarray analyses demonstrated that one third of all ET-induced genes is under positive transcriptional control of TGA factors. Interestingly, nearly all genes which are negatively affected by SA require TGA factors for being induced by ET. Collectively, these data support the idea that the positive function of TGA factors within the JA/ET pathway is targeted by SA to down-regulate the JA/ET-pathway.

Chapter IV takes up the question how the antagonistic affect of SA on the TGA function is executed. A likely candidate is the previously described glutaredoxin GRX480 which combines multiple criteria of a cross-talk mediator: It interacts with TGA factors, its expression is SA-inducible and its over-expression suppresses JA/ET-induced defense gene expression (Ndamukong et al., 2007). This list was extended by our findings that the GRX480-mediated suppression is integrated at the *ORA59* promoter. Loss-of-function evidence could not be provided, probably due to a functional redundancy within the glutaredoxin family. Seventeen plant-specific CC-type glutaredoxins were screened for their potential to suppress the *ORA59* promoter using a transient expressions system that allowed monitoring the negative effect of glutaredoxins on the *ORA59* promoter. Ten out of the 17 tested glutaredoxins revealed suppression capacity. Only these glutaredoxins contained the C-terminal ALWL motif which was previously described as crucial for glutaredoxins to mediate developmental processes in flowers (Li et al., 2009).

In Chapter V, the interplay between MYC2 and GRX480 was explored since both factors negatively regulate the JA/ET-pathway in a TGA-dependent manner. JA-induced *GRX480* expression was shown to be MYC2-dependent, giving rise to the hypothesis that MYC2 exerts its negative effect through GRX480. The down-regulation of the JA/ET-pathway as a result of the MYC2/GRX480 action removes the suppressive effect of the JA/ET-pathway on the JA-pathway. The hyper-stimulation of *MYC2* expression which is observed in plants ectopically expressing *GRX480*, can therefore be regarded as a result of a MYC2-driven feed-forward loop.

Collectively, the thesis has established a working model that envisions the *ORA59* promoter as a target site for the antagonism of SA and JA on the JA/ET pathway. Mechanistically, this antagonism is established through TGA factors that enhance *ORA59* promoter activity by synergistically interacting with EIN3 and a yet unknown JA-responsive transcription factor. TGA factors recruit JA- and SA-induced glutaredoxins that down-regulate *ORA59* promoter activity by redox-modification of a yet unknown target protein.

General introduction

Living plants are the striking result of evolution being driven by everlasting changes of environmental influences. They have evolved a sophisticated system to deal with a large number of stresses like drought, heat, pathogens and pests. To avoid successful pathogen attack, plants have developed multiple pre-invasive and post-invasive layers of defense. When pathogens which are well adapted to the plant defense machinery overcome the pre-invasive defense layer, a second very efficient layer of induced responses becomes active. Key molecules in these well-orchestrated induced defense programs are salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) (Dong, 1998; Van Loon et al., 2006). SA-induced defense responses are effective against pathogens with biotrophic lifestyles whereas the JA/ET pathway is mainly involved in defense responses against necrotrophic pathogens and the JA pathway is against insect herbivores (Glazebrook, 2005).

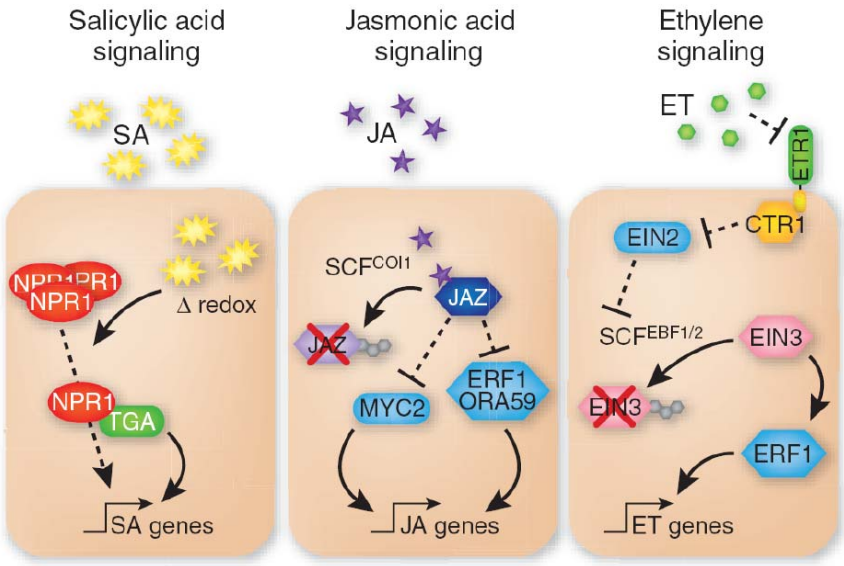

Figure 1. Simplified model of the major defense pathways (Pieterse et al., 2009)

Salicylic acid defense signalling

The SA defense program begins with a local defense response which subsequently spreads throughout the plant leading to a systemic immune response. This so-called systemic acquired resistance (SAR) is long lasting and very efficient against a broad range of pathogens (Durrant and Dong, 2004; Mishina and Zeier, 2007). Master regulator of the SAR is NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1) (Cao et al., 1994). The molecular action of NPR1 is tightly regulated on multiple levels. Redox-induced monomerization of cytosolic NPR1 oligomers allows the

entry of NPR1 into the nucleus where it activates its target genes (Mou et al., 2003). The redox homeostasis of NPR1 is controlled by S-nitrosylation and the action of TRXh5 (Tada et al., 2007). In addition to the redox regulation, the turnover of NPR1 is manipulated by phosphorylation and subsequent ubiquitination, determines the outcome of the SA response (Spoel et al., 2009). The information decoded in the NPR1 status needs to be transduced onto the target promoters. This step is mediated via the direct interaction of NPR1 with TGA transcription factors (Fig.1) leading to the formation of the NPR1-TGA enhanceosome which can activate its target genes (Rochon et al., 2006). The compromised establishment of the SAR in the *tga256* triple mutant, which is defective in all three subclass II TGA transcription factors, indicates a prominent role for these redundant TGA factors in the salicylic acid pathway (Zhang et al., 2003).

Ethylene signalling

Ethylene is a simple gas which controls a wide range of physiological processes including inhibition of cell elongation, seed germination, fruit ripening, organ senescence and pathogen responses (Schaller and Kieber, 2002). Ethylene is perceived by five ER (*endoplasmatic reticulum*)-membrane anchored receptors ETR1 (ETHYLENE RESPONSIVE1), ETR2 (ETHYLENE RESPONSIVE2), ERS1 (ETHYLENE RESPONSE SENSOR1), ERS2 (ETHYLENE RESPONSE SENSOR2) and EIN4 (ETHYLENE INSENSITIVE4) (Bleecker et al., 1988; Chang et al., 1993; Hua et al., 1995; Sakai et al., 1998; Chen et al., 2002) which are negative regulators of the pathway and get inactivated after ethylene recognition (Hua and Meyerowitz, 1998). Without ethylene perception, CTR1 (CONSTITUTIVE TRIPLE RESPONSE1), a Raf-like Ser/Thr kinase, assembles with the ethylene receptors and gets activated (Kieber et al., 1993; Gao et al., 2003). Active CTR1 represses the unknown function of EIN2 (ETHYLENE INSENSITIVE2), another ER-localized essential component of the ethylene pathway (Alonso et al., 1999), which subsequently leads to an inhibition of the $SCF^{EBF1/EBF2}$ (EIN3 BINDING F-BOX1,2) activity and promotion of stabilized EIN3 (ETHYLENE INSENSITIVE3) (Christians et al., 2008) (Fig.1). How the status of endomembrane localized EIN2 is transduced to EIN3 protein stability in the nucleus is not known. A recent study proposes a model of a bifurcate ethylene pathway downstream of CTR1 where a MKK9-activated MPK3-6 module circumvents the EIN2 node to phosphorylate EIN3, thereby stabilizing EIN3 (Yoo et al., 2008). Contrary to this, An and colleagues reported that ethylene induced EIN3 stabilization is only carried out by the action of EIN2 and not by MKK9 (An et al., 2010). EIN3 and the closest homolog EIL1 (ETHYLENE INSENSITIVE-like1) are transcription factors and their

stabilization defines the outcome of all the versatile ethylene responses (Guo and Ecker, 2003; Potuschak et al., 2003; Yanagisawa et al., 2003; Gagne et al., 2004). EIN3 functions predominantly as a transcriptional activator and targets for example as a dimer the promoter of the AP2/ERF transcription factor *ERF1* (*ETHYLENE RESPONSE FACTOR1*) (Solano et al., 1998). In addition to ERF1, other ERFs like ORA59 (OCTADECANOID–RESPONSIVE ARABIDOPSIS AP2/ERF59) are proposed as EIN3 targets and are of central importance for ethylene-responsive gene activation (Pre et al., 2008). Interestingly, the expression of these ERFs depends on a functional COI1 protein as well (Penninckx et al., 1998). Therefore, these ERFs and their target genes are classified as JA/ET-responsive genes. Target genes encode PR-proteins like ß-1,3-glucanases and plant defensins (Van Loon et al., 2006) explaining why a non-functional ethylene signalling pathway in *ein2* or *ora59rnai* mutants leads to a compromised resistance against the necrotrophic fungus *Botrytis cinerea* (Thomma et al., 1999; Pre et al., 2008), whereas the gain-of-function approach by over-expressing *ERF1* or *ORA59* enhances the resistance against *Botrytis cinerea* (Berrocal-Lobo et al., 2002, Pre et al., 2008).

Jasmonic acid signalling

JA/ET-induced defense mechanisms are established to counteract necrotrophic attackers. The central regulatory core module of the JA pathway is the complex between COI1 (CORONATINE INSENSITIVE1) and JAZ (JASMONATE ZIM-domain) which serves as the jasmonate receptor (Xie et al., 1998; Thines et al., 2007; Katsir et al., 2008; Fonseca et al., 2009; Katsir et al., 2010). Without jasmonic acid, the JAZ repressors interact with the partially redundant transcription factors MYC2, MYC3 and MYC4, all holding positive functions in the JA pathway (Boter et al., 2004; Lorenzo et al., 2004; Chini et al., 2007; Dombrecht et al., 2007; Fernandez-Calvo et al., 2011; Niu et al., 2011; Cheng et al., 2011). The inhibition of MYCs by JAZ repressors is established via the interaction with the general co-repressors TPL (TOPLESS) or TPR (TOPLESS-related) proteins through an interaction with the adaptor protein NINJA (NOVEL INTERACTOR of JAZ) (Pauwels et al., 2010). After recognition of its ligand, the jasmonate conjugate (+)-*7-iso*-jasmonoyl-L-isoleucine, the JAZ repressors get ubiquitinylated by the SCF^{COI1} complex and degraded through the 26S proteasome (Chini et al., 2007; Thines et al., 2007; Fonseca et al., 2009). The resulting liberation of the MYC transcription factors from the JAZ-NINJA-TPL repressor complex allows the activation of JA-responsive genes. In addition to the classical JA-responsive genes like *VSP2*, which are positively regulated by MYCs, the JA/ET responsive genes like *PDF1.2* are reciprocally regulated (Lorenzo et al., 2004). It needs to be determined

how the COI1-dependent JA/ET-responsive genes are activated upon JA-treatment independently of MYCs (Penninckx et al., 1998; Lorenzo et al., 2004) and how the negative effect of MYC2 on the JA/ET pathway is exerted.

TGA transcription factors

The family of TGA transcription factors, which binds to TGACG motifs, belongs to the superfamily of basic leucine zipper (bZIP) transcription factors (Lam and Lam, 1995, Jakoby et al., 2002; Johnson et al., 2003; Rochon et al., 2006). The TGA family consists of 10 members named TGA1-7, TGA9-10 and PAN (PERIANTHIA) (Jakoby et al., 2002). They are further subdivided into different subclasses. TGA1 and TGA4 form subclass I, TGA2, TGA5 and TGA6 form subclass II, TGA3 and TGA7 are in subclass III and subclass IV is comprised of TGA9 and TGA10 (Fig.2) (Kesarwani et al., 2007; Murmu et al., 2010). TGA1-TGA7, which are able to interact with NPR1, are involved in defense and general stress responses (Despres et al., 2003; Zhang et al., 2003; Kesarwani et al., 2007; Ndamukong et al., 2007; Fode et al., 2008; Müller et al., 2008; Zander et al., 2010) whereas TGA9, TGA10 and PAN have essential roles in developmental pathways. PAN is crucial for floral patterning (Running and Meyerowitz, 1996; Chuang et al., 1999) and TGA9 and TGA10 promote anther development probably due to an interaction with the glutaredoxins ROXY1 and ROXY2 (Murmu et al., 2010). Subclass I TGA transcription factors are known as redox-sensitive regulators. SA-induced reduction of critical cysteines allows the interaction with NPR1 *in planta* (Despres et al., 2003). More recent analyses have revealed that these cysteines are also subjected to S-nitrosylation (Lindermayr et al., 2010). The biological significance of this proposed redox module has still to be determined. Infections with virulent pseudomonades revealed that TGA1 and TGA4 have partially redundant positive functions in establishing basal immunity (Kesarwani et al., 2007).

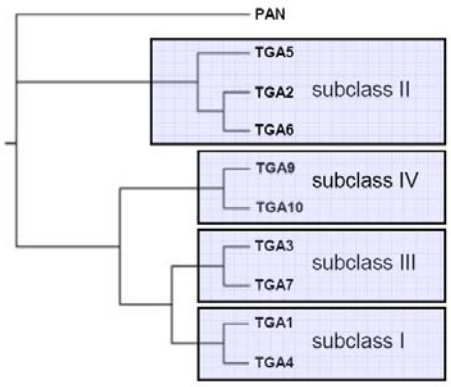

Figure 2. TGA transcription factor family in *Arabidopsis thaliana* (Hepworth et al., 2005 modified)

The compromised basal resistance against virulent pseudomonades of *tga3* and *tga7* mutants suggests an important of subclass III TGA transcriptions factors in plant immunity as well (Kesarwani et al., 2007; Song et al., 2011). TGA3 can interact with the cytokinin activated transcription factors ARR2 (ARABIDOPSIS RESPONSE REGULATOR2) thereby linking the cytokinin pathway, which promotes resistance against *Pseudomonas syringae*, with the salicylic acid pathway (Choi et al., 2010). Very recent results proposed a model where TGA7 is essential for recruitment of SSN2 (SUPPRESSOR of SNI1) to the *PR-1* promoter. This TGA7-facilitated SSN2 recruitment to the promoter reverses the SNI1-mediated transcriptional repression and allows the co-activator NPR1 to activate *PR-1* (Song et al., 2011). This contradicts with previous results where the SNI1 influence under inducing conditions was mapped to WRKY-boxes in a different promoter region (Pape et al., 2010).

Subclass II TGA transcription factors are the most frequently studied group within the TGA family. The SA-mediated translocation of NPR1 into the nucleus enables TGA2 to interact with NPR1 thereby forming an enhanceosome which triggers the expression of *PR-1* (Kinkema et al., 2000; Fan and Dong, 2002; Mou et al., 2003; Rochon et al., 2006). At least for *PR-1* is known that subclass II TGA factors act as repressors of basal expression (Zhang et al., 2003; Rochon et al., 2006). This negative function of TGA2 is abrogated after enhanceosome formation with NPR1 which in turn leads to gene activation (Rochon et al., 2006; Boyle et al., 2009). Conflicting data concerning the role of TGA2, TGA5 and TGA6 for *PR1*-expression have been communicated. Experiments with the *tga256* triple mutant carrying deletions of all three genes encoding subclass II TGA factors where all three TGA factors unraveled a loss of *PR1*-inducibility (Zhang et al., 2003) or a retarded induction (Blanco et al., 2009). However, the compromised SAR establishment in *tga256* mutant plants revealed a redundant but essential role of subclass II TGA transcription factors in SA-mediated plant immunity (Zhang et al., 2003). They have furthermore a prominent function in the negative cross-communication between the SA defense pathway and the JA/ET defense pathway (Ndamukong et al., 2007; Zander et al., 2010, Chapter II). How this SA-mediated antagonism is achieved is still unknown. The SA-inducible glutaredoxin GRX480, which interacts with TGA2 and suppresses JA-induced *PDF1.2* expression (Ndamukong et al., 2007) is proposed as a key player in the execution of the SA-mediated antagonism. In addition to the regulation of plant immunity, subclass II TGA factors are also involved in detoxification of xenobiotics (Müller et al., 2008; Fode et al., 2008). The transcriptional installation of a detoxification program which is activated after phytoprostane accumulation largely depends on TGA factors (Müller et al., 2008). In addition, complex formation of TGA2 with the GRAS protein SCL14 (*SCARECROW-*

like14) at stress-inducible promoters facilitates the activation of a detoxification network against xenobiotics (Fode et al., 2008). Altogether the family of TGA transcription factors displays a high degree of functional diversification with the focus on stress signal integration.

Glutaredoxins (GRXs)

Glutaredoxins are oxidoreductases which are capable to reduce disulfide bonds of their target proteins using glutathione as the electron donor. The GRX family in *Arabidopsis thaliana* is comprised of 31 members divided into three major groups according to their active site motifs (Lemaire, 2004). Beside the CPYC- and CGFS-type GRXs which are conserved in prokaryotes and eukaryotes, a third type specific for higher plants has evolved: the CC-type GRXs (Lemaire, 2004). The knowledge about this group is limited and restricted to ROXY1, ROXY2 and ROXY19/GRX480. ROXY1 is essential for functional petal primordia initiation (Xing et al., 2005) and acts redundantly with ROXY2 in anther development and microspore production (Xing and Zachgo, 2008). The redundant function of ROYX1/2 in anther development is again achieved via the interaction with TGA factors, this time TGA9 and TGA10 (Murmu et al., 2010). Whether TGA9 and TGA10 are directly redox-modified remains elusive. Contrary to the ROXY1/2-mediated developmental processes, GRX480 is involved in pathogen defense responses. GRX480 was initially found as an interacting protein of TGA2 (Ndamukong et al., 2007). Since the expression is SA-inducible and *GRX480* over-expression suppresses JA-induced *PDF1.2* induction, an important role in mediating the SA/JA antagonism was postulated (Ndamukong et al., 2007). It is remarkable that in all described glutaredoxin functions, TGA factors as interacting partners are required. It has been postulated that the TGA/glutaredoxin complex evolved early in evolution to protect the genome against oxidative stress during cell division.

Aim of the thesis

The aim of this thesis was to explore the molecular mechanism underlying the negative effect of SA on the JA/ET pathway. When starting the work it was known that class II TGA transcription factors are important for the suppression of the JA-induced expression of the *PDF1.2* gene. Furthermore, it was known that ectopic expression of the SA-induced glutaredoxin GRX480, which physically interacts with TGA factors, suppresses *PDF1.2* expression in wild-type but not in *tga256* plants (Ndamukong et al., 2007). However, the importance of glutaredoxins was not confirmed in the *grx480* mutant giving rise to the speculation that redundant members of this large gene family complement the mutant. Based on these preliminary data, the following questions were addressed:

Which of the JA/ET-induced genes represent direct target promoters for TGA factors?

Which of the 21 CC-type glutaredoxins are likely to be redundant to GRX480 and should be used for a multiple knock-out strategy?

What is the molecular mechanism underlying the negative effect of GRX480/TGA complex on JA/ET-induced gene expression?

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Chapter II

Arabidopsis thaliana class II TGA transcription factors are essential activators of jasmonic acid/ethylene-induced defense responses

Mark Zander[1], Sylvain La Camera[2], Olivier Lamotte[2,3], Jean-Pierre Métraux[2] and Christiane Gatz[1]

[1]Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Georg-August-Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany

[2]Université de Fribourg, Département de Biologie, Ch du Musée 10, CH-1700 Fribourg, Switzerland

Present address: [3]Olivier Lamotte, UMR CNRS 5184, INRA 1088, Plante-Microbe-Environnement, Université de Bourgogne, 17 rue Sully, F-21065 Dijon Cedex, France

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Abstract

The three closely related Arabidopsis basic leucine zipper (bZIP) transcription factors TGA2, TGA5 and TGA6 are required for the establishment of the salicylic acid (SA)-dependent plant defense response systemic acquired resistance which is effective against biotrophic pathogens. Here we show that the same transcription factors are essential for the activation of jasmonic acid (JA)- and ethylene (ET)-dependent defense mechanisms which counteract necrotrophic pathogens: The *tga256* triple mutant is impaired in JA/ET-induced *PDF1.2* and *b-CHI* expression which correlates with a higher susceptibility against the necrotroph *Botrytis cinerea*. JA/ET induction of the *trans*-activators *ERF1* and *ORA59*, which act upstream of *PDF1.2,* was slightly increased (*ERF1*) or unaffected (*ORA59*). *PDF1.2* expression can be restored in the *tga256* mutant by increased amounts of ORA59 as observed in the *tga256 jin1* quadruple mutant, which lacks the transcription factor JIN1/AtMYC2, which functions as a negative regulator of the JA/ET-dependent anti-fungal defense program. Whereas JA/ET-induced *PDF1.2* expression is strongly suppressed by SA in wild-type plants, no negative effect of SA on *PDF1.2* expression was observed in the *tga256 jin1* quadruple mutant. These results implicate that the antagonistic effects of TGA factors and JIN1/AtMYC2 on the JA/ET pathway are necessary to install SA-mediated suppression of JA/ET-induced defense responses.

Introduction

Plants are light-driven factories that supply organic carbon to our ecosystem. In the course of evolution, microorganisms have developed mechanisms to gain access to plant fixed carbon resources by either killing the plant (necrotrophic life style) or establishing structures to retrieve nutrients from living cells (biotrophic and symbiotic life style) (Glazebrook, 2005). Still, most plants are immune to the majority of pathogens and susceptible to only a relatively small number of adapted microbes. This is due to the efficient activation of inducible defense responses upon recognition of pathogen associated molecular patterns (PAMPs) or (a)virulence effectors (Chisholm et al., 2006).

Though being a simplified concept, it is generally recognized that defense responses mediated by the phytohormone salicylic acid (SA) are effective against biotrophic pathogens, whereas jasmonic acid (JA) and ethylene (ET) act as crucial signaling molecules that induce responses against necrotrophs (Glazebrook, 2005). Moreover, SA is necessary and sufficient for the inducible defense response systemic acquired resistance (SAR), which is established throughout the plant upon local infection with either avirulent or virulent pathogens (Durrant and Dong, 2004; Mishina and Zeier, 2007). This defense response is long-lasting and effective against a broad spectrum of (hemi)biotrophic pathogens, including viruses, bacteria, oomycetes and fungi.

To investigate the mechanisms of the signaling cascades and their mutual interactions, the induction of marker genes in the model plant *Arabidopsis thaliana* is often used as a readout. A crucial step leading to the activation of the SA marker gene *PATHOGENESIS-RELATED-1* (*PR-1*) and the establishment of SAR involves the SA-induced nuclear translocation of the ankyrin repeat protein NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (Mou et al., 2003)), which interacts with TGA transcription factors (Zhang et al., 1999). NPR1 is required to counteract the negative regulator SNI1 (SUPPRESSOR OF NPR1, INDUCIBLE1), as revealed by the re-establishment of SA-induced *PR-1* expression and SAR in the *sni1 npr1* double mutant (Li et al., 1999). Moreover, TGA2 and NPR1 have been postulated to form an enhanceosome at the *PR-1* promoter (Rochon et al., 2006), with NPR1 acting as a transcriptional co-activator (Spoel et al., 2009). Only simultaneous deletion of the closely related class II TGA factors TGA2, TGA5 and TGA6 impaired SAR, indicating that these factors act redundantly (Zhang et al., 2003). Conflicting data have been reported with respect to their role for induction of *PR-1* ranging from a complete loss of *PR-1* induction (Zhang et al., 2003) to a slightly delayed induction kinetics (Blanco et

al., 2009) upon treatment with either the SA analogue isonicotinic acid or SA, respectively. Increased basal *PR-1* transcript levels are observed in the *tga256* mutant in both reports (Zhang et al., 2003; Blanco et al., 2009). TGA2, but not TGA6, suppresses *PR-1* expression in the absence of SA (Kesarwani et al., 2007; Rochon et al., 2006).

Studies initiated to understand the mechanism of JA-induced gene expression have often made use of the genes *VSP2*, *LOX2*, *PDF1.2* and *b-CHI* (Lorenzo et al., 2004). Expression of these genes requires COI1 (CORONATINE INSENSITIVE1), an F-box protein which can form a ternary complex with JA-isoleucine and members of the JAZ repressor proteins (Thines et al., 2007; Yan et al., 2009). JAZ repressors inactivate transcription factor AtMYC2 at the protein level by direct protein-protein interactions (Chini et al., 2007). After COI1-mediated ubiquitinylation and subsequent degradation of JAZ proteins, AtMYC2 can activate its own gene, *VSP2* and *LOX2*. At the same time, AtMYC2 is a negative regulator of *PDF1.2* and *b-CHI* (Lorenzo et al., 2004).

In contrast to *VSP2* and *LOX2*, induction of *PDF1.2* and *b-CHI* requires a functional ET signaling cascade even if only JA is applied (Penninckx et al., 1998). In plants grown in soil, *PDF1.2* can be induced by either JA or ET, whereas in agar-grown plants, strong induction is only observed upon simultaneous application of both hormones. Upon activation of the ET signaling cascade the key ethylene response transcription factors EIN3 and EIN3-like 1 (EIL1) are no longer degraded through the 26S proteasome pathway (Kendrick and Chang, 2008). EIN3 and EIL1 regulate downstream targets of the ET signaling pathway like for instance ethylene response factor 1 (ERF1) (Solano et al., 1998). Ectopic expression of ERF1 and the related APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) ORA59 is sufficient for *PDF1.2* expression (Pre et al., 2008; Solano et al., 1998).

Activation of the SA or JA/ET pathway is not always initiated exclusively in response to either biotrophs or necrotrophs. For example, the hemi-biotrophic bacterial leaf-pathogen *Pseudomonas syringae* pv. *tomato* DC3000 can simultaneously induce synthesis of both SA and JA. However, elevated levels of SA eventually suppress JA accumulation so that the SA pathway is prioritized (Spoel et al., 2003). Pharmacological studies (Koornneef et al., 2008; Leon-Reyes et al., 2009; Spoel et al., 2003), analysis of pathogen-challenged mutants defective in either of the signaling pathways (Kloek et al., 2001), and experiments with plants simultaneously infected with biotrophs and necrotrophs (Koornneef et al., 2008; Spoel et al., 2007) have led to the concept that SA strongly antagonizes the JA and JA/ET pathways in *A. thaliana*. As revealed by mutant analysis, NPR1 and class II TGA factors are important for the SA-JA cross-talk (Ndamukong et al., 2007; Spoel et al., 2003). However, in the presence of

elevated levels of ET, NPR1 becomes dispensable (Leon-Reyes et al., 2009). In addition, experiments with ectopically expressed proteins suggest the TGA-interacting glutaredoxin GRX480 (Ndamukong et al., 2007) and transcription factor WRKY70 (Li et al., 2004) are involved in the SA-mediated suppression of JA-induced genes. However, the molecular mechanisms set in motion by these regulatory proteins have remained elusive.

Here we show that class II TGA transcription factors are essential for induction of *PDF1.2* transcription after infection with either *Botrytis cinerea* or *P. syringae* or in JA/ET-induced plants. This result establishes a so far unknown role for TGA factors in defense responses against necrotrophic pathogens. Moreover, we provide evidence that the integration of TGA factors into the JA/ET pathway establishes a molecular link that connects the SA and the JA/ET signaling networks.

Results

ET introduces the requirement of TGA factors for *PDF1.2* expression

TGA2, TGA5 and TGA6 form one clade within the TGA family of transcription factors (Xiang et al., 1997) and their simultaneous knock-out was required to detect their function as crucial activators of SAR (Zhang et al., 2003). The *tga256* mutant has resulted from the cross of two mutants obtained after fast neutron-deletion mutagenesis lacking the coding regions of *TGA6* and the two closely linked *TGA2* and *TGA5* alleles (Zhang et al., 2003). When infecting wild-type plants with the necrotrophic fungus *Botrytis cinerea*, almost 80% of the lesions were in the 3 to 8 mm range and only 10% were larger than 8 mm. In contrast, 40% of the lesions on *tga256* mutant plants were larger than 8 mm (Fig.1a), demonstrating that the fungus inflicts significantly more damage on the mutant than on wild-type plants.

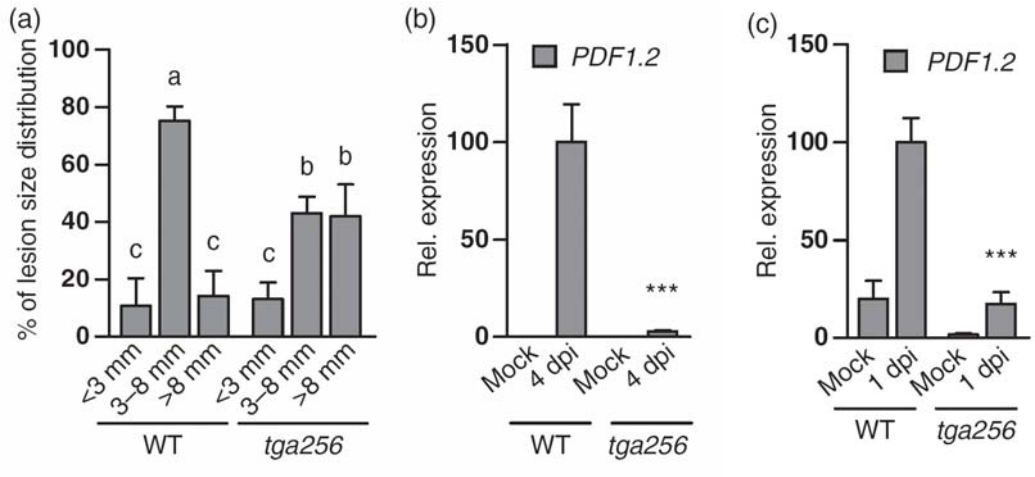


Figure 1. Symptom development and *PDF1.2* expression in wild-type and *tga256* mutant plants after pathogen infection

(a) Lesion sizes on wild-type and *tga256* mutant plants after 3 days of infection with *Botrytis cinerea*. 4-week-old soil grown plants were drop-inoculated with a *B. cinerea* spore solution (5 x 10^4 spores ml^{-1}) or with quarter-strength potato dextrose broth (mock). The diameters of at least 40 lesions per experiment were measured and grouped according to their size into the three indicated classes. The mean percent distribution (±SE) of the lesion sizes of three independent experiments is shown. Different letters denote significant differences (Student's *t* test; $P < 0.05$) between the relative abundances of lesion size classes in both genotypes.

(b) Quantitative real-time RT PCR analysis of relative *PDF1.2* transcript levels in wild-type and *tga256* mutant plants after 4 days of spray inoculation with *Botrytis cinerea*. 4-week-old soil grown plants were sprayed with a *B. cinerea* spore solution (2 x 10^5 spores ml^{-1}) or with quarter-strength potato dextrose broth (mock). The average of the relative *PDF1.2* transcript levels in 12 infected wild-type plants was set to 100%. The mean values (±SE) obtained from 12 individual wild-type and 12 individual *tga256* plants are shown. Asterisks represent significant differences between wild-type and *tga256* plants within a treatment (two-way ANOVA: ***, $P < 0.001$).

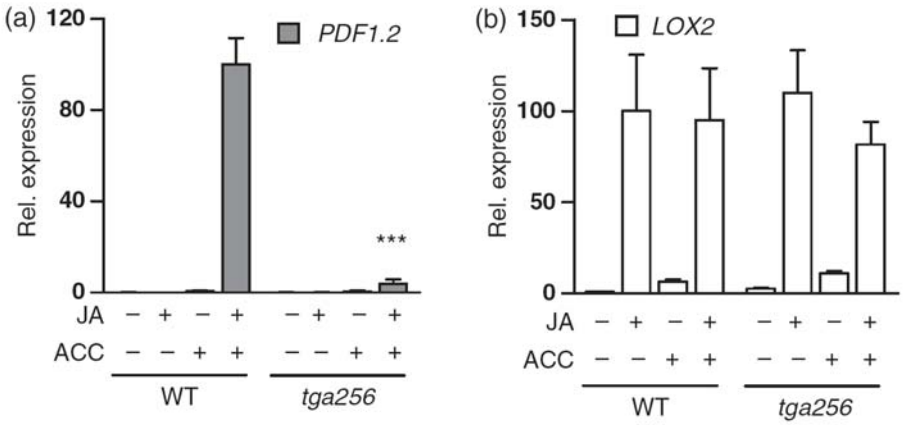
(c) Quantitative real-time RT PCR analysis of relative *PDF1.2* transcript levels in wild-type and *tga256* mutant plants at 1 day after dip inoculation with *Pseudomonas syringae* pv *maculicola*. 5-week-old soil grown plants were infected by immersing whole rosettes into bacterial suspensions of approximately 0.2 OD containing 0.02% (vol/vol) Silwet and 10 mM $MgCl_2$. The average of the relative *PDF1.2* transcript levels of 6 infected wild-type plants was set to 100%. The mean values (±SE) obtained from 6 individual wild-type and 6 individual *tga256* plants are shown. Asterisks represent significant differences between wild-type and *tga256* plants within a treatment (two-way ANOVA: ***, $P < 0.001$).

As activation of defense responses against *B. cinerea* requires functional JA and ET signaling cascades (AbuQamar et al., 2006), we tested whether the enhanced susceptibility correlates with a defect in these pathways using the defensin gene *PDF1.2* as a marker. As shown in Figure 1b, *PDF1.2* induction was impaired in the *tga256* mutant as compared to wild-type plants after infection with *B. cinerea*, indicating that class II TGA factors play an important role in the JA/ET-activated network. Lower

inducibility of *PDF1.2* as a result of deleted *TGA* alleles was also observed in plants infected with *P. syringae maculicola* ES4326 at 1 dpi (Fig.1c).

As *PDF1.2* expression is not affected in JA-treated *tga256* mutant plants (Ndamukong et al., 2007), we figured that ET, which is generated upon infection with necrotrophic fungi and *P. syringae* (De Vos et al., 2005), might introduce the requirement for class II TGA factors. Synergistic effects of JA and ET on *PDF1.2* expression are predominantly observed in seedlings grown on agar (Penninckx et al., 1998). Therefore, wild-type and *tga256* mutant plants were grown for 12 days on MS plates and treated for 48 hours with either the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC; 0.5 mM), methyl jasmonate (MeJA; 50 µM) or a combination of both chemicals. As observed before (Penninckx et al., 1998), ACC or JA only slightly induced *PDF1.2* expression, whereas a more than 1000-fold induction was observed after simultaneous application of both chemicals (Fig.2a). This induction was severely compromised in the *tga256* knock-out mutant. In contrast, *LOX2*, which is a marker gene for the ET-independent JA pathway (Lorenzo and Solano, 2005), was unaffected (Fig.2b). Similar results were obtained with the JA/ET-inducible gene *b-CHI* and the JA-inducible gene *VSP2* (Fig. S1).

To corroborate our conclusion that TGA factors play a crucial role in mediating *PDF1.2* expression only in the presence of ET, we investigated the effect of JA and ET in 4-week-old soil-grown plants. JA treatment (8 h; 4.5 µM gaseous MeJA) caused induction of *PDF1.2* independently of TGA factors (Fig.2c). In contrast, ACC treatment (48 h; 0.5 mM ACC) failed to stimulate *PDF1.2* expression in the *tga256* mutant (Fig.2d).



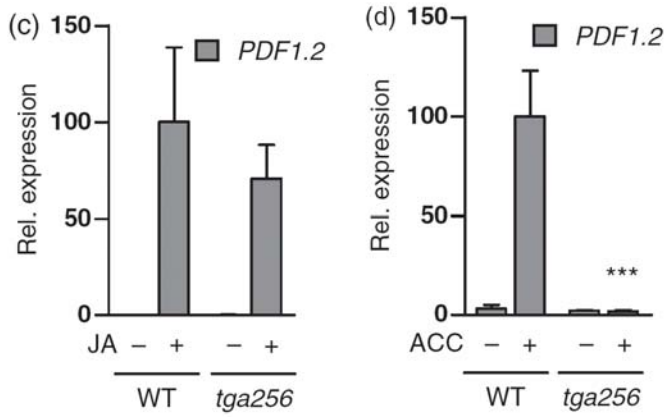


Figure 2. Expression of *PDF1.2* and *LOX2* in wild-type and *tga256* mutant plants after treatment with JA, ACC or JA and ACC

(a,b) 12-day-old wild-type and *tga256* mutant seedlings grown on Murashige and Skoog (MS) medium were transferred to medium supplemented with 0.01% ethanol (mock) or 50 µM MeJA/0.01% ethanol (JA). Transferred plants were sprayed with 0.5 mM of the ET precursor ACC. After 48 hours of treatment, approximately 50 seedlings were harvested for RNA extraction. *PDF1.2* (a) and *LOX2* (b) transcript levels were determined by quantitative real-time RT PCR analysis. Values from JA/ACC-(a) or JA-treated (b) wild-type plants were set to 100%. The mean values (±SE) from three independent experiments (1 plate with 50 seedlings/experiment) are shown. Asterisks represent significant differences between wild-type and *tga256* plants within a treatment (two-way ANOVA: ***, $P < 0.001$).
(c,d) 4-week-old wild-type and *tga256* mutant plants grown on soil were treated for 8 h with 4.5 µM MeJA or for 48 h with 0.5 mM ACC. Relative *PDF1.2* transcript levels were determined by quantitative real-time RT PCR analysis. Values from JA- (c) or ACC-treated (d) wild-type plants were set to 100%. The mean values (±SE) obtained from 6 individual wild-type and 6 individual *tga256* plants are shown. Asterisks represent significant differences between wild-type and *tga256* plants within a treatment (two-way ANOVA: ***, $P < 0.001$).

JA/ET induction of the *PDF1.2* promoter does not depend on the TGA binding site

TGA factors bind to the TGACG motif at position -399 to -395 relative to the predicted transcriptional start site within the *PDF1.2* promoter *in vitro* (Spoel et al., 2003). In order to analyze whether this motif is important for JA/ET-induced expression, the sequence TGACG was mutated to a stretch of five Ts and the wild-type and the mutant promoters (+1 to -931) were fused to the reporter gene *ß-glucuronidase* (*GUS*). The chimeric genes were transformed into the Arabidopsis genome by *Agrobacterium tumefaciens*-mediated gene transfer. Seedlings of 14 independent transformants of each construct were germinated on agar plates and subjected to either mock or JA/ACC treatment. As shown in Figure 3, the TGACG motif does not play an important

role for the JA/ACC inducibility of the *PDF1.2* promoter. GUS values of the independent transgenic lines are shown in Figure S2.

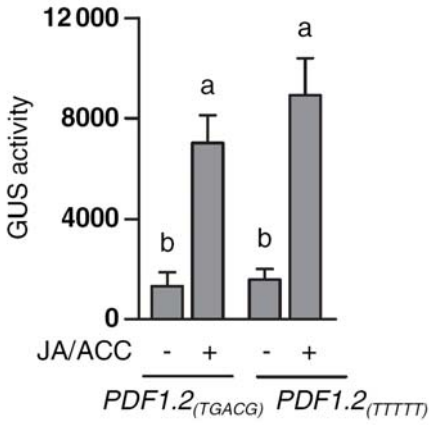


Figure 3. Expression of *PDF1.2*$_{(TGACG)}$*:GUS* and *PDF1.2*$_{(TTTTT)}$*:GUS* after treatment of plants with JA and ACC.

959 bps of the *PDF1.2* promoter were cloned upstream of the ß-glucuronidase reporter gene. In construct *PDF1.2*$_{(TTTTT)}$, the TGACG motif at position -397 to -392 was replaced b 5 Ts. Col-0 plants were transformed with these constructs and approximately 50 F2 plants from 14 independent transformants of each construct were grown on MS medium for 12 days, transferred to MS plates containing 0.01% ethanol (mock) or 50 µM MeJA/0.01% ethanol (JA), sprayed with 0.5 mM ACC and harvested for quantitative GUS expression analysis after 48 hours. Values indicate the mean GUS activities (pmol methylumbelliferyl glucuronide/mg protein) of 14 independent F2 lines of each construct (±SE) (see Figure S2 for values of the individual lines). Different letters denote significant differences between treatments within a *PDF1.2:GUS* construct (Student's *t* test; $P < 0.05$).

JA/ET-induced expression of *ORA59* or *ERF1* is not severely affected in the *tga256* knock-out mutant

Next, we tested whether expression of the two known transcription factors ERF1 and ORA59, which act upstream of *PDF1.2* (Lorenzo et al., 2004; Pre et al., 2008), was affected in the *tga256* mutant. As transcriptional activation of regulatory factors might precede the regulation of their target genes, a time course experiment was performed with JA/ACC-treated seedlings. Under these conditions, the synergistic effect of JA and ACC on expression of *PDF1.2* was observed after 48 hours (Fig.4a).

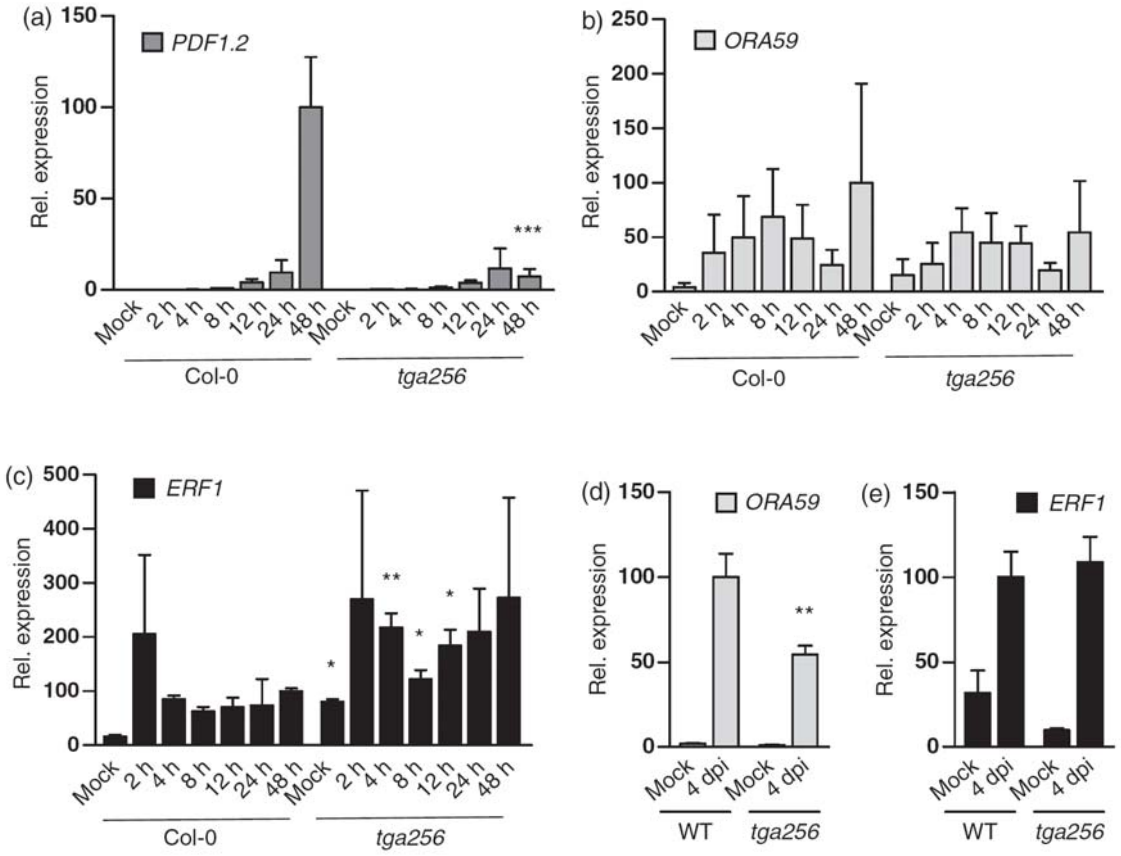


Figure 4. Time-course of *PDF1.2*, *ORA59* and *ERF1* expression after treatment with JA and ACC in wild-type and *tga256* mutant plants

12-day-old wild-type and *tga256* mutant seedlings grown on MS medium were transferred to fresh MS medium containing 50 µM MeJA/0.01% ethanol (JA) and subsequently sprayed with 0.5 mM ACC. Approximately 50 seedlings were harvested for RNA extraction after the indicated hours. The mock value is from plants transferred for 48 hours to MS plates containing 0.01% ethanol. *PDF1.2* (a), *ORA59* (b) and *ERF1* (c) transcript levels were determined by quantitative real-time RT PCR analysis. The relative expression in wild-type plants after 48 hours of JA/ACC treatment was set to 100%. The mean values (±SE) of two samples from two independent experiments are shown. To demonstrate the effect of the *tga256* mutations on *ORA59* (d) and *ERF1* (e) transcript levels after *B. cinerea* infections, the same cDNAs were used as in Figure 1b. Asterisks represent significant differences between wild-type and *tga256* plants (two-way ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

In contrast, transcription of *ERF1* and *ORA59* was induced already after 2 hours (Fig.4b,c). As judged from two biological replicates, expression of *ORA59* was not affected at any of the analyzed time points in the *tga256* knock-out mutant. *ERF1* expression was significantly enhanced in mock- and JA/ACC-treated samples of the *tga256* mutant at 4, 8 and 12 hours. However, this enhancement did not lead to increased *PDF1.2* expression. We therefore assume that TGA factors activate *PDF1.2* expression by influencing the expression of other regulatory factors. In *B. cinerea*-

infected *tga256* plants (4 dpi), expression of *ORA59* was reduced by a factor of two when compared to wild-type plants (Fig.4d). Under these conditions, expression levels were approximately 20-fold higher than in the pharmacological experiments (Fig.S3). Thus, TGA factors might be needed for high level of *ORA59* expression. Expression of *ERF1* was unaffected (Fig.4e).

The *tga256 jin1* mutant shows induced *PDF1.2* transcript levels that cannot be suppressed by SA

Another regulatory gene that influences *PDF1.2* expression is *JASMONATE-INSENSITIVE1* (*JIN1*) which encodes the transcription factor AtMYC2 (Lorenzo et al*.,* 2004). AtMYC2 activates JA-responsive genes like *VSP2* and *LOX2*, but has a negative effect on JA/ET- inducible genes like *PDF1.2* and *b-Chi*. In order to define the genetic interaction between TGA factors and AtMYC2, the *tga256* mutant was crossed with the *jin1-1* mutant, which encodes a truncated AtMYC2 protein (Nickstadt, 2005). Seedlings of the homozygous *tga256 jin1* quadruple mutant and the parental lines were grown on MS medium and treated with JA and ACC. As displayed in Figure 5a, *PDF1.2* expression was about 40-fold enhanced in the *jin1-1* mutant as compared to the induced *PDF1.2* transcript levels in wild-type plants, confirming the previously described strong negative effect of AtMYC2 on the JA/ET pathway (Lorenzo et al*.,* 2004). Simultaneous inactivation of class II TGA factors and AtMYC2 resulted in plants that induce *PDF1.2* expression to wild-type levels (Fig.5a). Thus, with respect to *PDF1.2* expression, the *jin1-1* allele is a strong suppressor of the *tga256*-mediated phenotype and vice versa.

As TGA factors have been described as regulatory components of the SA signaling network (Zhang et al*.,* 2003), we speculated that the positive effect of TGA factors on JA/ET-induced *PDF1.2* expression might be regulated by SA, thus establishing a molecular link between the two competing defense programs. In order to challenge this hypothesis, we analyzed the effect of exogenously applied SA on JA/ACC-induced *PDF1.2* expression in the *tga256 jin1* quadruple mutant. As described before (Leon-Reyes et al*.,* 2009), application of SA impaired *PDF1.2* expression in wild-type plants after JA/ACC treatment (Fig.5b). Likewise, the JA/ACC-treated *jin1-1* mutant showed 20-fold lower activation of *PDF1.2* after treatment with SA as compared to the JA/ACC-induced levels. The remaining *PDF1.2* transcript levels were still in the same range as in the JA/ACC-treated wildtype and *tga256 jin1* plants.

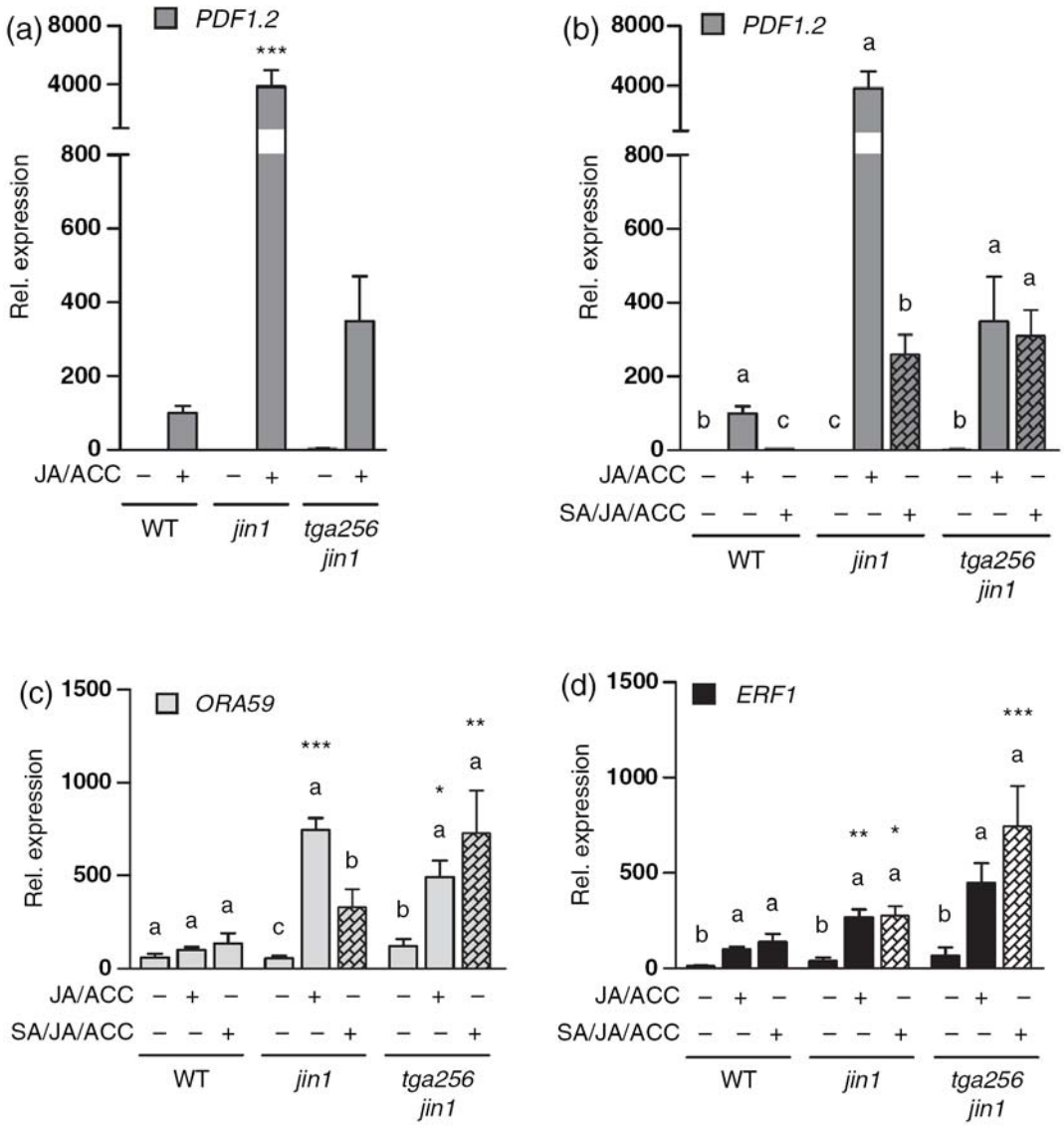


Figure 5. Analysis of *PDF1.2*, *ORA59* and *ERF1* transcript accumulation in wild-type, *jin1-1* and *tga256 jin1* mutant plants after treatment with JA/ACC and SA

Quantitative real-time RT PCR analysis of relative *PDF1.2* (a,b), *ORA59* (b) and *ERF1* (c) transcript levels in wild-type, *jin1-1* and *tga256 jin1* mutant plants. Plantlets were germinated on MS medium, transferred after 12 days to MS plates containing 50 µM MeJA/0.01% ethanol (JA) which were supplemented with 200 µM SA when indicated, sprayed with 0.5 mM ACC and incubated for 48 hours. Mock control plants were transferred to MS plates containing 0.01% ethanol. Approximately 50 plantlets/sample were harvested for RNA extraction after 48 hours. Transcript levels were determined by quantitative real-time RT PCR analysis. Values of wild-type plants after JA/ACC induction were set to 100%. The mean values (±SE) of three independent experiments with one to five independent replicates each are shown. Different letters in (b,c,d) indicate significant differences among treatments within a genotype (Student's *t* test; $P < 0.05$). Asterisks in (a,c,d) represent significant differences between the genotypes within a treatment compared to wild-type (two-way ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Thus, SA treatment of the *jin1-1* mutant diminished *PDF1.2* expression to the same extent as deletion of the *TGA* genes. In the JA/ACC-treated *tga256 jin1* quadruple mutant, expression was not affected by SA in 6 out of 8 samples (Fig.5) and enhanced 10-fold in two samples (Fig.S4). Despite these fluctuations, this analysis indicates that - at least in the *jin1-1* mutant- TGA factors are necessary for mediating the negative effect of exogenous SA on *PDF1.2* expression.

In order to investigate whether AtMYC2 represses *PDF1.2* expression indirectly by negatively regulating expression of the corresponding upstream factors, *ORA59* and *ERF1* transcription was analyzed using the same cDNAs as in Figure 5b. This analysis indicates that JA/ACC-induced *ORA59* transcript levels increase in the absence of AtMYC2 and that this increase is reduced twofold by SA (Fig.5c). Likewise, mutations of the *TGA* alleles in the *jin1-1* mutant background significantly reduced *ORA59* expression (Student's *t* test; $P < 0.05$). Thus, SA and deletion of the *TGA* genes had the same effect, as observed above for *PDF1.2* expression. However, the effects on *ORA59* transcript levels were only 2-fold as compared to 20-fold in the case of *PDF1.2.* Again, no cross-talk was observed in the *tga256 jin1* quadruple mutant. *ERF1* transcript levels were not as strongly affected by the *jin1-1* allele (Fig.5d). SA had no effect. Variable *ERF1* expression was observed in the *tga256 jin1* quadruple mutant after JA/ACC/SA treatment, which most likely accounts for the highly induced *PDF1.2* expression levels in the two samples that were not considered for calculating the means (Fig.S4).

PDF1.2 transcription activated by ectopic expression of *ORA59* is subject to the SA cross-talk

Our data demonstrate that AtMYC2 negatively affects *ORA59* transcription (Fig.5c), suggesting that enhanced *PDF1.2* levels in *jin1-1* mutant plants are due to increased ORA59 levels. Though SA and TGA factors had some influence on *ORA59* transcription at least in the *jin1-1* mutant, their effect on *PDF1.2* was much stronger. In order to obtain independent supportive evidence for the idea that SA can affect *PDF1.2* expression without affecting *ORA59* expression, we made use of transgenic plants expressing *ORA59* under the control of an estradiol-inducible promoter (Pre et al*.,* 2008). SA treatment reduced ORA59-induced expression of *PDF1.2*, when we applied 10 nM estradiol which induces *PDF1.2* expression to approximately the same levels as JA and ACC (Fig.6). Although the cross-talk was not as stringent as in JA/ACC/SA-treated wild-type plants, this experiment provides evidence that SA can work through a mechanism that does not involve suppression of *ORA59* transcription.

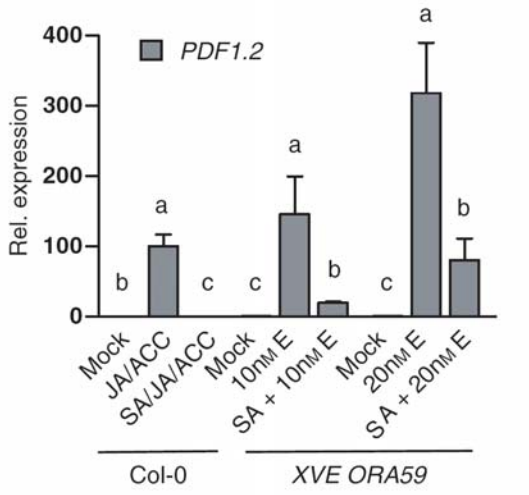


Figure 6. Effect of salicylic acid on *PDF1.2* expression in plants ectopically expressing *ORA59*

Seedlings of wild-type and transgenic plants expressing *ORA59* under the control of an ß-estradiol-inducible promoter (*XVE:ORA59*) were grown for 12 days on MS medium. Transgenic *XVE:ORA59* plants were transferred to MS plates containing 10 or 20 nM ß-estradiol with or without 200 µM SA. Mock control plants were transferred to MS plates containing 0.01% ethanol. Wild-type plants were treated as described in Figure 2. Approximately 50 plantlets were harvested for RNA extraction after 48 hours of incubation. Transcript levels were determined by quantitative real-time RT PCR analysis. The mean value of three independent samples of JA/ACC-treated wild-type plants was set to 100%. The mean values (±SE) of three independent samples are shown. Different letters indicate significant differences among treatments within wild-type plants, *XVE:ORA59* plants treated with 10 nM ß-estradiol (E) and *XVE:ORA59* plants treated with 20 nM ß-estradiol (Student's *t* test; $P < 0.05$).

Increased expression of TGA5 leads to hyper-induction of *PDF1.2*

In order to test, whether TGA2, TGA5 and TGA6 act redundantly with respect to their ability to promote *PDF1.2* expression, the *tga25* and *tga6* mutants were analyzed. As displayed in Figure 7a, *PDF1.2* expression was reduced in the *tga25* mutant, indicating that endogenous amounts of TGA6 cannot promote transcription. The *tga6* mutant behaved like wild-type, revealing that TGA6 is not required. Next, the cDNAs of *TGA2*, *TGA5* and *TGA6* were placed under the control of the *Cauliflower Mosaic Virus* (CaMV) *35S* promoter and transformed into the *tga256* mutant. All three factors were able to support activation of *PDF1.2* expression in the presence of JA and ACC (Fig.7b). Western blot analysis showed that lines with similar amounts of different TGA proteins yielded different amounts of *PDF1.2* transcript levels (Fig.7c). TGA5 revealed the highest capacity to promote transcription. In these lines, *ORA59* expression was constitutively enhanced (Fig.7d). However, this did not lead to significantly elevated *PDF1.2* transcript levels in the absence of JA and ACC, suggesting that TGA2 or TGA6 might be necessary to support the activating capacity of ORA59. In the presence of

JA/ACC, a hyper-induction of *PDF1.2*, which was similar to the hyper-induction in the *jin1-1* mutant, was observed. Apparently, TGA5 is sufficient to support this effect.

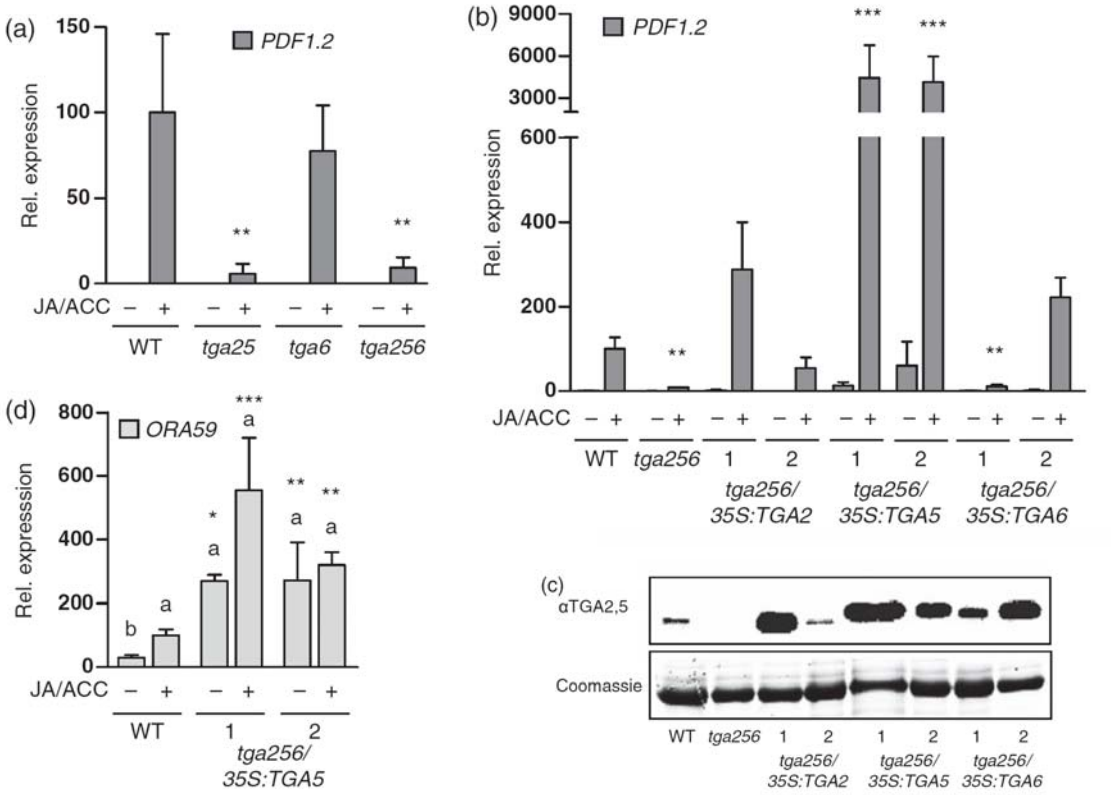


Figure 7. Analysis of *PDF1.2* and *ORA59* expression in *tga25* and *tga6* mutants and in plants ectopically expressing either TGA2, TGA5 or TGA6

12-day-old wild-type, *tga256*, *tga25* and *tga6* mutant seedlings or transgenic lines ectopically expressing TGA2, TGA5 or TGA6 in the *tga256* mutant background were grown on Murashige and Skoog (MS) medium and transferred to medium supplemented with 0.01% ethanol (mock) or 50 µM MeJA/0.01% ethanol (JA). Transferred plants were sprayed with 0.5 mM of the ET precursor ACC. After 48 hours of treatment, approximately 50 seedlings were harvested for RNA or protein extraction. *PDF1.2* (a,b) and *ORA59* (d) transcript levels were determined by quantitative real-time RT PCR analysis. Values of JA/ACC-treated wild-type plants were set to 100%. The mean values (±SE) of three independent biological replicates are shown. (c) Western blot analysis of the transgenic lines analyzed in (b) using the antibody generated against the C-termini of TGA2 and TGA5 (Fode *et al.*, 2008). The samples for protein extraction were taken from untreated seedlings grown in the same experiments as for RNA extraction. Asterisks represent significant differences between the genotypes within a treatment compared to wild-type (two-way ANOVA: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Different letters in (d) indicate significant differences between treatments within a genotype (Student's *t* test; $P < 0.05$).

Discussion

TGA transcription factors TGA2, TGA5 and TGA6 are essential regulators of the SA-dependent defense response systemic acquired resistance (Zhang et al., 2003). Moreover, they are crucial for the activation of a detoxification pathway upon chemical stress (Fode et al., 2008c; Mueller et al., 2008). This study assigns another function to these factors: they are indispensable for the induction of JA-inducible genes like *PDF1.2* and *b-Chi* under conditions of increased ET levels and contribute to the defense against the necrotrophic pathogen *B. cinerea*. Moreover, genetic evidence suggests that this activity is negatively modulated by SA.

TGA factors are required to antagonize the strong negative effect of AtMYC2

Up to now, the two transcriptional activators ERF1 and ORA59 have been described as integrators of the signaling events elicited by JA and ET. Both factors belong to the family of AP2/ERF domain proteins and their transcript levels are synergistically activated by JA and ET (Lorenzo et al., 2004; Pre et al., 2008). The role of TGA factors as essential activators of *PDF1.2* expression has not been described before. However, TGA factors are only necessary for *PDF1.2* expression, when increased ET levels come into play, like under conditions of infection with *B. cinerea* and *P. syringae*, simultaneous application of JA and ACC to MS-grown plantlets, and ACC treatment of soil-grown plants (Fig.1 and 2). A similar modulating effect of signaling cascades by ET has been recently reported with respect to the NPR1 dependency of the SA-JA cross-talk (Leon-Reyes et al., 2009). Whereas the suppression of JA-induced *PDF1.2* expression by SA depends on NPR1, NPR1 is not required when ET levels are elevated.

As revealed by the *tga256 jin1* quadruple mutant, TGA factors are dispensable for *PDF1.2* induction when the negative regulator AtMYC2 is mutated (Fig.5a). In this mutant, transcript levels of *ORA59* are elevated, apparently compensating for the lack of TGA factors (Fig.5c). We propose that TGA factors and AtMYC2 act as antagonistic modulators of the JA/ET pathway, with ERF1 and ORA59 functioning as JA/ET-induced core regulators (Fig.8). TGA factors and AtMYC2 act at different levels (Fig.5b,c): AtMYC2 has a so far unexplored negative effect on the expression of the *ORA59* gene which acts upstream of *PDF1.2*. In contrast, TGA factors do not influence *ORA59* transcript levels after JA/ACC treatment (Fig.4b). Still, they do not function directly at the *PDF1.2* promoter, as the only TGACG binding site within this promoter can be deleted without affecting promoter activity (Fig.3). Thus, it seems more likely that TGA factors act indirectly by regulating transcription of a yet unknown protein that

controls *PDF1.2* promoter activity. Given, that more than 24 hours are needed until the synergistic effect of JA and ACC can be observed (Fig.4a), it seems plausible that TGA-dependent ET-induced transcriptional changes have to occur to ensure efficient induction of *PDF1.2*. None of the three TGA factors was transcriptionally induced by JA/ACC treatment (Fig.S5) and it might well be that they act synergistically with transcriptional regulators connected to the ET pathway. A major goal of the future is to find the direct target genes of class II TGA factors which are necessary for *PDF1.2* expression in the presence of elevated ET levels.

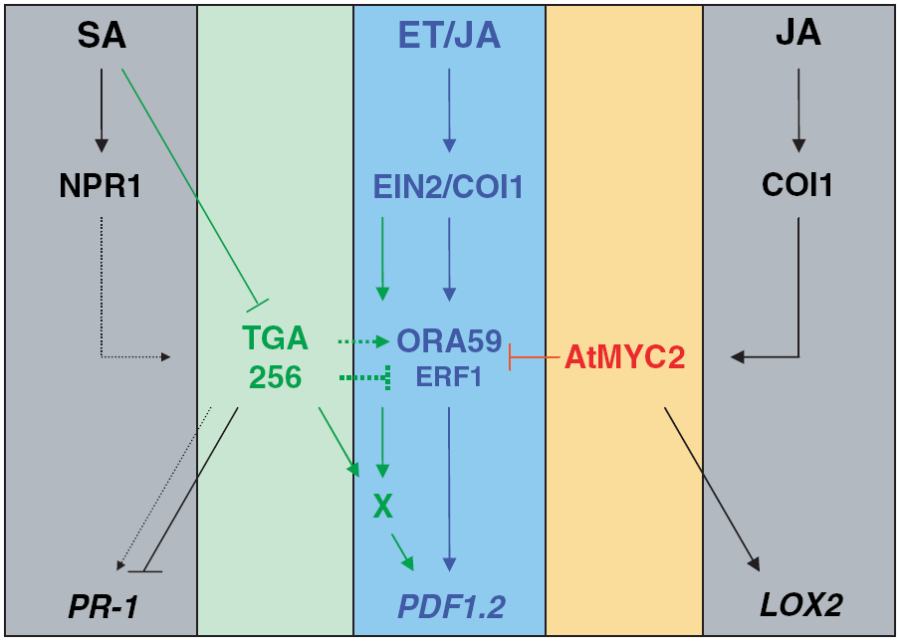


Figure 8. Schematic representation of the role of AtMYC2 and TGA factors for the regulation of *PDF1.2* promoter activity

Under conditions of elevated levels of JA and ET, the expression of the two AP2 transcription factors *ORA59* and *ERF1*, which are activators of *PDF1.2* expression (Lorenzo *et al.,* 2004; Pre *et al*., 2008), is induced through an COI1/EIN2 dependent process (blue box; Penninckx *et al*., 1998). *ORA59* and – to a lesser extent – *ERF1* expression is repressed by AtMYC2, which is transcriptionally induced by elevated JA levels and positively regulates genes like *LOX2* (grey box on the right; Lorenzo *et al*., 2004). TGA factors counteract the negative effect of AtMYC2 by promoting *PDF1.2* expression (green arrows) through regulation of an unknown target gene (represented by X). TGA factors might synergistically interact with signalling components activated by the ET-signalling cascade. Their positive influence on *ORA59* expression (dashed arrow) and their negative influence on *ERF1* expression (dashed line) is of lower importance. As deduced from the SA insensitivity of JA/ET-induced *PDF1.2* expression in the absence of TGA256 and AtMYC2, we speculate that the positive function of TGA factors with respect to the JA/ET pathway is abolished in the presence of SA. As the SA-JA/ET antagonism is independent from NPR1 (Leon-Reyes *et al*., 2009), we assume that SA regulates the activity of TGA factors in this context through a yet unexplored mechanism. At the same time, TGA2 represses basal levels of *PR-1* in the absence of SA (Rochon *et al*., 2006; Kesarwani *et al.,* 2007). This inhibitory effect is turned into a positive effect in the presence of SA through NPR1 (Cao *et al.*, 1997) (left grey box). As the activating role of the three TGA factors is not observed under all conditions (Blanco *et al.*, 2009), we used dashed lines for this pathway.

In addition to controlling this yet unknown regulator of *PDF1.2* expression, TGA factors can contribute to activation of the *ORA59* promoter. This becomes evident after infection with *B. cinerea*, when *ORA59* transcript levels are activated to a higher degree than after single application of the hormones (Fig.S3). TGA factors are involved in this high level of expression, as revealed by 2-fold lower *PDF1.2* transcript levels in the *tga256* mutant (Fig.4d). In addition, high *ORA59* transcription in the *jin1-1* mutant partially depends on class II TGA factors (Fig.5c). Consistently, plants expressing high levels of TGA5 show increased activation of *ORA59* (Fig.7d). As the *ORA59* promoter contains a TGACG motif, it might well be that it represents one of the direct target promoters of TGA5.

TGA factors are required to mediate the SA-JA/ET cross-talk in the *jin1-1* mutant background

In wild-type Arabidopsis plants, SA has a strong negative effect on JA/ET-induced *PDF1.2* expression. As deletion of the *TGA256* genes also has a negative effect, one might speculate that SA modulates the activity of TGA factors in the context of the JA/ET network. Evidence in favour of this hypothesis was obtained by analysis of the impact of either SA treatment or deletion of the *TGA256* alleles in the *jin1-1* mutant background (Fig.5b). High induction of *PDF1.2* in the *jin1-1* mutant was reduced to the same level, irrespective of whether SA was applied or whether the *jin1-1* allele was crossed into the *tga256* mutant background. Consistently, the SA-JA/ET cross-talk was abolished in the *tga256 jin1* mutant. Though we cannot exclude that TGA factors are only important for the SA-JA/ET cross-talk in the *jin1-1* mutant background, we propose that the modulation of the JA/ET pathway by TGA factors serves to install the SA sensitivity in wild-type plants. As *ORA59* transcript levels are only slightly affected by either SA or the *tga256* alleles (Fig.5c), the SA-JA/ET cross-talk most likely targets a yet unknown direct target of the TGA factors. One possible scenario is that TGA factors repress EAR-ERFs, which are ERF transcription factors that negatively regulate transcription and might displace ORA59 at the *PDF1.2* promoter. Previously, AtERF4 has been suggested as a mediator of the SA-JA/ET cross-talk (McGrath et al., 2005). Either mutation or SA-mediated inhibition of TGA factors would lead to constitutive expression of theses EAR-ERFs which would block *PDF1.2* expression. Results obtained with transgenic plants expressing *ORA59* under the control of an estradiol-inducible promoter are consistent with this idea. In these plants, *PDF1.2* expression can be triggered in the absence of the hormones by estradiol-induced elevation of *ORA59* transcript levels and this activation can be suppressed by SA (Fig.6).

How the activity of TGA factors in the context of the JA/ET pathway is influenced by SA is not known. NPR1, which confers the SA sensitivity on the *PR-1* promoter, is not involved in the cross-talk (Leon-Reyes et al., 2009). We have previously presented evidence that the SA-induced glutaredoxin GRX480, which interacts with TGA factors, might be involved suppression of *PDF1.2* expression by SA (Ndamukong et al., 2007). However, as GRX480 is induced efficiently by SA only in the presence of NPR1, we consider it unlikely that it plays a major role in the NPR1-independent SA-JA/ET cross-talk described here. In conclusion, we have shown that TGA factors, which are essential for the SA-dependent establishment of SAR, play a pivotal role in the activation of the JA/ET pathway, both after pathogen infection and hormone treatment. Under these conditions, they serve to counteract the repressing activity of AtMYC2 in an SA-dependent manner.

Material and methods

Plant material, growth conditions, and chemical treatments

Arabidopsis thaliana (accession Columbia [Col-0]) was used. Mutants (*tga25*, *tga6*, *tga256* (Zhang et al*.,* 2003) and *jin1-1* (Berger et al*.,* 1996)) were obtained from Y. Zhang (University of British Columbia, Vancouver, Canada) and from S. Berger (Julius-Maximilians University, Würzburg, Germany), respectively. Line XVE-*ORA59* (Pre et al*.,* 2008) was obtained from J. Memelink (University of Leiden, The Netherlands). Plants were vertically grown under controlled environmental conditions (21/19°C, 16-h-light/8-h-dark cycle, 60% relative humidity) on agar plates containing Murashige and Skoog (MS) medium. After 12 days, plants were transferred to MS-plates containing 0.01% ethanol or 50 µM MeJA (Sigma-Aldrich, St. Louis, Mo, USA) with or without 200 µM SA (Merck, Darmstadt, Germany). For ET induction, plants were sprayed after transfer with 0.5 mM of the ethylene precursor 1-aminocyclopropane-carboxylic acid (ACC) (Sigma-Aldrich, St. Louis, Mo, USA). Plants grown for four weeks in soil (21/19°C, 16-h-light/8-h-dark cycle, 60% relative humidity) were sprayed with 0.5 mM ACC. JA induction of four-weeks old soil-grown plants was performed in closed glass boxes, where 4.5 µM MeJA was applied via the gaseous phase. Plant material was harvested after 48 and 8 hours, respectively. XVE-*ORA59* plants were grown as described above and transferred to MS plates containing 10 or 20 nM ß-estradiol (Sigma-Aldrich, St. Louis, Mo, USA) with or without 200 µM SA. If not indicated otherwise, plant material was harvested after 48 hours.

Pathogen infections

For *B. cinerea* infection experiments, wild-type and *tga256* mutant plants were grown on a pasteurized soil mix of humus/perlite (3:1) under controlled environmental conditions (20-22/16-18°C h 12-h-light/12-h-dark cycle, 60–70% relative humidity). *Botrytis cinerea* strain BMM, provided by Brigitte Mauch-Mani (University of Neuchâtel, Switzerland), was grown on potato dextrose agar (Merck, Darmstadt, Germany). After harvesting of the spores and subsequent filtration through glass wool to remove hyphae, the spores were diluted in quarter-strength potato dextrose broth. For assessment of symptom development, droplets of 5 µl of spore suspension (5×10^4 spores ml^{-1}) were deposited on leaves of 4-week-old plants. The diameter of the lesions was measured after 3 days. *PDF1.2* expression analysis was done with plants which had been spray-inoculated for 4 days (2×10^5 spores ml^{-1}). Quarter-strength potato dextrose broth served as mock for drop and spray inoculations of control plants.

For infection of wild-type and *tga256* mutant plants with *Pseudomonas syringae* pv *maculicola* ES4326, plants were grown on soil for 5 weeks under controlled environmental conditions (20-22/16-18°C; 8-h-light/16-h-dark cycle, 60–70% relative humidity). Infections were done using dip inoculation with a bacterial culture diluted to OD 0.02 in 10 mM $MgCl_2$, 0.02% (vol/vol) Silwet L-77 (OSi Specialties, Crompton Europe S.A., Geneva, Switzerland) (Laurie-Berry *et al.,* 2006). *PDF1.2* expression analysis was carried at one day post infection.

Genetic analysis

The *tga256* mutant was crossed into the *jin1-1* background. The F1-progeny was allowed to self-fertilize and the resulting F2 generation was screened for homozygosity. The *tga2-1 tga5-1* allele results from a deletion induced by fast neutron bombardment (Zhang et al*.,* 2003) and was detected using primers P1, P2 and P3 (for primer sequences see Table S1). The *tga6-1* allele was confirmed by PCR with primers P4 and P5. In addition, quantitative real-time RT PCR analysis was performed to confirm the lack of expression of *TGA2*, *TGA5* and *TGA6* in the respective mutants. Primers P6, P7 and P8 were used to detect the mutant *jin1-1* allele. Homozygosity was further confirmed by Northern blot analysis which allows detection of the truncated *jin1-1* transcript.

Binary vectors and plant transformation

Gateway technology (Invitrogen, Karlsruhe, Germany) was used to generate binary vectors for analysis of the *PDF1.2* promoter. The promoter region from position -959 to -1 relative to the predicted transcriptional start site of the *PDF1.2* (At5g44420) gene was amplified using primers P9 and P10 (for primer sequences see Table S1), which add GATEWAY recombination sites to the promoter fragment. Genomic DNA extracted from Col-0 plants using the DNeasy Plant Mini Kit (Quiagen, Valencia, CA, USA) was used as a template. The fragment was inserted into pDONR223 (Invitrogen, Karlsruhe, Germany) resulting in pDONR223/*PDF1.2-Pro*. Mutation of the TGACG motif at position -397 to -392 to 5 T´s was achieved by PCR using primer pairs P11/P12 and P13/14 and pDONR223/*PDF1.2-Pro* as a template resulting in two fragments which subsequently served as templates for overlapping PCR with primers P11 and P13. Both promoter fragments were recombined upstream of the *ß-glucuronidase* gene in the binary vector pBGWFS7 (http://www.psb.ugent.be/gateway/).

The coding region of TGA5 and TGA6 was amplified from cDNA fragments with primer P15/ P16 and P17/P18 and cloned into the pDONR223 vectors (Invitrogen, Karlruhe,

Germany). The resulting pDONR223/*TGA5*, pDONR223/*TGA6* and pDONR201/*TGA2* were incubated with the binary destination vector pB2GW7 (http://www.psb.ugent.be/gateway/), thus placing them under the control of the CaMV35S promoter. To generate transgenic plants the binary plasmids were electroporated (GenePulser II, Bio-Rad) into *Agrobacterium tumefaciens* strain GV3101 (pMP90). These Agrobacteria were used to transform Col-0 plants with pGBWFS7 derivatives for the promoter analysis and to transform *tga256* mutant plants for ectopic expression of *TGA2*, *TGA5* and *TGA6* (Clough and Bent, 1998). Transgenic lines expressing TGA2, TGA5 or TGA6 were identified by protein gel blot analysis using the αTGA25 antiserum (Fode et al., 2008a).

Quantitative GUS assay

Crude protein extracts were prepared from at least 50 seedlings from 14 individual T2-transformants. Growth conditions and hormone treatments were as described above. Quantitative GUS assays using 4-methyl-umbelliferyl-ß-D-glucuronide (Sigma-Aldrich, St Louis, MO, USA) as a substrate was done in microtitre plates (Jefferson et al., 1987). The released fluorescence was measured with a Cyto Fluor Series 4000plate reader (Perspektive Biosystems, Hertford, Hertfordshire, UK). The total amount of protein was determined using a commercial Bradford assay solution (CARL ROTH, Karlsruhe, Germany).

Quantitative real-time RT-PCR analysis

RNA extraction and real-time RT PCR analysis was performed as described (Fode et al., 2008b). Calculations were done according to the $2^{-\Delta C_T}$ method (Livak and Schmittgen, 2001). *UBQ5* served as a reference (Kesarwani et al., 2007)**.** Primers used to amplify and quantify the cDNA are indicated in Table S1 (*PDF1.2* (At5g44420), *VSP2* (At5g24770), *UBQ5* (At3g62250)). QuantiTect Primers to amplify mRNA for *TGA2* (At5g06950), *TGA5* (At5g06960), *TGA6* (At3g12250), *b-CHI* (At3g12500), *LOX2* (At3g45140), *ORA59* (At1g06160) and *ERF1* (At3g23240) were obtained from Quiagen, Düsseldorf, Germany.

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Supplementary material

Figure S1. Expression of *b-CHI* and *VSP2* in wild-type and *tga256* mutant plants after treatment with JA, ACC or JA and ACC

Figure S2. Expression of *PDF1.2$_{(TGACG)}$:GUS* and *PDF1.2$_{(TTTTT)}$:GUS* in independent plant lines after treatment with JA and ACC

Figure S3. Comparison between the relative *ORA59* transcript levels in wild-type plants after infection with *Botrytis cinerea* or treatment with JA and ACC

Figure S4. Expression of *PDF1.2*, *ORA59* and *ERF1* in wild-type and *tga256 jin1* mutant plants after treatment with JA/ACC and SA/JA/ACC

Figure S5. Time-course of *TGA2*, *TGA5* and *TGA6* expression after treatment with JA and ACC

Table S1. List of primers used for genotyping, cloning and real-time RT PCR analysis

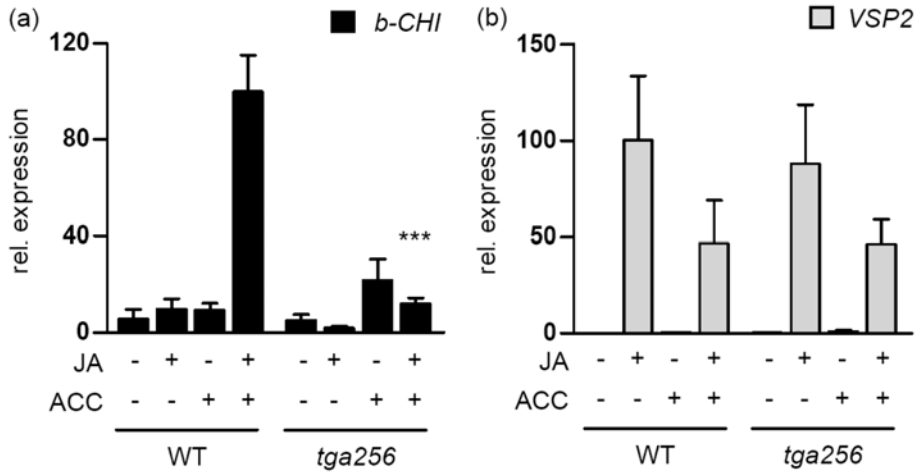

Figure S1. Expression of *b-CHI* and *VSP2* in wild-type and *tga256* mutant plants after treatment with JA, ACC or JA and ACC

12-day-old wild-type and *tga256* mutant seedlings were treated as indicated in Figure 2a. *b-CHI* (a) and *VSP2* (b) transcript levels were determined by quantitative real-time RT PCR analysis. Values of JA/ACC- (a) or JA-treated (b) wild-type plants were set to 100%. The mean values (+/- SE) obtained from three samples from three independent experiments are shown. Asterisks represent significant differences between wild-type and *tga256* plants within a treatment (two-way ANOVA: ***, $P < 0.001$).

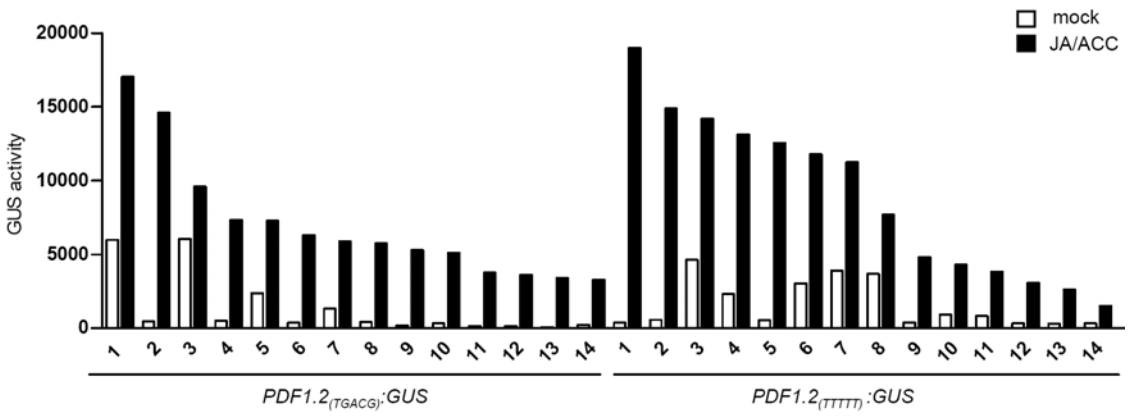

Figure S2. Expression of *PDF1.2(TGACG):GUS* and *PDF1.2(TTTTT):GUS* in independent plant lines after treatment with JA and ACC

GUS activities (pmol methylumbelliferyl glucuronide/mg protein) of 14 independent F2 lines encoding *PDF1.2$_{(TGACG)}$:GUS* and *PDF1.2$_{(TTTTT)}$:GUS* plants as indicated are shown. The means are displayed in Figure 3.

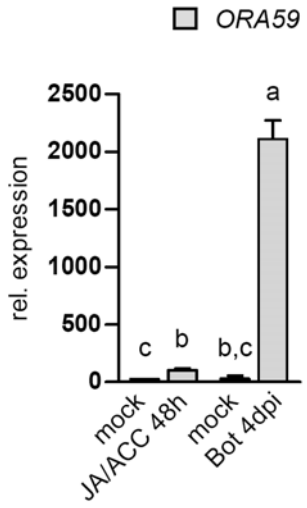

Figure S3. Comparison between the relative *ORA59* transcript levels in wild-type plants after infection with *Botrytis cinerea* or treatment with JA and ACC

Quantitative real-time RT PCR analysis of relative *ORA59* transcript levels in wild-type plants after 48 h of JA/ACC treatment or 4 days of spray inoculation with *B. cinerea*. The cDNAs were taken from the experiments described in Figure 1 (*B. cinerea*) and Figure 5 (pharmacological assay) and analyzed together in one PCR run. Mean values of the relative *ORA59* expression (+/- SE) of JA/ACC-.treated wild-type plants from three independent experiments with one of five independent replicates each were set to 100%. The mean values (+/- SE) of 12 individual infected wild-type plants are shown. Different letters denote significant differences among treatments (Student's *t* test; $P < 0.05$).

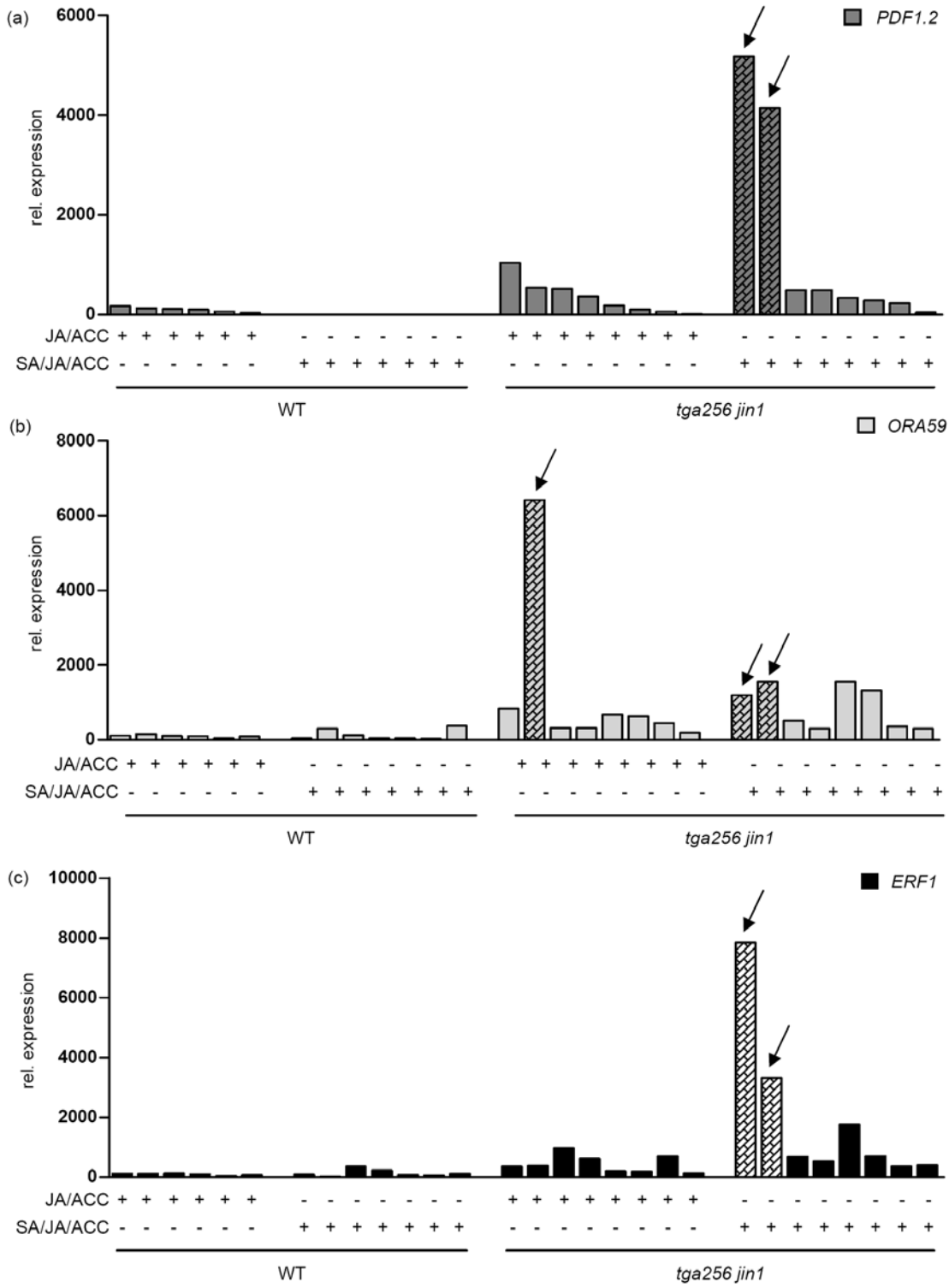

Figure S4. Expression of *PDF1.2*, *ORA59* and *ERF1* in wild-type and *tga256 jin1* mutant plants after treatment with JA/ACC and SA/JA/ACC

Relative *PDF1.2* (a), *ORA59* (b) and *ERF1* (c) transcript levels of three independent experiments with 1 to 5 samples each are shown. The mean values of JA/ACC-treated wild-type plants from each experiment were set to 100%. The single values of JA/ACC and SA/JA/ACC- treated wild-type and *tga256 jin1* plants were calculated accordingly. Arrows indicate values that were not considered for calculation of the means in Figure 5.

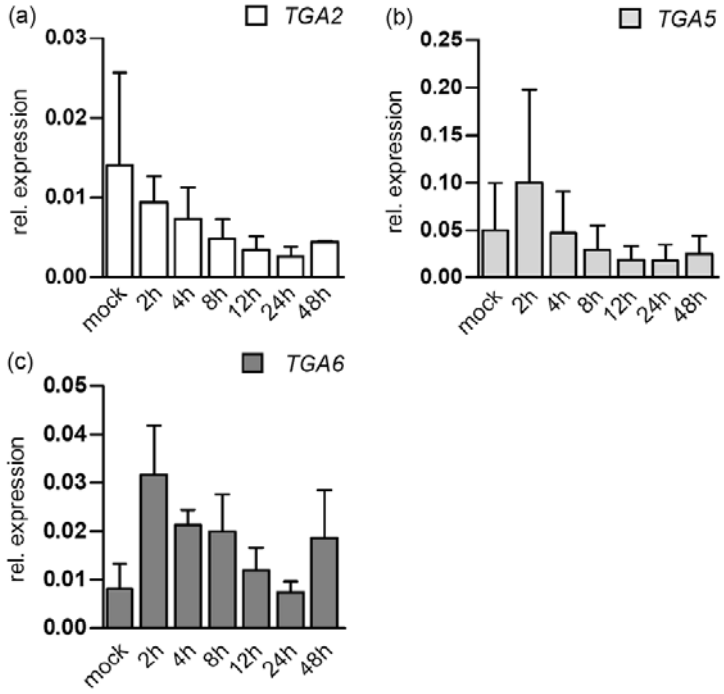

Figure S5. Time-course of *TGA2*, *TGA5* and *TGA6* expression after treatment with JA and ACC

12-day-old wild-type seedlings were treated with JA and ACC as described in Figure 2a. Plants were harvested for RNA extraction after the indicated hours. The mock value is from plants transferred for 48 hours to MS plates containing 0.01% ethanol. *TGA2* (a), *TGA5* (b) and *TGA6* (c) transcript levels were determined by quantitative real-time RT PCR analysis. The mean values of the relative expression (+/- SE) of two samples from two independent experiments are shown. Statistical analysis yielded no significant differences between the treatments (one-way ANOVA, $P < 0.05$).

Table S1: List of primers used for genotyping, cloning and real-time RT-PCR Analysis

Abbr.	Description	Sequence
P1	TGA25 fwd.	GTC AAT CCG GTT TCA TAT TCT CCT C
P2	TGA25 rev.	CCG CAT AAA CAA TAA ACC AAG AGA G
P3	*tga25* rev.	GAG CGA CAA CTC CTT TCA ACT CAT C
P4	TGA6 fwd.	TTC TCA CTT TGT GAT TTG CCT TTG G
P5	TGA6 rev.	TGG GCA ATC TTG CTA TGA TTT CAA G
P6	AtMYC2 fwd.	CAG AGA AAC TCC AAA TCA AGA ACC A
P7	AtMYC2 rev.	CGA TTT TTG AAA TCA AAC TTG CTC T
P8	*jin1-1* rev.	TCT ACG CGA GAA GAG CTG AAG AAT A
P9	PDF1.2-Pro. fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CTG ATC TCT TAT AAT GTT CGG TTT TTC C
P10	PDF1.2-Pro. rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC GCA TGT GAA GAA ATA AGC CAA AGA TCA ACG
P11	pDONR fwd.	TCG CGT TAA CGC TAG CAT GGA TCT C
P12	PDF1.2 – Pro. overlap rev.	CTG TGC AGT TCA TAG TCT GAC CTT AAA AAT CGT CGA ACA AAT ACA ACA TTT TTC
P13	pDONR rev.	GTA ACA TCA GAG ATT TTG AGA CAC
P14	PDF1.2 –Pro. overlap fwd.	CGA TTT TTA AGG TCA GAC TAT GAA CTG CAC AG
P15	TGA5 cDNA fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGG AGA TAC TAG TCC AAG AAC ATC
P16	TGA5 cDNA rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC ATG GTT CAC TCT CTT GGT CTG G
P17	TGA6 cDNA fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGC TGA TAC CAG TTC AAG GAC
P18	TGA6 cDNA rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TCA CTC TCT TGG CCG GGC A
P19	PDF1.2 fwd.	CTT GTT CTC TTT GCT GCT TTC
P20	PDF1.2 rev.	CAT GTT TGG CTC CTT CAA G
P21	VSP2 fwd.	CAA ACT AAA CAA TAA ACC ATA CCA TAA
P22	VSP2 rev.	GCC AAG AGC AAG AGA AGT GA
P23	UBQ5 fwd.	GAC GCT TCA TCT CGT CC
P24	UBQ5 rev.	GTA AAC GTA GGT GAG TCC A

Chapter III

Suppression of the ethylene defense response by salicylic acid is mediated at the *ORA59* promoter through class II TGA bZIP transcription factors

Mark Zander[1] and Christiane Gatz[1]

[1]Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Georg-August-Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany

Abstract

Plants are constantly exposed to different pathogens to which they react with a battery of induced defense mechanisms. The corresponding signal transduction pathways are highly interconnected so that cellular responses can be modulated depending on the combination of attackers with different life styles. Here we show that bZIP transcription factors of the TGA family directly activate the promoter of the *ORA59* gene which encodes the global regulator of the ethylene (ET)-induced defense program. In the *tga256* mutant, which lacks the closely related class II TGA factors TGA2, TGA5 and TGA6, ET-induced transcription of the *ORA59* promoter is compromised. Chromatin immunoprecipitation experiments and analyses of transgenic lines carrying different $ORA59_{Pro}$*:GUS* fusions collectively suggest that class II TGA factors are recruited to the TGACG binding site of the *ORA59* promoter under conditions of enhanced ET levels. Microarray analysis of wild-type and *tga256* mutant plants treated with the ET precursor ACC yielded 193 ACC-regulated genes that did not respond to ACC in the *tga256* mutant. To challenge the hypothesis whether the strong negative effect of SA on ET-induced genes involves TGA factors, we compared the transcriptomes of wild-type and *tga256* mutants after the combined treatment with ACC and SA. This documented that all the ACC-induced genes that are suppressed by SA require TGA proteins for activation. Unlike wild-type plants, *tga256* mutant plants did not react with a SA-induced increase in susceptibility towards *Botrytis cinerea* underpinning the notion that TGA factors provide the molecular link between SA and the plant defense program against necrotrophic pathogens.

Introduction

Plants live in complex environments in which they have to combat a broad range of microbial pathogens and pests. This selective pressure led to the evolution of a highly sophisticated defense system. After recognition of the pathogen or pathogen-induced damage, appropriate defense responses are activated, which are mainly coordinated by the phytohormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). SA-mediated responses are evoked by biotrophic pathogens, JA/ET-dependent responses are induced by necrotrophic pathogens and herbivore attack triggers JA responses (Glazebrook, 2005). Simultaneous activation of these three major stress responses is not observed, suggesting that it might constitute an evolutionary disadvantage. Instead, a strong mutual antagonism between the three pathways exists.

The JA/ET pathway requires the key regulator of JA signalling, the JA-Ile receptor COI1, and components of the ET signalling pathway, irrespective of whether it is induced by either JA or ET alone (Penninckx et al., 1998). If only JA is applied, the SA antagonism depends on two interacting proteins of the SA pathway: the ankyrin repeat protein NPR1 and at least one of the highly redundant class II bZIP transcription factors TGA2, TGA5 or TGA6 (Spoel et al., 2003; Ndamukong et al., 2007). As soon as JA is applied in combination with ET, induction and cross-talk are realized by different molecular components. Now, class II TGA transcription factors become important for the activation of the pathway and the negative effect of SA is independent from NPR1 (Leon-Reyes et al., 2009; Zander et al., 2010; Chapter 2). Mutation of the negative regulator of the JA/ET pathway, MYC2 (Lorenzo et al., 2004), re-establishes the capacity to induce the pathway in the *tga256* mutant. Importantly, induction in the *tga256 myc2* quadruple mutant is not suppressed any more by SA (Zander et al., 2010; Chapter 2). The SA-JA/ET cross-talk was therefore explained by the SA-mediated inhibition of the activating function of TGA256 within the JA/ET pathway. Since expression of the AP2/ERF transcription factor *ORA59*, a key activator of the JA/ET pathway (Pre et al., 2008) was not significantly altered by SA or by the *tga256* alleles, the target site of TGA factors as mediators of the SA antagonism had remained elusive.

Here, we investigated the function of TGA factors in the presence of ET alone. Under these conditions, TGA factors integrate the SA signal directly at the *ORA59* promoter which explains the SA/ET antagonism at a global scale.

Results

TGA factors are essential positive components of the ethylene defense signalling pathway

Previous analysis has shown that TGA factors are required for the induction of the defense gene *PDF1.2* after application of the ET precursor ACC (Zander et al., 2010). Since TGA factors are involved in the induction of genes upon chemical stress (Fode et al., 2008) we aimed to exclude that TGA factors would only participate in the regulation of ET-induced genes if ethylene levels were generated by chemical treatment. Therefore, we analyzed the function of TGA factors in the *ctr1-1* mutant which is characterized by a constitutively active ET signalling cascade (Kieber et al., 1993).

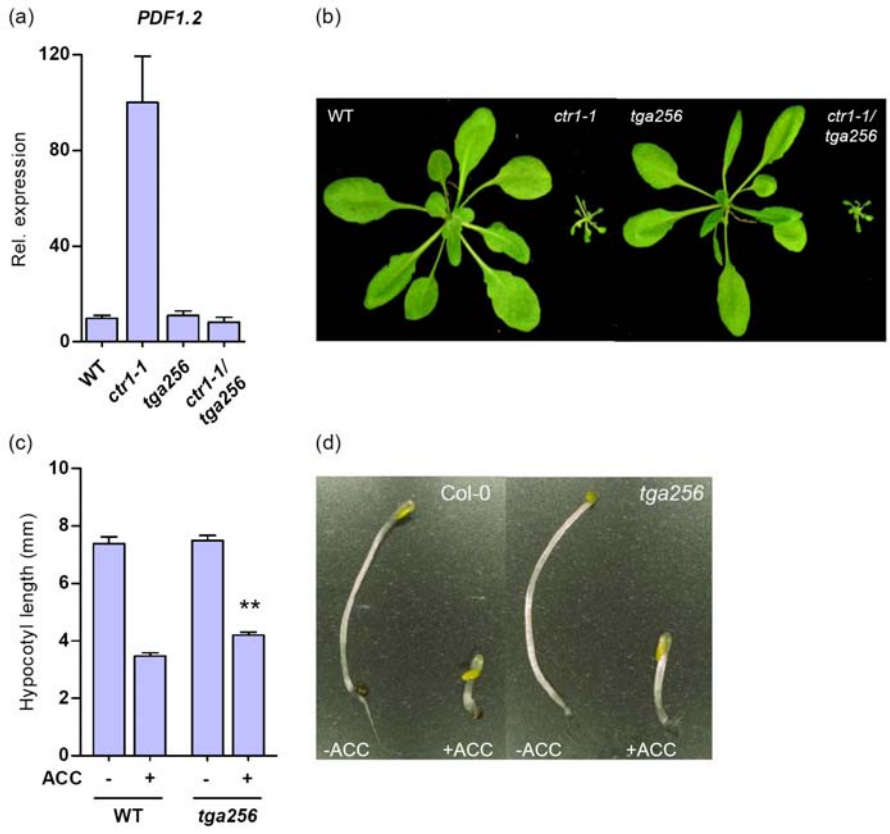

Figure 1. *PDF1.2* expression and growth phenotype of *ctr1-1/tga256* mutant plants

(a) Quantitative real-time RT-PCR analysis of *PDF1.2* transcript levels in untreated four-week old soil-grown wild-type, *ctr1-1*, *tga256* and *ctr1-1/tga256* mutant plants. The average of the relative *PDF1.2* transcript levels in eight *ctr1-1* plants were set to 100%. The mean values (±SE) from eight individual wild-type, *ctr1-1, tga256* and *ctr1-1/tga256* plants are shown.

(b) Phenotype of four-week old soil-grown wild-type, *ctr1-1, tga256* and *ctr1-1/tga256* plants.

(c) Quantification of the triple response of three-day old etiolated wild-type and *tga256* seedlings grown on MS plates with or without 10 µM ACC. Bars represent the average hypocotyl length of 20 seedlings (±SE). Asterisks represent significant differences between wild-type and *tga256* mutants (two-way ANOVA**$P < 0.01$).

(d) Triple response phenotype of three-day old etiolated wild-type and *tga256* seedlings.

In the *ctr1-1 tga256* quadruple mutant, the 10-fold increased *PDF1.2* transcription, which is observed in untreated *ctr1-1* plants, was reverted to background levels (Fig.1a) indicating that TGA factors are required for the activation of *PDF1.2* by a de-regulated ET signalling network. In contrast to the *ein3/eil1* alleles, which revert the characteristic dwarf phenotype of the *ctr1-1* mutant (Alonso et al., 2003); the *tga256* alleles did not rescue this phenotype (Fig.1b). This indicates that TGA factors are not integrated into those ET responses that cause these developmental changes. Consistently, *tga256* mutant seedlings exhibit the triple response after ACC treatment, albeit a small but significant insensitivity was detected (Fig.1c,d).

ACC-induced *ORA59* expression depends on TGA factors at late time points

The AP2/ERF transcription factor ORA59 is required for *PDF1.2* expression after treatment of plants with either ET or JA or ET and JA (Pre et al., 2008). To analyze whether TGA factors influence the activation of *PDF1.2* through modulation of *ORA59* expression, *ORA59* transcript levels were determined using real-time RT-PCR analysis of RNA collected from ACC-treated soil-grown wild-type and *tga256* mutant plants in a time course experiment (Fig.2a). In wild-type plants, expression of *ORA59* increased after 4 hours and reached a plateau after 8 hours (Fig.2a). In the *tga256* mutant, *ORA59* transcripts accumulated to higher than wild-type levels after 4 hours, but subsequently decreased showing a difference to the wild-type control already after 8 hours. After 12 and 24 hours, ACC-induced *ORA59* expression was hardly higher than in mock-treated plants, suggesting an important role of TGA factors in regulating *ORA59* transcription at later time points. The initial TGA-independent activation of *ORA59* transcription is unlikely to be not sufficient for the activation of *PDF1.2* which is induced only in the wild-type after 24 hours (Fig.2b). As expected, transcriptional activation of *PDF1.2* and *ORA59* depends on either of the two redundant transcriptional activators EIN3 or EIL1 even at late time points (Fig.2c,d).

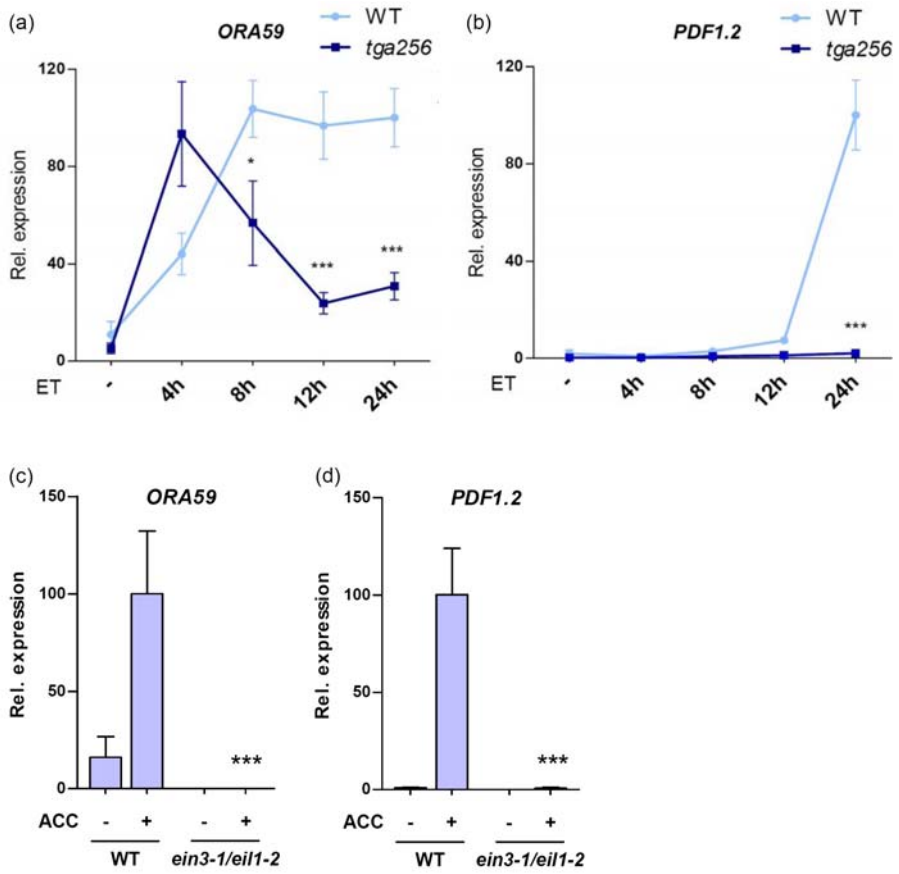

Figure 2. Time course of *ORA59 and PDF1.2* expression after ACC treatment in *tga256* mutant plants

Four-week old soil-grown wild-type and *tga256* plants were sprayed with 1 mM ACC for the indicated hours. The mock value (-) is from plants sprayed for 24 hours with water. The relative *ORA59* (a) and *PDF1.2* (b) transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in wild-type plants after 24 hours were set to 100%. The mean values (±SE) from three independent experiments, each consisting of 3-6 biological replicates are shown.

ORA59 and PDF1.2 expression after ACC treatment in *ein3-1/eil1-2* mutant plants. Four-week old soil-grown wild-type and *ein3-1/eil1-2* mutant plants were sprayed with 1 mM ACC for 24 hours. The relative *ORA59* (c) and *PDF1.2* (d) transcript levels were determined by quantitative real-time RT-PCR analysis. The mean values (±SE) from two independent experiments, each consisting of 3-4 biological replicates are shown. Asterisks represent significant differences between wild-type and *tga256* mutant plants or *ein3-1eil1-2* mutant plants (two-way ANOVA, **P* < 0.05, ****P* < 0.001).

Class II TGA factors directly target the *ORA59* promoter

The *ORA59* promoter contains one TGACG motif between base pair positions -360 and -356 upstream of the transcriptional start site raising the possibility that TGA factors are directly recruited to this motif. To test for *in vivo* binding of TGA factors to the *ORA59* promoter, chromatin immunoprecipitation (ChIP) analyses were performed using an antiserum against TGA2 and TGA5 (Ndamukong et al., 2007). In mock-treated wild-type plants, a two-fold enrichment of the *ORA59* promoter fragment (amplified between bp position –437 and –302) compared to *tga256* mutant plants was

detectable (Fig.3a). After ACC treatment, a further 2.5-fold enrichment was visible, whereas no ACC effect was observed when the chromatin of *tga256* mutant plants was analyzed. This indicates that class II TGA factors bind to the *ORA59* promoter *in vivo* and that binding is increased after 24 hours of ACC treatment.

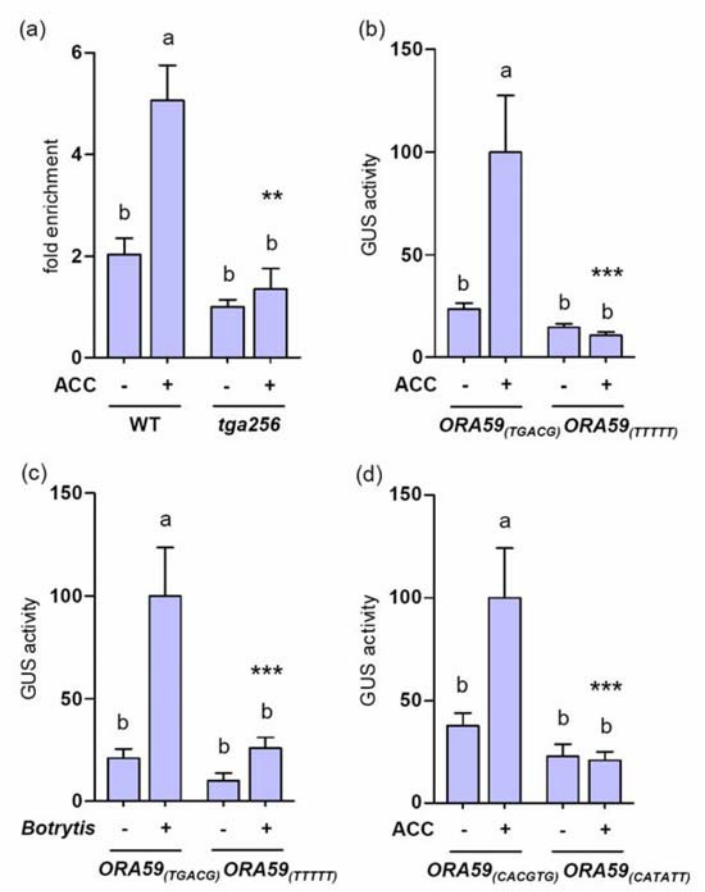

Figure 3. The ACC-induced binding of TGA factors to the *ORA59* promoter determines the crucial importance of the TGACG motif

(a) Chromatin immunoprecipitation analysis using αTGA2,5 antiserum was performed with four-week old soil-grown wild-type and *tga256* mutant plants. Plants were treated with 1 mM ACC or water for 24 hours. Eight plants were pooled per treatment and per experiment. The co-immunoprecipitated DNA was recovered and analysed with quantitative real-time RT-PCR using primers against the *ORA59* promoter and a genomic fragment of *Actin8* for normalization. This values were again normalized against the reference gene *GES* (At1g61120). The values of water-treated *tga256* plants were set to 1. Based on this the fold enrichment was calculated. Values (±SE) from two independent experiments are shown.

(b,c) A 975-bp fragment of the *ORA59* promoter was cloned upstream of the *GUS* reporter gene. Wild-type plants were transformed with this *ORA59*$_{(TGACG)}$*:GUS* construct or a *ORA59*$_{(TTTTT)}$*:GUS* construct where the TGACG motif from bp position -360 to -356 was mutated to TTTTT. For both constructs 13-15 independent F2-lines were tested as a pool. Each line treated with 1 mM ACC for 24 hours (b) or spray-inoculated with *Botrytis cinerea* (c) for 72 hours consists of 25 three-week old plants grown on soil in one pot. In each experiment the values of ACC or *Botrytis cinerea* treated *ORA59*$_{(TGACG)}$*:GUS* lines were set to 100%. The values (±SE) from two independent experiments for each treatment are shown.

(d) Additionally transgenic lines were generated where the G-box (CACGTG) of the *ORA59* promoter at bp position -333 to -328 was mutated to CATATT. Together with the wild-type constructs 15 independent

ORA59$_{(CATATT)}$:GUS F2-lines were tested as a pool. Each line treated with 1 mM ACC for 24 hours consists of 25 three-week old plants grown on soil in one pot. In each experiment the values of ACC treated lines were set to 100%. The values (±SE) from two independent experiments for each treatment are shown. Different letters indicate differences among treatments within a genotype (Student's *t*-test, $P < 0.05$). Asterisks represent significant differences between wild-type and *tga256* plants (two-way ANOVA, $^{**}P < 0.01$, $^{***}P < 0.001$).

To determine if the TGACG motif is crucial for the activation of the *ORA59* promoter, we generated transgenic lines carrying either the wild-type promoter (from -1 to -975) or a TGACG-deficient promoter fused to the *ß-glucuronidase* (*GUS*) gene (*ORA59$_{(TTTTT)}$:GUS*). To assess the importance of the MYC2 binding site at base-pair positions –333 and –328, a third construct was generated where the MYC2 binding site CACGTG was mutated to CATATT (*ORA59$_{(CATATT)}$:GUS*). Analysis of fifteen independent transgenic lines of each construct showed that the wild-type *ORA59$_{Pro}$:GUS* construct was inducible by a factor of 2 to 3 after ACC, whereas no induction of the *ORA59$_{(TTTTT)}$:GUS* and the *ORA59$_{(CATATTT)}$:GUS* construct constructs was observed (Fig.3b,d). The importance of the TGA binding sites within the *ORA59* promoter was further confirmed by the analysis of the GUS activities in transgenic plants infected with *Botrytis cinerea* (Fig.3c). Collectively, our data indicate that recruitment of TGA factors to the TGACG element of the *ORA59* promoter constitutes a crucial step for continued expression of promoter activity under conditions of elevated ethylene levels. In addition, the *ORA59* promoter activity requires a functional basal activity of the JA pathway which is probably installed via the MYC2 binding site.

Salicylic acid-mediated suppression of ACC-induced *ORA59* expression depends on TGA factors

Next, we tested whether ACC-induced expression of *ORA59* was subject to the SA cross-talk and how this was affected by the *tga256* alleles. In wild-type plants, *ORA59* transcript levels were 10-fold elevated at 24 hours after treatment. This induction was suppressed by SA (Fig.4a). In *tga256* mutant plants, ACC induction leads to a severely compromised but still detectable induction of *ORA59* transcription (Fig.4a). However, no suppressive effect after SA treatment on *ORA59* transcription was observed (Fig.4a). This indicates that the SA-mediated antagonism is based on the interference of SA with the positive function of TGA factors within the ET or JA/ET pathway.

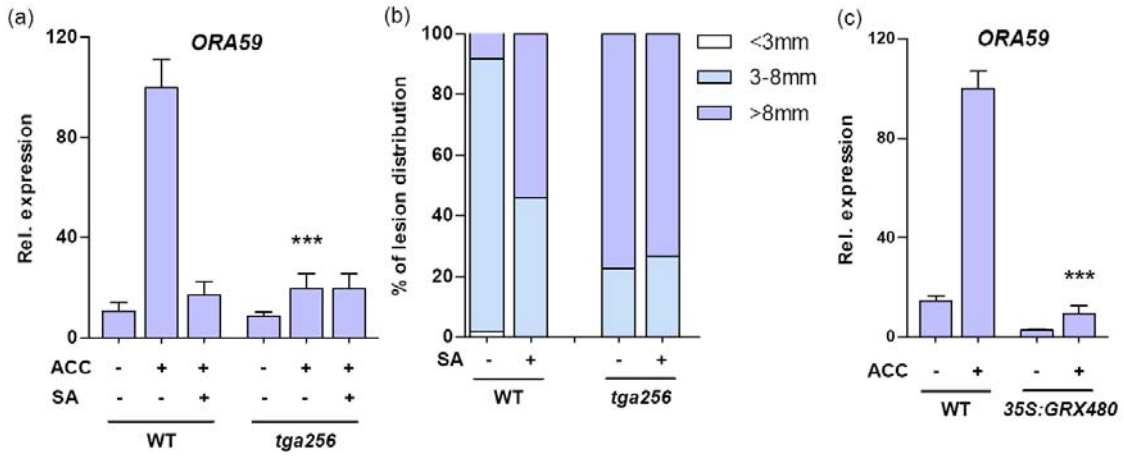

Figure 4. The *tga256* mutant is insensitive to SA-mediated *ORA59* suppression and susceptibility against *Botrytis cinerea*

(a) Four-week old soil-grown wild-type and *tga256* mutant plants were sprayed with 1 mM ACC or a combined treatment of 1 mM ACC and 1 mM SA for 24 hours. Water spraying serves as a control. The relative *ORA59* transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in ACC-treated wild-type plants were set to 100%. The mean values (±SE) from two independent experiments, each consisting of 3-6 biological replicates are shown.

(b) Four-week old soil-grown wild-type and *tga256* plants were pre-treated with 1 mM SA for 24 hours and then drop-inoculated with a *Botrytis cinerea* spore solution ($5x10^4$ spores ml^{-1}) or with quarter-strength potato dextrose broth as the mock control. The diameters of at least 35 lesions per experiment were measured and grouped into the indicated different size classes. The result from one representative experiment is shown. One further experiment was performed with similar results.

(c) Four-week old soil-grown wild-type and *35S:GRX480* mutant plants were sprayed with 1 mM ACC for 24 hours. Water spraying serves as a control. The relative *ORA59* transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in ACC-treated wild-type plants were set to 100%. The mean values (±SE) from five individual plants are shown. Asterisks represent significant differences between wild-type and *tga256* plants (two-way ANOVA, ****P* < 0.001).

To obtain further evidence for the notion that the *tga256* mutant is insensitive to the SA-ET antagonism, SA treatment was combined with a *Botrytis cinerea* infection. Wild-type and *tga256* mutant plants were pre-treated with 1 mM SA for 24 hours and subsequently challenged with *Botrytis cinerea* for further 72 hours. Measurement of lesion sizes revealed that SA application prior to infection leads to the formation of larger lesions as compared to mock-treated plants (Fig.4b): Without SA application nearly 90% of the lesion diameters varied between 3 and 8 mm. In the presence of SA, 50% of the lesion diameters were bigger than 8 mm. The *tga256* mutant plant was more susceptible than wild-type plants: 70% of the lesions diameters were bigger than 8 mm. Importantly, lesion size was not influenced by SA. This result highlights that SA antagonizes the defense response against necrotrophic pathogens by targeting the activating function of TGA factors. As previous results had shown that the SA-induced

glutaredoxin GRX480, which interacts with TGA factors, represses JA-induced *PDF1.2* expression (Ndamukong et al., 2007), we asked whether GRX480 would also suppress *ORA59* expression after ACC treatment. Indeed in *35S:GRX480* mutant plants the ACC-induced *ORA59* expression was nearly completely abolished (Fig.4c). Thus GRX480 is a candidate to mediate suppression of the JA/ET pathway by binding to TGA factors at the *ORA59* promoter and inhibiting their function.

TGA factors are involved in ACC-induced transcriptional reprogramming

The negative effect of SA on the expression of the key regulator *ORA59* should affect its direct and indirect target genes. As TGA factors represent the regulatory node for the SA antagonism at the *ORA59* promoter, we asked the question whether all ACC-induced genes that are subject to the SA antagonism are TGA-dependent or whether a TGA-independent SA antagonism merging on other genes exists. Microarray analyses were carried out, using ATH1 whole-genome arrays. The experimental design included four treatments (mock, ACC, ACC and SA) of 8 plants per experiment and genotype (wild-type, *tga256*). This setup was repeated three times with independently grown plants. Twelve hours was chosen as the time point for harvest as differences in regulatory genes and their target genes might be already detectable. Off the more than 22 000 *Arabidopsis* genes on the Affymetrix ATH1 gene chip, 374 genes were more than 2-fold ($p < 0.05$) up-regulated in wild-type plants after ACC treatment (Table S1) and 238 genes were more than 2-fold ($p < 0.05$) down-regulated (Table S5).

From the 374 ACC-induced genes, 136 genes were 2-fold ($p < 0.05$) less expressed in the ACC-treated *tga256* mutant than in the ACC-treated wild-type plants and are thus classified as TGA-dependent. In contrast, expression of 258 genes is TGA-independent as revealed by less than 2-fold or $p > 0.05$ difference in expression between ACC-treated wild-type and *tga256* plants and were thus classified as TGA-independent. 86 genes were significantly suppressed by SA.

Visualization of the relative abundance of the 144 transcripts that were either down-regulated by SA (2-fold; $p < 0.05$) or affected in the *tga256* mutant (2-fold; $p < 0.05$) was done according to the MarVis clustering tool (Kaever et al., 2009) (Fig.5). Cluster I contains 74 genes which are suppressed by SA (Table S2). The majority of these genes (62 out of 74) is not expressed in the *tga256* mutant. From the 12 remaining genes, eight just barely missed the criterion to be TGA-dependent: at least 2-fold ($p < 0.05$) less expressed in *tga256* plants as compared to wild-type plants (Table S2). We deduce from these findings that the expression of a SA cross-talk responsive gene has to be TGA-dependent. Cluster II comprise 70 genes that were less affected by SA (less

than 2-fold different) and but which were still TGA-dependent at least 2-fold ($p < 0.05$) less expressed in *tga256* plants as compared to wild-type plants (Table S3). It is concluded that genes of cluster II are induced by ACC through a mechanism that requires class II TGA factors though they are not subject to the clear negative SA-driven feedback mechanism. Unexpectedly, cluster II genes showed somewhat higher levels of expression in the SA/ACC treated *tga256* mutant than in the ACC treated *tga256* plants thus re-establishing the transcript pattern observed in SA/ACC-treated wild-type plants. A probable explanation is that SA-activated transcription factors can compensate for the class II TGA factors to re-install the expression levels found in SA/ACC treated wild-type plants. The ACC-induced genes whose expression were neither TGA-dependent nor responsive to SA were grouped in cluster III (Table S4).

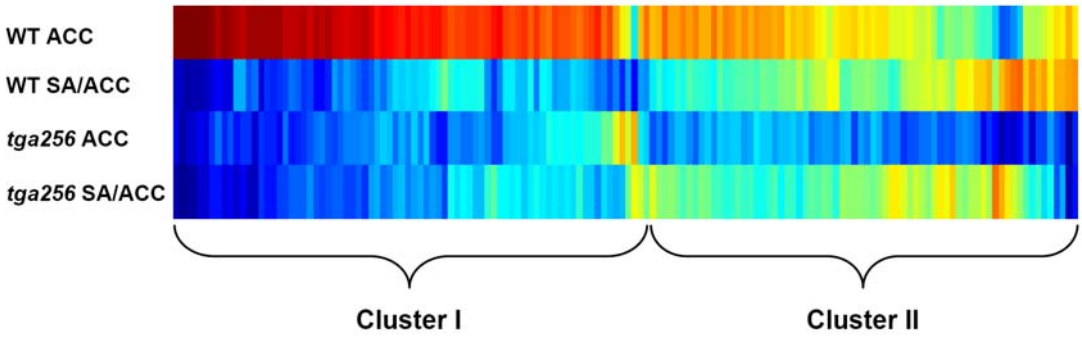

Figure 5. Visualized cluster analyses of ACC-induced genes which are SA-responsive or TGA-dependent.

For cluster analysis and visualization MarVis software was used (Kaever et al., 2009). Those genes were selected whose expression were more than 2-fold ($p < 0,05$) less in SA-treated wild-type plants compared to ACC-treated wild-type plants (74 genes). In addition genes whose ACC-induced expression in *tga256* mutant plants is more than 2-fold ($p < 0,05$) less compared to ACC-treated wild-type plants were included (136 genes). Due to the large overlap 148 genes were in sum included in the cluster analyses.

Motif sampler analysis (http://www.arabidopsis.org/tools/bulk/motiffinder/index.jsp) indicated that 25% of the ACC-induced genes contain a putative ERF binding site (GCC) within the 1000 bp sequence upstream from the annotated transcriptional start site, irrespective whether they belong to cluster I or cluster II or whether they were expressed independently from TGA factors (cluster III). This is consistent with the notion that ERF transcription factors play a major role in the ET signal transduction cascade. Table 1 lists the expression data from ACC-induced ERF transcription factors and other regulatory genes. From the 14 ERF/AP2-like genes induced after ACC-

treatment, 4 group IX ERF transcription factors (*ORA59*, *AtERF15*, *ERF1* and *At5g43410*) fall into cluster I. This group has been shown before to be involved in ET-responsive processes (Berrocal-Lobo et al., 2002; McGrath et al., 2005; Nakano et al., 2006; Onate-Sanchez et al., 2007; Bethke et al., 2009).

Cluster II contains only group II transcription factor CEJ1 (*cooperatively regulated by ethylene and jasmonate 1*), which does not recognize a GCC box (Tsutsui et al., 2009). Group IX transcription factor ERF14, which had been described as an essential regulator of *PDF1.2* transcription after ET-treatment (Onate-Sanchez et al., 2007), was not represented on the ATH1 gene chip. Therefore, real time RT-PCR analysis was performed yielding its classification into the list of ACC-induced genes that are not dependent on TGA factors (Fig.S1). Cluster III contains ERF genes especially from group VIII namely *AtERF8*, *AtERF9* and *AtERF11,* which contain an EAR- (*ERF-associated amphiphilic repression*) domain and are thought to be negative regulators (Ohta et al., 2001; Nakano et al., 2006).

Except for ERF transcription factors, other ACC-activated regulators that might function upstream of ERF transcription factors in the ethylene signalling network were not induced by ACC in the *tga256* mutant including genes of the MAPK signalling cascade like MPK7, MAPKKK19 and MAPKKK20 and JAZ8, a member of the repressors of JA signalling (Table 1, cluster I).

Table 1 ACC-induced regulatory genes grouped into three clusters according to their SA-responsiveness and TGA-dependency.

				Reduced expression (x-fold) compared to ACC-treated WT plants in ...					
Cluster I		ACC-induced in WT	*p*-value	WT SA/ACC	*p*-value	*tga256* ACC	*p*-value	*tga256* SA/ACC	*p*-value
AT1G06160	ORA59	6,2	8E-04	4,1	7E-03	6,3	8E-04	4,5	5E-04
AT2G31230	ATERF15	2,8	3E-06	2,3	5E-05	4,3	2E-08	3,1	1E-04
AT5G43410	ethylene-responsive factor	3,7	1E-07	3,5	2E-07	3,7	1E-07	3,8	4E-04
AT3G23240	ERF1	9,6	1E-09	2,5	3E-04	3,0	5E-05	2,9	7E-04
AT1G66370	MYB113	6,1	7E-08	4,4	1E-06	3,1	5E-05	4,7	2E-04
AT5G67080	MAPKKK19	7,5	2E-07	3,3	2E-04	4,5	1E-05	2,6	6E-03
AT3G50310	MAPKKK20	6,5	6E-11	5,5	3E-10	5,4	4E-10	7,3	5E-06
AT1G18350	ATMKK7	11,4	2E-12	2,9	1E-06	2,7	3E-06	3,3	9E-05
AT1G30135	JAZ8	6,3	1E-09	5,3	7E-09	4,2	7E-08	4,7	1E-04
Cluster II									
AT3G50260	CEJ1	2,1	2E-03	1,5	8E-02	2,1	2E-03	1,6	3E-02
AT5G04340	ZAT6	5,1	9E-07	2,1	5E-03	4,1	7E-06	1,5	3E-02
AT1G27730	STZ (salt tolerance zinc finger)	3,9	9E-06	1,3	3E-01	2,8	3E-04	1,3	8E-02
AT3G01970	WRKY45	6,9	2E-05	1,6	2E-01	2,6	1E-02	1,7	1E-02
AT1G06180	ATMYB13	3,5	5E-05	1,0	9E-01	2,8	5E-04	1,0	9E-01
AT3G15500	ANAC055	3,0	8E-04	1,6	9E-02	2,2	1E-02	1,3	2E-01
AT4G34590AT 4G34588	GBF6, bZIP11	3,7	5E-07	1,9	2E-03	2,6	3E-05	1,4	2E-03
Cluster III									
AT5G47220	ATERF-2	2,7	2E-02	1,2	6E-01	2,1	7E-02	1,1	6E-01
AT4G17500	ATERF-1	2,6	8E-03	1,1	8E-01	1,9	5E-02	0,9	5E-01
AT5G44210	ERF9	2,7	1E-05	1,3	2E-01	1,3	1E-01	1,3	4E-02
AT1G28370	ERF11	3,1	2E-06	1,3	1E-01	1,9	1E-03	1,9	1E-03
AT5G58620	zinc finger (CCCH-type) protein	2,1	3E-05	0,9	4E-01	1,9	2E-04	1,5	1E-02
AT5G53980	ATHB52	2,2	2E-04	1,1	6E-01	0,3	2E-06	1,0	1E+00
AT1G66390	MYB90	3,9	3E-03	0,5	9E-02	0,9	7E-01	0,6	2E-01
AT2G47190	MYB2	3,2	2E-04	1,1	7E-01	1,6	9E-02	1,2	3E-01
AT5G13330	Rap2.6L (related to AP2 6L)	3,1	3E-03	0,6	1E-01	1,7	1E-01	0,5	8E-04
AT3G25730	AP2 domain transcription factor	2,1	3E-05	1,1	7E-01	1,6	4E-03	1,5	5E-03
AT1G44830	AP2 domain transcription factor	4,1	7E-06	1,0	9E-01	0,6	3E-02	0,8	3E-01
AT1G53170	ERF8	2,2	1E-06	1,5	1E-03	1,5	2E-03	1,8	1E-03
AT4G39780	AP2 domain transcription factor	2,3	1E-07	1,6	2E-04	1,7	6E-05	2,1	5E-04
AT4G23810	WRKY53	2,3	4E-02	0,5	6E-02	1,1	8E-01	0,7	2E-02
AT2G38470	WRKY33	2,0	2E-02	0,7	3E-01	1,7	7E-02	0,6	3E-02
AT5G13080	WRKY75	5,5	7E-03	1,1	9E-01	2,1	2E-01	0,9	7E-01
AT1G80840	WRKY40	5,2	4E-04	0,8	5E-01	1,7	2E-01	0,8	3E-01
AT1G75390AT 1G75388	CPuORF5	3,1	3E-06	1,3	1E-01	1,0	8E-01	1,2	1E-01
AT3G02550	LBD41	2,2	4E-04	1,5	5E-02	1,6	2E-02	1,2	3E-02
AT1G10585	transcription factor	9,1	2E-04	0,6	3E-01	1,9	2E-01	0,3	2E-03
AT1G73500	MKK9	2,2	4E-03	1,4	2E-01	1,8	2E-02	1,6	2E-03
AT1G19180	JAZ1	2,3	3E-03	1,1	7E-01	1,4	2E-01	0,9	6E-01

TGA factors are involved in ACC-repressed transcriptional reprogramming

From the 238 genes that were more than 2-fold ($p < 0.05$) down-regulated in wild-type plants (Table S5) 57 genes were at least 2-fold ($p < 0.05$) different either upon SA treatment or in the *tga256* alleles. As observed with the ACC-induced genes, almost all the genes that were responsive to SA were regulated by class II TGA-factors whereas not all the genes regulated by class II TGA factors were responsive to SA (Fig.6). Genes in cluster I are down-regulated by ACC in the wild-type background and their expression is further suppressed by SA (Table S6). In the *tga256* mutant these gene maintain their expression levels independent of ACC treatment revealing that the *tga256* mutant is ET-insensitive concerning this subset of genes. The gene expression pattern of the SA/ACC-treated *tga256* mutant is similar to the expression pattern of the ACC treated wild-type indicating that SA-activated factors in the *tga256* mutant can complement for the responsiveness to ACC but cannot perceive the SA signal. Genes of the second cluster do not respond to SA in the ACC-treated wild-type, but are expressed to higher levels in the ACC-treated *tga256* mutant. Like in the wild-type, SA has no influence on these genes, neither in the wild-type nor in the *tga256* mutant.

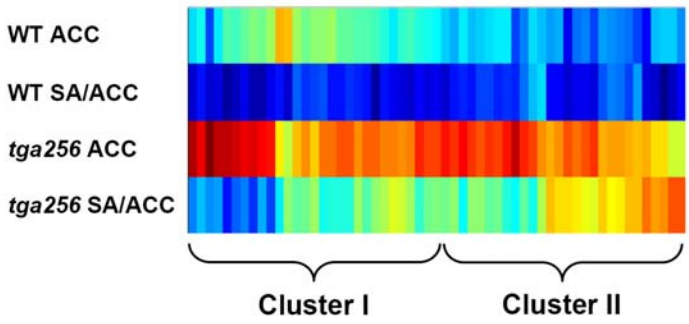

Figure 6. Visualized cluster analyses of ACC-repressed genes which are SA-responsive or TGA-dependent

For cluster analysis and visualization MarVis software was used (Kaever et al., 2009). Those genes were selected whose expression were more than 2-fold ($p < 0,05$) less in SA-treated wild-type plants compared to ACC-treated wild-type plants (32 genes). In addition genes whose ACC-induced expression in *tga256* mutant plants is more than 2-fold ($p < 0,05$) higher compared to ACC-treated wild-type plants were included (24 genes). 57 genes were in sum analyzed.

Interestingly, the ACC-repressed group of genes pinpoints a very pronounced negative crosstalk of ethylene on genes related to auxin responses (Table S5). Furthermore, the glucosinolate pathway is negatively targeted by the ethylene pathway. The indolic and the aliphatic branch of this pathway are suppressed most likely due to the negative

regulation of *ATR1* and *HAG1* by ET (Table S2). Both are MYB transcription factors which control the homeostasis of indolic and aliphatic glucosinolates, respectively (Celenza et al., 2005; Gigolashvili et al., 2007). Their ACC-mediated down-regulation is compromised in *tga256* mutants establishing a link between TGA factors and the glucosinolate pathway.

TGA-dependent and TGA-independent genes represent different functional categories

Next, we took a global view on the distributions of ET-induced genes among different functional groups as defined by Mapman using Pageman software (http://mapman.mpimp-golm.mpg.de/general/ora/ora.shtml) (Usadel et al., 2006). Five overrepresented bins were found for all ACC-induced genes, namely: “miscellaneous”, hormone metabolism, amino acid metabolism, stress and secondary metabolism (Table 2). Next, cluster I, cluster II and the TGA-independent genes (cluster III) were separately analyzed. In the TGA-dependent and SA-responsive cluster I three bins are overrepresented: “miscellaneous” (peroxidases), hormone metabolism (ethylene) and stress (biotic). This group of genes probably forms the defense cluster which is effective against *Botrytis cinerea*, as indicated by the enhanced susceptibility of *tga256* mutants (Zander et al., 2010, Chapter 2) and the SA-mediated susceptibility of wild-type plants against *Botrytis cinerea*. Enriched bins in cluster II are “miscellaneous” (cytochromes P450) and amino acid metabolism (synthesis) whereas in cluster III the bins “miscellaneous” (nitrilases), hormone metabolism (ethylene and jasmonate), amino acid metabolism (synthesis, degradation) and secondary metabolism (flavonoids) are over-represented (Table 2).

Table 2 PageMan analysis of the four indicated groups with the over-represented bins.

ACC-induced genes	fold over-represented	*p*-value
Misc	3,1	2E-15
peroxidases	7,3	1E-05
nitrilases	6	2E-04
Hormone metabolism	3,6	1E-08
ethylene	9,1	4E-10
Amino acid metabolism	5,3	4E-09
synthesis	5,4	9E-07
Stress	2	1E-03
biotic	3	2E-05
Secondary metabolism	3	8E-05
flavonoids	5,9	7E-05
Cluster I		
Misc	4,2	2E-07
peroxidases	17,5	8E-05
Hormone metabolism	5	2E-04
ethylene	12,4	3E-04
Stress	3	5E-03
biotic	5	5E-04
Cluster II		
Misc	3,3	1E-04
cytochrome P450	4,8	2E-02
Amino acid metabolism	6	5E-03
synthesis	8,8	1E-03
Cluster III		
Misc	2,4	6E-06
nitrilases	5,5	6E-03
Hormone metabolism	3,4	2E-05
ethylene	7,4	´5E-05
jasmonate	8,0	5E-03
Amino acid metabolism	5,9	4E-07
synthesis	5,4	1E-04
degradation	8,5	8E-05
Secondary metabolism	3,1	10E-04
flavonoids	6	2E-03

Discussion

Loss of function and gain of function experiments have shown that the AP2/ERF transcription factor ORA59 is an essential integrator of the JA and ET signal transduction pathways (Pre et al., 2008). Since *ORA59* is regulated at the transcriptional level by JA and ET, control of its promoter has profound effects on its target genes which collectively mount a detectable resistance against necrotrophic fungi like *Botrytis cinerea*. Here we show that class II TGA transcription factors and their binding motif TGACG have an essential function for the activation of the promoter after ET treatment. This activating function is antagonized by SA which explains the negative cross-talk of SA on a large set of ET-induced genes.

The *ORA59* promoter contains functional *cis* elements that respond to the three major stress pathways

Transcription of *ORA59* is induced by either ET, JA, or a combination of JA and ET (Pre et al., 2008). ET-induced *ORA59* transcription is suppressed by SA. In addition the *ORA59* promoter is JA-induced independent of MYC2 but concomitantly negative-regulated by JA-activated MYC2. Consistently, binding sites for crucial regulators representing the different pathways are found in the *ORA59* promoter: a putative EIN3 binding site at base pair positions between -570 and -560, an ERF binding site (GCCGCC) at positions -369 and -364, a TGA binding site (TGACG) between positions -360 and -356 and a MYC2 binding site (CACGTG) between -333 and -328.

Since the *ORA59* promoter is completely inactive in the *ein3-1/eil1-2* mutant (Fig.2c), it seems likely that under conditions of increased ET levels, stabilized EIN3 binds to the promoter and initiates transcription. This idea is supported by transient assays with reporter gene constructs that contain the *ORA59* promoter upstream of the luciferase gene. This promoter is activated by co-transfection with an effector plasmid encoding the EIN3 cDNA under the control of the *CaMV 35S* promoter (Fig.S2). When the putative *EIN3* binding site was deleted, expression was compromised (Fig.S2). The residual activation might be due to EIN3-activated ERF-transcription factors binding to the GCCGCC motif.

In addition to EIN3, the JA-Ile receptor COI1 is crucial for promoter activity even if only ET is applied (Pre et al., 2008). The importance of the MYC2 binding site between -333 and -328 for promoter activity (Fig.3d) suggests that the JA signal is integrated at this site. Since the key regulator of the JA response, MYC2, is a negative regulator of *ORA59* promoter activity we postulate a yet unknown MYC factor to contribute to *ORA59* promoter activation. Based on the finding that dominant negative JAZ repressors interfere with the activation of the JA/ET pathway we speculate that the classical COI1/JAZ/MYC regulatory module functions at this site. According to our array data (Table 1, cluster I), the ACC-induced TGA dependent module MYB113/JAZ8 might function at the *ORA59* promoter. Since ET alone can activate the promoter, a basal turn-over of JAZ proteins seems to be necessary and sufficient for promoter activation. As a consequence of this model we postulate that the negative effect of MYC2 on the JA-induced *ORA59* promoter is indirect.

The third critical *cis* element within the *ORA59* promoter is the binding site for the TGA factors, TGACG. Chromatin immunoprecipitation experiments indicated direct binding of TGA factors at the *ORA59* promoter (Fig.3a). This result is consistent with the strong hyper-activation of the promoter in plants ectopically expressing TGA5 (Zander et al.,

2010). Although the initial activation of the promoter by EIN3 is TGA-independent, TGA factors start to become important after 12 hours (Fig.2a). We speculate that TGA factors compensate at later time points for the decreasing amounts of EIN3 which might undergo protein degradation after its initial stabilization. It remains unknown how the ACC-induced binding of TGA factors is facilitated. One option would be that ORA59 or other ERF transcription factors that are activated by EIN3 bind to the GCCGCC motif which is located directly adjacent to the TGACG motif. This might lead to a more efficient recruitment of TGA factors to the promoter. TGA factors and ERFs might then be responsible for the continued *ORA59* expression even when EIN3 levels revert to basal levels.

When the pathway is activated by the simultaneous treatment of JA and ET, TGA factors are not involved in the regulation of *ORA59* promoter activity (Zander et al., 2010). This can be explained by the JA-Ile-enhanced destabilization of JAZ repressor proteins. This might release the unknown MYC factor at the critical MYC box. At the same time, MYC2 is induced. MYC2 indirectly inhibits the function of TGA factors so that they do not contribute to the activation. Evidence for the antagonistic effect of MYC2 on the activating function of TGA factors was obtained from expression analysis of the *ORA59* promoter in the *myc2* mutant: *ORA59* promoter was hyper-induced after JA/ET treatment, but this activity was reduced by either the *tga256* alleles or SA treatment (Zander et al., 2010).

Based on the findings that TGA factors physically interact with the SA-inducible plant glutaredoxin GRX480 (Ndamukong et al., 2007) and that ectopically expressed *GRX480* suppresses ET-induced *ORA59* activity (Fig.4c), we hypothesize that the TGA-GRX480 complex interferes with promoter activation and thus explains the SA-ET antagonism at the *ORA59* promoter. Consistent with the TGA-independent activation of the promoter, SA does not inhibit *ORA59* promoter activity under conditions of an activated JA signalling cascade. When the pathway is activated by *Botrytis cinerea*, *ORA59* expression is not as efficiently compromised in the *tga256* mutant as after ET treatment but it was not as insensitive to the *tga256* alleles as after JA treatment (Zander et al., 2010; Fig.2a). This indicates that pathogen induction leads to a JA/ET ratio that partially involves the TGA factors for induction. SA pre-treatment enhanced susceptibility against *Botrytis cinerea* to a similar degree as the *tga256* alleles (Fig.4b) indicating that also under these conditions TGA factors provide the target for the SA-mediated suppression of resistance against necrotrophic pathogens.

SA-mediated inhibition of TGA function at the *ORA59* promoter might explain the SA-mediated suppression of ACC-induced genes at a global scale

A well-studied marker gene of the JA/ET response is the defensin *PDF1.2*, which is directly regulated by *ORA59* (Zarei et al., 2011). So far, we have not been able to identify whether the 4-fold reduced expression of *ORA59,* which is observed after SA treatment and in the *tga256* mutant, completely explains the 100-fold lower *PDF1.2* expression. It still might be that *PDF1.2* expression is synergistically activated by ORA59 and another regulator that would also be under the control of the TGA-mediated activation mechanism. Table 1 lists other regulatory proteins that are expressed like *ORA59* as identified by microarray analysis. These include three further ERF transcription factors, three kinases, one MYB transcription factor and one JAZ protein. *ERF15* and *AtMKK7* have been listed as being up-regulated in plants expressing *ORA59* under the control of an estradiol inducible promoter (Pre, 2006) indicating that these genes are regulated through ORA59. ERF1 and ERF96 (At5g43140) are also subject to the TGA-mediated regulatory circuit and are repressed by SA and not up-regulated upon *ORA59* expression and might therefore be regulated independently from ORA59. However, although the At5g43410 contains a TGACG binding site in the promoter, this fragment was not enriched in chromatin immunoprecipitation analysis using the TGA2,5 antibody (data not shown). Expression analysis of these genes in *ora59rnai* plants under our conditions will show whether they genes are targets of ORA59 or whether their expression is regulated through TGA factors in a more direct way.

Visualization of the expression data of TGA-dependent genes indicated that cluster II genes was not affected in a significant manner by SA. These should be controlled by a master-regulator that should respond to EIN3 and would require TGA factors for expression. Candidate transcription factors are CEJ1, ZAT6, STZ, WRKY45, ANAC055 and bZIP11 (Table 1, cluster II). Since our model postulates that SA mediates its negative effect through the induction of GRX480 that would interact with TGA factors, it remains elusive if why this postulated TGA-GRX repressosome is not functional at these promoters. It remains to be determined whether COI1 is required for the expression of the genes of cluster II. If not, it might well be that GRX480 interferes with the ET induction by modifying activating compounds within this pathway.

TGA-dependent genes that are less expressed after ACC treatment can be further down-regulated by SA

When analyzing the effect of SA on ACC-induced genes in the wild-type and *tga256* mutants we found that SA has the same effect as the *tga256* alleles leading to the concept that SA inactivates the positive function of TGA factors. Therefore, it was unexpected that SA and *tga256* alleles had opposing effects on down-regulated genes: Upon ACC treatment, cluster I genes were down-regulated in wild-type but not in *tga256* mutants. Still, SA further suppressed these genes. This mode of regulation makes it unlikely that ACC induces a repressive factor in a TGA-dependent SA-sensitive manner. Since EIN3 can also work as a direct negative protein (Chen et al., 2009), we rather suggest that this negative action is operational on a key regulator of cluster I genes. This negative regulation seems to depend on TGA factors. Upon addition of SA, this negative effect is even more pronounced which might be due to the negative action of GRX480. Cluster II of the ACC-repressed genes does not respond to SA though they need TGA factors to respond to the ACC signal. The master negative regulator of this cluster might be induced in a TGA-dependent manner and should be part of cluster II of the ACC-induced genes.

SA treatment leads to the activation of transcription factors that complement the class II TGA factors.

Visualization of the microarray data obtained from ACC, SA and SA/ACC-treated wild-type and *tga256* plants demonstrated that in SA/ACC-treated *tga256* samples, wild-type responses could be restored. A plausible explanation is that SA treatment leads to the activation of most likely another group of TGA factors that complement the *tga256* mutant with respect to its function as a regulator of the ET response. If we assume that this factor contributes to the regulation of all four clusters of TGA-regulated genes that we defined, we envision the following scenario: In SA/ACC-treated *tga256* plants, the target genes of TGA256 are now under the control of the SA-induced TGA factor that is sensitive to SA when regulating cluster I genes like ORA59 but which cannot perceive the SA signal at the promoter of the key regulator of cluster II. However, when being involved in the down-regulation of genes after ACC treatment it differs from TGA256 that it cannot perceive the SA signal that leads to further suppression of these genes. These results give rise to the consideration that after SA treatment, the function of TGA256 is generally replaced by this alternative group of TGA factors. Chromatin immunoprecipitation experiments of SA/ACC-treated plants will show whether TGA2,5 remain bound to the promoter.

In conclusion, this study has established that class II TGA factors directly act as positive factors at the ACC-induced *ORA59* promoter. After SA treatment, this positive function is abolished. As this mechanism explains the effect of SA on the entire ET defense pathway, the next crucial step will be the understanding how the function of TGA factors at the *ORA59* promoter are modulated by SA.

Material and method

Plant material, growth conditions, and chemical treatments

Arabidopsis thaliana (accession Columbia [Col-0]) was used. The *tga256* triple mutant (Zhang et al., 2003) was obtained from Y. Zhang (University of British Columbia, Vancouver, Canada) and the *ctr1-1* mutant (Kieber et al., 1993) was obtained from NASC (N8057). *ein3-1eil1-2* mutants were kindly provided from Richard Vierstra (University of Wisconsin, USA). Plants were grown on soil under controlled environmental conditions (21/19°C, 16-h-light/8-h-dark cycle, 60% relative humidity). For ET induction, four-week old soil-grown plants were sprayed with 1mM of the ethylene precursor 1-aminocyclopropane-carboxylic acid (ACC) (Sigma-Aldrich, St. Louis, Mo, USA). For the SA/ACC treatment, 1mM SA (Merck, Darmstadt, Germany) was applied together with the ACC. Water spraying serves as the mock control. For the triple response assay surface-sterilized wild-type and *tga256* mutant seeds were sowed on agar plates containing Murashige and Skoog (MS) medium supplemented with or without 10µM ACC. After four days at 4°C in the dark the seedlings were transferred for two hours into the light and then again for two days in the dark at 22°C. Protoplasts assay was carried out as already described (Yoo et al., 2007) using *Arabidopsis thaliana* wild-type plants. 7,5µg of effector plasmid and 5µg of the reporter plasmid pBGWL7:ORA59$_{Pro.-975}$ or pBGWL7:ORA59$_{\Delta EIN3}$ were transfected. For normalization 1µg of the p70S plasmid containing the *Renilla* LUC gene was co-transfected.

Pathogen infections

For *B. cinerea* infection experiments, five-week old soil-grown wild-type and *tga256* mutant plants were grown on a under controlled environmental conditions (20-22/16-18°C h 12-h-light/12-h-dark cycle, 60–70% relative humidity). *Botrytis cinerea* strain BMM, provided by Brigitte Mauch-Mani (University of Neuchâtel, Switzerland), was grown on potato dextrose agar (Merck, Darmstadt, Germany). After harvesting of the spores and subsequent filtration through glass wool to remove hyphae, the spores were diluted in quarter-strength potato dextrose broth. Before infection plants were sprayed with 1mM SA or water which serves as control. After 24h of induction, droplets of 5 µl of spore suspension (5 x 10^4 spores ml^{-1}) were deposited on leaves of five-week-old plants. For assessment of symptom development the diameter of the lesions was measured after 3 days and grouped into three size categories (<3mm, 3-8mm, >8mm). The diameter of the lesions was measured after 3 days. Spray-inoculation for 3 days (2 x 10^5 spores ml^{-1}) was performed with three-week old soil grown transgenic

ORA59:GUS plants. Quarter-strength potato dextrose broth served as mock for drop and spray inoculations of control plants.

LUC assays

After 16 hours of incubation the protoplasts were harvested. To measure the LUC activities the dual LUC reporter system from Promega was used with the subsequent analyses in the TD20/20 luminometer from TurnerBiosystems.

Genetic analysis

The *tga256* mutant was crossed into the *ctr1-1* background. The F1-progeny was allowed to self-fertilize and the resulting F2 generation was screened for homozygosity. The dwarfed growth phenotype of *ctr1-1* mutant plants was used for screening the mutant *ctr1* allele. In homozygous *ctr1-1* plants the *tga2-1 tga5-1* allele was detected using primers P1, P2 and P3 (for primer sequences see Table S9). The *tga6-1* allele was confirmed by PCR with primers P4 and P5.

Binary vectors and plant transformation

Gateway technology (Invitrogen, Darmstadt, Germany) was used to generate binary vectors for analysis of the *ORA59* promoter. The promoter region from position -975 to -1 relative to the predicted transcriptional start site of the *ORA59* (At1g06160) gene was amplified using primers P6 and P7 (for primer sequences see Table S1), which add GATEWAY recombination sites to the promoter fragment. Genomic DNA extracted from Col-0 plants using the DNeasy Plant Mini Kit (Quiagen, Valencia, CA, USA) was used as a template. The fragment was inserted into pDONR223 (Invitrogen, Darmstadt, Germany) resulting in pDONR223/*ORA59 Pro.* Mutation of the TGACG motif at position -360 to -356 to 5 T´s was achieved by PCR using primer pairs P8/P9 and P10/11 and pDONR223/ *ORA59Pro* as a template resulting in two fragments which subsequently served as templates for overlapping PCR with primers P8 and P10. The CACGTG motif at position -333 to -328 was mutated to CATATT using primer pairs P8/P12 and P10/P13 and pDONR223/ *ORA59Pro* as a template. The putative EIN3 binding site between positions -570 to -560 binding was deleted using at first the primer pairs P8/P14 and P10/P15 and the pDONR223/*ORA59 Pro* as the template. Subsequently the deletion was generated via overlapping PCR using primers P8 and P10 and. The WT fragment and the fragments where the TGACG or the CACGTG motif are mutated were recombined upstream of the *ß-glucuronidase* gene in the binary

vector pBGWFS7 (http://www.psb.ugent.be/gateway/). For transient assays in protoplasts the wild-type promoter fragment and the fragment where the EIN3 binding site was deleted were recombined into the binary vector pBGWFL7 (http://www.psb.ugent.be/gateway/). EIN3 was amplified from cDNA using primers P16/P17which add GATEWAY recombination sites and subsequently recombined into pDONR207 (Invitrogen, Darmstadt, Germany). The resulting pDONR207 derivatives were recombined into the binary vector pB2GW7-HA for protoplast assays. For the new generation of *35:GRX480* plants the *GRX480* cDNA from pDONR207/GRX480 (Ndamukong et al., 2007) was recombined into the binary plasmid pB2GW7-HA. To generate transgenic plants the binary plasmids were electroporated (GenePulser II, Bio-Rad) into *Agrobacterium tumefaciens* strain GV3101 (pMP90). These Agrobacteria were used to transform Col-0 plants with the two pGBWFS7 derivatives.

Microarray analyses

Four-week old soil-grown wild-type and *tga256* mutant plants were treated for 12h with 1m ACC, or a combined treatment with 1mM ACC and 1mM SA or water as mock control. The experiment was repeated three times. In each experiment eight plants per treatment and genotype were pooled resulting in three independent samples per treatment and genotype. RNA extraction was carried out using the RNeasy Plant Mini Kit (Quiagen, Valencia, CA, USA). Microarray analysis were performed with Arabidopsis ATH1 genome arrays and done by the NASC´s International Affymetrix Service. For data analyses and statistics the Robin software was used (Lohse et al., 2010). Cluster analysis was performed with MarVis (Kaever et al., 2009) and bin over-representation analyses was done with PageMan (Usadel et al., 2006). For *cis*-element enrichment analyses Motif Analyses from TAIR was used.

Chromatin immunoprecipitation (ChIP) analyses

Four-week old soil-grown wild-type and *tga256* mutant plants were treated for 24h with 1mM ACC or water. 10 plants per treatment and genotype were pooled and cross-linked. Cross-linking and chromatin isolation were performed as described in Fode et al., 2008. The immunoprecipitation was carried out as previously described (Saleh et al., 2008) using a TGA2,5 antiserum. For pre-clearing, the chromatins were incubated with pre-immune serum for 2 hours at 4°C and subsequently incubated with Protein A Dynabeads (Invitrogen, Darmstadt, Germany) for further 2 hours at 4°C. The chromatins were incubated overnight with 5µl of the TGA2,5 antiserum and afterwards for 2 hours at 4°C with Protein A Dynabeads (Invitrogen, Darmstadt, Germany). After

washing, elution and precipitation, the DNA was subjected to quantitative real-time RT-PCR analyses. Calculations were done according to the $2^{-\Delta C_T}$ method (Livak and Schmittgen, 2001). An amplified genomic fragment of *Actin8* was used for normalization. This values were further normalized against the second reference gene *GES* (At1g61120) leading to the calculation of the fold enrichment. The mock-treated *tga256* sample was set to 1. Used primers are depicted in Table S1 (*Actin8* P18-19, *GES* P20-21, *ORA59* P22-23).

Quantitative GUS assay

Protein extracts were prepared from *ORA59:GUS* plants. For both constructs 13-15 independent F2-lines were tested as a pool. Each line was treated with 1mM ACC for 24hours or spray-inoculated with *Botrytis cinerea* for 72h. Quantitative GUS assays using 4-methyl-umbelliferyl-ß-D-glucuronide (Sigma-Aldrich, St Louis, MO, USA) as a substrate was done in microtitre plates (Jefferson et al., 1987). The released fluorescence was measured with a Cyto Fluor Series 4000plate reader (Perspektive Biosystems, Hertford, Hertfordshire, UK). The total amount of protein was determined using a commercial Bradford assay solution (CARL ROTH, Karlsruhe, Germany).

Quantitative real-time RT-PCR analysis

RNA extraction and real-time RT PCR analysis was performed as described (Fode et al., 2008). Calculations were done according to the $2^{-\Delta C_T}$ method (Livak and Schmittgen, 2001). *UBQ5* served as a reference (Kesarwani et al., 2007). Primers used to amplify and quantify the cDNA are indicated in Table S1 (*PDF1.2* (At5g44420) P24-25, *UBQ5* (At3g62250) P26-27). QuantiTect primers to amplify mRNA for *ORA59* (At1g06160), *ERF14* (At1g40370 were obtained from Quiagen, Düsseldorf, Germany.

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Supplementary material

Figure S1. *ERF14* expression after ACC and SA/ACC treatment in wild-type and *tga256* mutant plants

Figure S2. EIN3 directly activates the *ORA59:LUC* promoter construct in wild-type *Arabidopsis* protoplasts

Table S1. List of ACC-induced genes in wild-type plants

Table S2. Cluster I genes of ACC-induced genes according to MarVis analysis

Table S3. Cluster II genes of ACC-induced genes according to MarVis analysis

Table S4. Cluster III genes of ACC-induced genes not included into MarVis analysis

Table S5. List of ACC-repressed genes in wild-type plants

Table S6. Cluster I genes of ACC-repressed genes according to MarVis analysis

Table S7. Cluster II genes of ACC-repressed genes according to MarVis analysis

Table S8. Cluster III genes of ACC-repressed genes not included into MarVis analysis

Table S9. List of primers used for genotyping, cloning and real-time RT PCR analysis

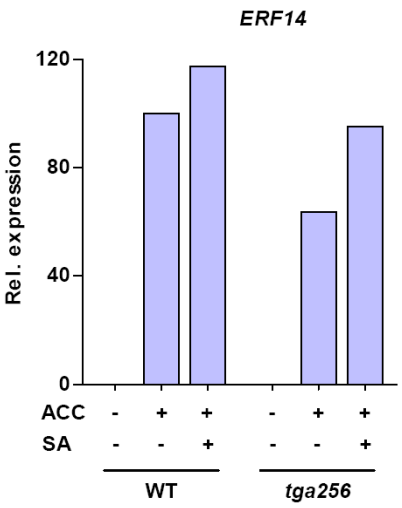

Figure S1. *ERF14* expression after ACC and SA/ACC treatment in wild-type and *tga256* mutant plants.

The relative *ERF14* transcript levels were determined by quantitative real-time RT-PCR analysis. The used cDNA derived from the RNA which was used for microarrays. The relative expression values in ACC-treated wild-type plants were set to 100%.

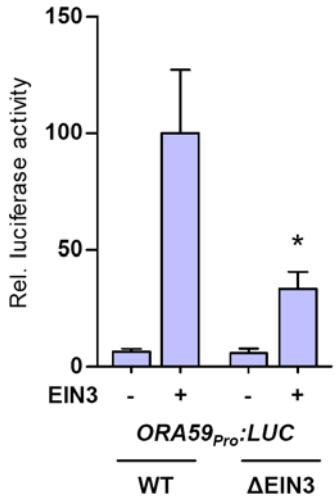

Figure S2. EIN3 directly activates the *ORA59:LUC* promoter construct in wild-type *Arabidopsis* protoplasts.

A 975-bp fragment of the *ORA59* promoter was cloned upstream of the *LUC* reporter gene. Additionally a second construct was analyzed where the putative EIN3 binding site between base pair positions between -570 and -560 was deleted. Expression was analyzed in mesophyll protoplasts derived from either wild-type plants in the presence of effector plasmids encoding EIN3 under the control of the *CaMV 35S* promoter. Relative LUC activities are expressed in arbitrary luminescence units, normalized to Renilla LUC activity. *ORA59* promoter activity in the presence of EIN3 was set to 100. Values are means of three replicates (±SE).

Table S1 List of ACC-induced genes (2-fold, p-value < 0,05) in wild-type plants

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Photosystem		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT4G18360	(S)-2-hydroxy-acid oxidase	6,2	1E-06	1,8	3E-02	3,2	3E-04	1,1	6E-01
AT2G45290	transketolase	2,1	1E-06	1,5	2E-03	1,6	4E-04	1,5	8E-03
Cell wall									
AT1G30620	MUR4 (MURUS 4); UDP-arabinose 4-epimerase	2,1	1E-03	1,2	4E-01	1,8	7E-03	0,9	7E-01
AT5G44480	DUR (DEFECTIVE UGE IN ROOT)	7,1	4E-06	3,1	2E-03	3,7	4E-04	1,8	1E-02
AT4G24000	ATCSLG2; cellulose synthase	2,1	7E-03	0,8	4E-01	1,3	4E-01	0,7	5E-02
AT3G29810	COBL2 (COBRA-LIKE PROTEIN 2 PRECURSOR)	2,2	1E-02	0,9	6E-01	1,5	2E-01	1,1	4E-01
AT2G22470	AGP2 (ARABINOGALACTAN PROTEIN 2)	2,8	6E-05	1,5	5E-02	2,1	1E-03	1,7	2E-02
AT1G76930	ATEXT4 (EXTENSIN 4)	3,8	1E-03	1,0	1E+00	3,1	5E-03	3,3	3E-03
AT5G12880	proline-rich family protein	2,6	1E-05	1,6	1E-02	1,9	7E-04	1,7	4E-03
AT5G06860	PGIP1 (POLYGALACTURONASE INHIBITING PROTEIN 1)	3,5	3E-04	1,2	6E-01	3,3	5E-04	1,0	8E-01
AT2G43870	Polygalacturonase	2,4	1E-04	1,2	3E-01	0,6	7E-03	1,0	9E-01
AT5G04310	pectate lyase family protein	2,9	3E-06	3,0	2E-06	2,8	6E-06	2,7	2E-04
AT5G04310	pectate lyase family protein	2,1	1E-06	2,1	1E-06	1,9	2E-05	2,4	3E-05
AT3G48580	xyloglucosyl transferase	3,4	4E-06	1,6	3E-02	2,1	1E-03	1,1	7E-01
AT3G43270	pectinesterase family protein	2,0	3E-02	1,0	1E+00	1,0	1E+00	1,1	7E-01
AT3G09410	unknown protein	2,4	4E-03	0,7	3E-01	2,1	1E-02	0,9	8E-01
Lipid metabolism									
AT1G04220	KCS2 (3-KETOACYL-COA SYNTHASE 2)	2,7	7E-03	1,2	5E-01	0,7	3E-01	1,5	2E-01
AT5G27600	LACS7 (LONG-CHAIN ACYL-COA SYNTHETASE 7)	2,4	1E-03	0,8	4E-01	2,9	3E-04	1,3	2E-01
AT5G07010	ST2A (SULFOTRANSFERASE 2A)	13,8	2E-06	5,0	5E-04	4,6	8E-04	1,7	2E-03
AT5G07000	ST2B (SULFOTRANSFERASE 2B)	3,6	5E-07	2,3	1E-04	1,9	2E-03	1,2	3E-01
AT1G27980	DPL1; carboxy-lyase	2,2	4E-04	0,7	8E-02	1,2	3E-01	0,8	7E-02
AT1G48320	thioesterase family protein	2,2	8E-04	0,6	1E-02	1,0	1E+00	0,7	2E-03
N-metabolism									
AT5G07440	GDH2 (GLUTAMATE DEHYDROGENASE 2)	2,3	9E-04	1,4	1E-01	2,2	1E-03	1,3	3E-01
Amino acid metabolism									
AT1G50110	branched-chain amino acid aminotransferase 6	3,3	5E-06	1,3	2E-01	1,0	8E-01	1,2	7E-02
AT1G10070	ATBCAT-2	4,1	4E-05	2,2	8E-03	2,9	6E-04	2,1	4E-04
AT1G17745	PGDH (3-PHOSPHOGLYCERATE DEHYDROGENASE)	2,6	4E-05	0,8	3E-01	1,7	7E-03	1,1	6E-01
AT4G34200	EDA9 (embryo sac development arrest 9)	4,2	1E-06	1,7	2E-02	2,7	1E-04	1,3	1E-01
AT4G35630	PSAT; O-phospho-L-serine:2-oxoglutarate aminotransferase	2,6	5E-05	1,4	6E-02	2,2	5E-04	1,2	1E-01
AT4G23600	CORI3 (CORONATINE INDUCED 1)	2,4	1E-02	2,9	3E-03	1,2	6E-01	2,2	4E-03
AT3G29200	CM1 (CHORISMATE MUTASE 1)	2,2	2E-06	1,3	3E-02	1,5	5E-03	1,1	4E-01
AT3G44720	ADT4 (arogenate dehydratase 4)	2,1	1E-03	0,8	2E-01	1,9	5E-03	0,9	5E-01
AT5G05730	ASA1 (ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1)	2,1	5E-03	1,2	5E-01	1,9	1E-02	0,9	6E-01
AT1G25155 AT5G57890 AT1G25083 AT1G24909 AT1G25220 AT1G24807	anthranilate synthase beta subunit	2,4	3E-05	1,1	5E-01	1,9	1E-03	1,0	8E-01
AT2G04400	indole-3-glycerol phosphate synthase	2,2	5E-03	0,9	8E-01	1,5	1E-01	0,7	5E-02
AT3G54640	TSA1 (TRYPTOPHAN SYNTHASE ALPHA CHAIN)	2,5	2E-04	0,9	8E-01	2,0	1E-03	1,0	7E-01
AT5G54810 AT4G27070	TSB1 (TRYPTOPHAN SYNTHASE BETA-SUBUNIT 1)	2,3	3E-06	1,8	2E-04	2,3	2E-06	1,8	5E-03
AT5G14760	AO (L-ASPARTATE OXIDASE)	2,1	2E-03	0,8	3E-01	1,0	9E-01	0,7	9E-02
AT5G38710	proline oxidase	2,9	4E-02	1,0	1E+00	1,8	3E-01	0,8	3E-01
AT5G46180	delta-OAT; ornithine-oxo-acid transaminase	2,1	8E-04	1,0	1E+00	1,7	2E-02	1,2	5E-01
AT3G08860	alanine--glyoxylate aminotransferase	2,4	7E-03	0,8	5E-01	1,0	1E+00	0,8	5E-01

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Amino acid metabolism		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G21400 AT5G34780	dehydrogenase E1 component family protein	2,1	7E-04	0,7	9E-02	1,4	1E-01	1,0	1E+00
AT1G08630	THA1 (Threonine Aldolase 1)	9,8	7E-12	3,2	4E-07	4,9	3E-09	3,4	2E-04
Metal handling									
AT2G24850	TAT3 (TYROSINE AMINOTRANSFERASE 3)	2,6	5E-02	0,4	5E-02	1,8	2E-01	0,5	4E-02
AT3G15352	ATCOX17; copper chaperone	2,2	6E-03	1,1	8E-01	1,7	5E-02	0,9	5E-01
Secondary metabolism									
AT1G33030	O-methyltransferase family 2 protein	3,4	2E-04	1,2	5E-01	3,0	5E-04	1,5	6E-02
AT3g50280	transferase family protein	2,1	9E-04	1,2	4E-01	1,3	2E-01	1,0	7E-01
AT5G01210	transferase family protein	6,0	5E-05	0,9	9E-01	1,1	7E-01	0,9	5E-01
AT1G76470	3-beta-hydroxy-delta5-steroid dehydrogenase	19,5	8E-09	1,8	6E-02	3,2	1E-03	1,9	1E-02
AT4G37790	ELI3-2 (ELICITOR-ACTIVATED GENE 3-2)	2,1	4E-03	0,9	7E-01	1,2	4E-01	1,0	9E-01
AT4G34230	ATCAD5 (CINNAMYL ALCOHOL DEHYDROGENASE 5)	3,6	1E-05	1,9	8E-03	2,4	8E-04	1,6	2E-02
AT3G51440	strictosidine synthase family protein	2,1	2E-02	0,5	3E-02	1,7	1E-01	0,7	3E-02
AT5G57220	CYP81F2	3,8	2E-03	2,5	2E-02	4,1	1E-03	2,6	3E-03
AT5G48180	NSP5 (NITRILE SPECIFIER PROTEIN 5)	2,1	3E-03	0,8	3E-01	1,1	8E-01	0,6	1E-02
AT5G22300	NIT4 (NITRILASE 4)	5,6	1E-05	1,4	3E-01	3,1	1E-03	1,3	2E-01
AT5G05600	oxidoreductase, 2OG-Fe(II) oxygenase family protein	2,8	2E-03	1,7	1E-01	2,2	1E-02	1,8	3E-02
AT2G39980	transferase family protein	4,4	1E-06	0,6	4E-02	1,1	7E-01	1,0	9E-01
AT5G61160	AACT1 (anthocyanin 5-aromatic acyltransferase 1)	36,8	1E-07	12,3	2E-05	6,9	3E-04	7,8	3E-04
AT5G39050	Transferase	2,8	1E-02	0,6	2E-01	1,5	3E-01	0,4	6E-05
AT1G59950	aldo/keto reductase	3,5	9E-05	3,6	7E-05	3,1	4E-04	4,2	1E-02
AT1G17020	SRG1 (SENESCENCE-RELATED GENE 1)	4,6	4E-04	0,6	1E-01	1,9	9E-02	0,7	2E-01
AT5G63580	FLS2 (FLAVONOL SYNTHASE 2)	2,6	2E-04	2,2	1E-03	1,1	8E-01	1,2	3E-01
AT4G39230	isoflavone reductase	2,9	3E-06	1,0	9E-01	1,8	3E-03	1,4	2E-02
AT5G52810	ornithine cyclodeaminase/mu-crystallin family protein	2,1	1E-02	0,5	2E-02	1,6	8E-02	0,6	3E-02
Hormone metabolism									
AT5G59220	protein phosphatase 2C	2,2	4E-02	0,5	4E-02	0,8	6E-01	0,4	8E-03
AT1G51760 AT1G51780	IAR3 (IAA-ALANINE RESISTANT 3)	4,6	1E-07	1,6	2E-02	2,7	4E-05	1,5	4E-02
AT1G60730	aldo/keto reductase family protein	2,3	7E-03	0,7	2E-01	0,5	2E-02	0,7	9E-04
AT4G34770	auxin-responsive family protein	3,3	2E-04	2,0	2E-02	1,3	3E-01	1,4	2E-02
AT1G53700	WAG1 (WAG 1)	3,6	7E-06	2,9	7E-05	1,6	5E-02	2,0	2E-03
AT2G28085	auxin-responsive family protein	3,1	3E-06	3,9	2E-07	6,2	2E-09	4,2	2E-05
AT1G74360	leucine-rich repeat transmembrane protein kinase	2,1	9E-03	0,6	5E-02	1,4	2E-01	0,6	2E-03
AT4G11280	ACS6	3,8	2E-06	1,7	1E-02	3,6	3E-06	1,9	2E-03
AT1G62380	ACO2 (ACC OXIDASE 2)	2,8	5E-06	1,3	1E-01	1,0	8E-01	1,1	5E-01
AT5G47220	ATERF-2	2,7	2E-02	1,2	6E-01	2,1	7E-02	1,1	6E-01
AT4G17500	ATERF-1	2,6	8E-03	1,1	8E-01	1,9	5E-02	0,9	5E-01
AT3G50260	CEJ1	2,1	2E-03	1,5	8E-02	2,1	2E-03	1,6	3E-02
AT1G06160	ORA59	6,2	8E-04	4,1	7E-03	6,3	8E-04	4,5	5E-04
AT5G44210	ERF9	2,7	1E-05	1,3	2E-01	1,3	1E-01	1,3	4E-02
AT1G04310	ERS2 (ETHYLENE RESPONSE SENSOR 2)	3,3	9E-06	1,1	7E-01	1,0	1E+00	0,9	3E-01
AT2G31230	ATERF15	2,8	3E-06	2,3	5E-05	4,3	2E-08	3,1	1E-04
AT1G28370	ERF11	3,1	2E-06	1,3	1E-01	1,9	1E-03	1,9	1E-03
AT3G23150	ETR2 (ethylene response 2)	4,3	7E-07	1,3	2E-01	1,0	9E-01	1,1	5E-01
AT5G43410	ethylene-responsive factor	3,7	1E-07	3,5	2E-07	3,7	1E-07	3,8	4E-04
AT3G23240	ERF1	9,6	1E-09	2,5	3E-04	3,0	5E-05	2,9	7E-04
AT4G37150	MES9 (METHYL ESTERASE 9)	2,4	8E-04	0,8	4E-01	1,2	5E-01	1,3	4E-01
AT1G55020	LOX1; lipoxygenase	3,0	1E-07	2,8	4E-07	1,9	1E-04	2,7	2E-04
AT3G25780	AOC3 (ALLENE OXIDE CYCLASE 3)	3,0	2E-04	0,9	6E-01	1,7	4E-02	0,9	6E-01

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Hormone metabolism		**x-fold ind.**	***p-value***	**x-fold repr.**	***p-value***	**x-fold repr.**	***p-value***	**x-fold repr.**	***p-value***
AT5G56300	GAMT2 (GIBBERELLIC ACID METHYLTRANSFERASE 2)	2,2	1E-02	0,8	5E-01	0,7	1E-01	0,9	8E-01
AT3G44860 AT3G44870	FAMT (farnesoic acid carboxyl-O-methyltransferase)	2,5	9E-04	1,2	5E-01	2,4	1E-03	1,4	5E-02
AT1G66690 AT1G66700	S-adenosyl-L-methionine:carboxyl methyltransferase family protein	39,5	6E-10	9,1	2E-06	6,3	2E-05	5,6	6E-05
Co-factor and vitamine metabolism									
AT1G67810	SUFE2 (SULFUR E 2)	6,9	1E-05	2,0	5E-02	6,9	2E-05	3,4	2E-04
Major CHO metabolism									
AT4G34860	beta-fructofuranosidase	3,0	2E-06	1,4	4E-02	2,3	7E-05	1,9	4E-03
AT4G15210	BAM5 (BETA-AMYLASE 5)	6,4	4E-02	4,9	7E-02	0,7	7E-01	2,3	2E-01
Stress									
AT2G43580	chitinase	80,2	2E-10	20,1	9E-08	25,2	3E-08	65,2	2E-08
AT3G47540	chitinase	2,1	2E-02	0,8	4E-01	1,5	2E-01	0,6	4E-02
AT2G43620	chitinase	3,0	7E-03	0,5	5E-02	0,8	6E-01	1,1	6E-01
AT4G19810	glycosyl hydrolase family 18 protein	2,6	3E-03	1,0	9E-01	1,6	1E-01	1,5	1E-01
AT3G04720	PR4 (PATHOGENESIS-RELATED 4)	2,6	7E-05	2,0	2E-03	1,7	1E-02	1,8	4E-03
AT2G15120 AT2G15220	pseudogene, disease-resistance family protein	2,6	4E-05	1,7	1E-02	1,8	4E-03	1,1	4E-01
AT2G43590	chitinase	16,8	6E-09	13,9	2E-08	8,3	5E-07	19,0	2E-06
AT3G12500	ATHCHIB	15,5	3E-09	3,8	7E-05	2,7	1E-03	4,1	2E-04
AT4G33720	pathogenesis-related protein	25,3	3E-11	24,8	3E-11	12,5	2E-09	23,7	7E-06
AT1G19670	ATCLH1	2,4	3E-03	4,6	9E-06	1,7	4E-02	2,5	1E-04
AT5G47910	RBOHD (RESPIRATORY BURST OXIDASE HOMOLOGUE D)	2,6	1E-06	1,7	9E-04	1,9	2E-04	2,0	3E-04
AT3G28930	AIG2 (AVRRPT2-INDUCED GENE 2)	2,8	2E-04	1,3	3E-01	2,3	2E-03	1,5	1E-02
AT3G13650	disease resistance response	2,1	3E-02	3,9	6E-04	2,1	3E-02	3,2	2E-06
AT2G32680	AtRLP23	2,3	2E-02	0,8	6E-01	1,8	8E-02	2,0	9E-04
AT1G72900	disease resistance protein (TIR-NBS class)	2,8	1E-02	0,7	4E-01	2,5	2E-02	0,7	2E-02
AT4G04220	AtRLP46	2,0	1E-02	0,5	3E-02	0,7	2E-01	0,9	3E-01
AT1G58170	disease resistance-responsive protein-related	2,9	4E-04	1,1	6E-01	1,2	5E-01	1,2	2E-01
AT1G64160	disease resistance-responsive family protein	2,4	5E-05	1,4	4E-02	1,6	1E-02	0,9	6E-01
AT4g13900 At4g13920	AtRLP49	2,4	4E-03	0,5	4E-02	1,4	2E-01	1,0	8E-01
AT5G44420	PDF1.2	14,4	3E-04	21,7	6E-05	10,9	8E-04	26,7	3E-05
AT2G26020	PDF1.2b	9,5	2E-04	22,6	5E-06	8,3	4E-04	12,0	3E-05
AT1G73260	trypsin and protease inhibitor family protein	3,6	1E-03	1,3	5E-01	1,9	8E-02	1,6	1E-01
AT3G01420	DOX1; lipoxygenase	2,3	3E-02	0,5	8E-02	0,8	6E-01	0,5	2E-02
AT4G11650	ATOSM34 (osmotin 34)	14,9	4E-06	7,7	1E-04	4,4	2E-03	6,2	8E-06
AT5G52640	ATHSP90.1 (HEAT SHOCK PROTEIN 90.1)	2,4	3E-02	0,5	1E-01	1,6	3E-01	0,7	2E-01
AT3G12580	HSP70 (heat shock protein 70)	3,2	6E-03	1,0	9E-01	2,1	5E-02	1,0	1E+00
AT5G38940 AT5G38930	manganese ion binding / nutrient reservoir	2,4	1E-03	1,5	1E-01	1,7	4E-02	1,2	3E-01
Redox regulation									
AT2G16060	AHB1 (ARABIDOPSIS HEMOGLOBIN 1)	2,5	3E-03	1,1	6E-01	5,5	1E+00	0,9	3E-01
Polyamine metabolism									
AT4G34710	ADC2 (ARGININE DECARBOXYLASE 2)	2,1	7E-04	0,8	4E-01	1,6	2E-02	0,8	2E-01
AT2G16500	ADC1 (ARGININE DECARBOXYLASE 1)	3,2	1E-08	2,2	6E-06	2,6	4E-07	2,1	2E-04
Nucleotide metabolism									
AT2G19570	CDA1 (CYTIDINE DEAMINASE 1)	2,6	1E-04	0,8	4E-01	1,3	2E-01	0,9	2E-01
AT4G29700	type I phosphodiesterase	2,4	9E-05	3,9	3E-07	1,0	9E-01	2,1	1E-05
Biodegradation of xenobiotics									
AT1G49660	AtCXE5 (Arabidopsis thaliana carboxyesterase 5)	2,3	4E-07	1,2	6E-02	1,1	4E-01	1,3	1E-02

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Biodegradation of xenobiotics		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G80160	lactoylglutathione lyase family protein	2,5	7E-03	0,7	3E-01	1,6	2E-01	0,8	5E-01
C1-metabolism									
AT2G21550	bifunctional dihydrofolate reductase-thymidylate synthase	2,0	5E-05	1,6	4E-03	1,8	5E-04	2,0	3E-04
AT5G14780	FDH (FORMATE DEHYDROGENASE)	2,2	2E-03	1,0	1E+00	1,2	4E-01	1,1	1E-01
Misc									
AT2G34500	CYP710A1	3,1	4E-02	0,6	3E-01	1,7	3E-01	0,3	3E-03
AT4G37370	CYP81D8	2,3	4E-02	0,5	1E-01	1,9	1E-01	0,3	1E-03
AT4G37430	CYP91A2	2,1	5E-03	0,7	2E-01	1,0	9E-01	0,9	6E-01
AT3G14620	CYP72A8	2,2	1E-03	1,1	7E-01	2,2	8E-04	1,3	3E-02
AT5G67310	CYP81G1	4,0	2E-04	1,6	1E-01	2,2	2E-02	1,4	2E-01
AT2G30770	CYP71A13	11,6	1E-04	0,8	6E-01	4,4	9E-03	0,9	7E-01
AT4G37310	CYP81H1	2,0	4E-05	1,0	1E+00	1,3	4E-02	1,1	6E-01
AT3G26830	PAD3 (PHYTOALEXIN DEFICIENT 3)	11,5	2E-05	1,4	5E-01	4,0	5E-03	1,4	9E-02
AT5G36220	CYP81D1	3,0	3E-07	1,3	1E-01	1,1	4E-01	1,3	1E-02
AT3G26200	CYP71B22	7,6	2E-08	9,1	7E-09	5,8	2E-07	9,7	3E-06
AT1G64710	alcohol dehydrogenase	2,7	4E-04	3,1	1E-04	2,2	3E-03	2,3	7E-04
AT4G37530 AT4G37520	peroxidase	4,2	1E-03	0,8	7E-01	3,1	8E-03	1,6	1E-01
AT5G05340	peroxidase	3,4	5E-04	1,1	9E-01	1,3	3E-01	1,2	5E-01
AT4G11290	peroxidase	6,0	1E-04	5,4	2E-04	6,0	1E-04	13,8	4E-07
AT3G49120 AT3G49110	PRXCB (PEROXIDASE CB)	2,0	6E-05	1,0	8E-01	1,6	2E-03	1,5	2E-02
AT5G64120	peroxidase	7,1	5E-06	0,8	5E-01	0,9	8E-01	1,1	7E-01
AT5G58390	peroxidase	5,8	6E-08	3,2	2E-05	3,5	8E-06	4,7	2E-06
AT4G08770	peroxidase	83,3	3E-10	3,3	5E-03	5,8	1E-04	7,4	5E-07
AT4G08780	peroxidase	88,9	8E-13	3,3	3E-04	7,5	5E-07	12,0	2E-06
AT3G17790	PAP17; acid phosphatase/ phosphatase/ protein serine/threonine phosphatase	2,1	5E-04	0,9	5E-01	1,2	3E-01	0,9	3E-01
AT5G18470	lectin family protein	3,4	2E-03	0,9	7E-01	2,0	6E-02	1,6	8E-03
AT3G16530	legume lectin family protein	4,2	2E-05	2,5	2E-03	2,7	8E-04	2,1	6E-04
AT1G78850 AT1G78860	lectin family protein	2,2	5E-07	2,1	1E-06	2,9	5E-09	2,2	2E-04
AT1G62760	invertase/pectin methylesterase inhibitor family protein	7,6	1E-06	3,1	9E-04	3,4	4E-04	3,7	1E-03
AT5G53870	plastocyanin-like domain-containing protein	2,2	2E-02	0,8	4E-01	1,0	9E-01	1,7	2E-02
AT5G20230	ATBCB (ARABIDOPSIS BLUE-COPPER-BINDING PROTEIN)	4,3	7E-04	0,9	9E-01	2,4	2E-02	1,5	9E-02
AT2G44790	UCC2 (UCLACYANIN 2)	12,5	2E-09	2,5	1E-03	3,6	3E-05	3,1	4E-06
AT4G34131 AT4G34135	UGT73B3 (UDP-glucosyl transferase 73B3)	2,2	1E-02	0,9	8E-01	4,0	1E-04	2,1	1E-04
AT4G15490	UGT84A3	2,9	3E-03	1,1	8E-01	2,6	5E-03	1,1	8E-01
AT4G15550	IAGLU (INDOLE-3-ACETATE BETA-D-GLUCOSYLTRANSFERASE)	2,6	9E-04	0,9	8E-01	1,8	2E-02	1,7	4E-03
AT2G30140	UDP-glucoronosyl/UDP-glucosyl transferase family protein	2,8	8E-04	0,8	4E-01	2,1	8E-03	1,0	7E-01
AT5G12890	UDP-glucoronosyl/UDP-glucosyl transferase family protein	2,0	2E-06	1,1	6E-01	1,4	3E-03	1,3	4E-02
AT2G43840	UGT74F1	4,9	6E-10	3,9	9E-09	3,5	3E-08	4,2	2E-05
AT1G07260	UGT71C3	24,4	5E-11	13,9	1E-09	10,0	1E-08	11,1	3E-06
AT4G12480	pEARLI 1	3,1	9E-03	0,9	9E-01	1,5	3E-01	1,9	1E-02
AT3G18280	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2,2	6E-04	0,8	2E-01	1,1	6E-01	0,9	6E-01
AT1G62790	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2,4	3E-05	1,0	9E-01	1,6	7E-03	1,0	9E-01
AT4G13180	short-chain dehydrogenase/reductase (SDR) family protein	2,1	4E-03	1,0	9E-01	24,2	2E-11	13,0	9E-08
AT3G04000	short-chain dehydrogenase/reductase (SDR) family protein	2,1	3E-03	0,8	4E-01	1,5	6E-02	0,4	3E-03
AT2G39030	GCN5-related N-acetyltransferase (GNAT) family protein	14,5	3E-07	5,5	1E-04	6,4	4E-05	6,6	2E-04

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Misc		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT3G60140	DIN2 (DARK INDUCIBLE 2)	2,5	4E-02	1,5	3E-01	1,0	1E+00	0,6	1E-01
AT2G44480	BGLU17 (BETA GLUCOSIDASE 17)	2,2	2E-04	2,4	5E-05	1,97	8E-04	2,2	5E-03
AT1G02850	BGLU11 (BETA GLUCOSIDASE 11)	4,2	7E-05	0,6	1E-01	2,7	2E-03	0,4	9E-06
AT4G27830	BGLU10 (BETA GLUCOSIDASE 10)	2,4	4E-05	0,8	3E-01	1,8	2E-03	0,9	2E-01
AT5G56870	BGAL4 (beta-galactosidase 4)	4,7	1E-04	2,4	1E-02	2,6	8E-03	2,5	2E-03
AT3G57240	BG3 (BETA-1,3-GLUCANASE 3)	2,6	7E-03	0,3	4E-03	1,7	1E-01	0,8	7E-02
AT2G27500	glycosyl hydrolase family 17 protein	2,5	2E-03	1,6	7E-02	1,9	2E-02	1,1	6E-01
AT4G16260	catalytic/ cation binding / hydrolase	17,9	3E-09	2,6	3E-03	2,9	1E-03	2,7	1E-03
AT3G26820 AT3G26840	esterase/lipase/thioesterase family protein	3,5	1E-04	0,9	6E-01	2,1	1E-02	0,9	8E-01
AT4G38540	monooxygenase	2,8	8E-03	1,2	5E-01	2,4	2E-02	1,2	3E-01
AT1G12200	flavin-containing monooxygenase family protein	2,0	7E-03	0,7	1E-01	2,0	9E-03	0,8	1E-01
AT4G12290 AT4G12280	copper amine oxidase family protein	2,1	1E-03	0,7	4E-02	1,6	3E-02	1,2	4E-01
AT2G17720	oxidoreductase	2,2	1E-04	0,9	6E-01	1,8	2E-03	1,0	9E-01
AT1G26420	FAD-binding domain-containing protein	2,4	3E-02	0,5	1E-01	1,9	1E-01	0,9	4E-01
AT4G20830	FAD-binding domain-containing protein	2,2	5E-03	0,7	1E-01	1,8	2E-02	1,0	1E+00
AT2G29350	SAG13; alcohol dehydrogenase/ oxidoreductase	6,3	2E-03	1,1	9E-01	2,5	9E-02	1,1	8E-01
AT1G26380	FAD-binding domain-containing protein	5,1	8E-04	2,0	1E-01	3,8	4E-03	2,1	3E-02
AT5G44390	FAD-binding domain-containing protein	2,9	3E-04	1,2	6E-01	1,3	3E-01	1,4	3E-02
AT1G17170	ATGSTU24	3,3	2E-02	0,5	1E-01	4,0	8E-03	0,3	3E-03
AT1G69920	ATGSTU12	2,0	1E-03	1,5	5E-02	1,5	4E-02	1,3	2E-01
AT2G29460	ATGSTU4	7,0	1E-03	0,9	8E-01	2,9	5E-02	0,7	2E-01
AT1G27130	ATGSTU13	2,5	6E-04	2,8	1E-04	2,2	2E-03	2,1	2E-03
AT2G29470	ATGSTU3	9,3	8E-05	1,1	8E-01	1,5	4E-01	0,9	7E-01
AT2G29440	ATGSTU6	3,2	3E-05	1,8	1E-02	1,5	9E-02	1,6	9E-03
RNA									
AT4G16265	NRPB9B	2,1	3E-04	1,6	9E-03	1,7	4E-03	1,6	3E-03
AT1G27730	STZ (salt tolerance zinc finger)	3,9	9E-06	1,3	3E-01	2,8	3E-04	1,3	8E-02
AT5G04340	ZAT6	5,1	9E-07	2,1	5E-03	4,1	7E-06	1,5	3E-02
AT5G58620	zinc finger (CCCH-type) family protein	2,1	3E-05	0,9	4E-01	1,9	2E-04	1,5	1E-02
AT5G53980	ATHB52 (ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 52)	2,2	2E-04	1,1	6E-01	0,3	2E-06	1,0	1E+00
AT1G66390	MYB90	3,9	3E-03	0,5	9E-02	0,9	7E-01	0,6	2E-01
AT2G47190	MYB2	3,2	2E-04	1,1	7E-01	1,6	9E-02	1,2	3E-01
AT1G06180	ATMYB13	3,5	5E-05	1,0	9E-01	2,8	5E-04	1,0	9E-01
AT1G66370	MYB113	6,1	7E-08	4,4	1E-06	3,1	5E-05	4,7	2E-04
AT3G15500	ANAC055	3,0	8E-04	1,6	9E-02	2,2	1E-02	1,3	2E-01
AT5G13330	Rap2.6L (related to AP2 6L)	3,1	3E-03	0,6	1E-01	1,7	1E-01	0,5	8E-04
AT3G25730	AP2 domain-containing transcription factor	2,1	3E-05	1,1	7E-01	1,6	4E-03	1,5	5E-03
AT1G44830	AP2 domain-containing transcription factor TINY	4,1	7E-06	1,0	9E-01	0,6	3E-02	0,8	3E-01
AT1G53170	ERF8	2,2	1E-06	1,5	1E-03	1,5	2E-03	1,8	1E-03
AT4G39780	AP2 domain-containing transcription factor	2,3	1E-07	1,6	2E-04	1,7	6E-05	2,1	5E-04
AT4G23810	WRKY53	2,3	4E-02	0,5	6E-02	1,1	8E-01	0,7	2E-02
AT2G38470	WRKY33	2,0	2E-02	0,7	3E-01	1,7	7E-02	0,6	3E-02
AT5G13080	WRKY75	5,5	7E-03	1,1	9E-01	2,1	2E-01	0,9	7E-01
AT1G80840	WRKY40	5,2	4E-04	0,8	5E-01	1,7	2E-01	0,8	3E-01
AT3G01970	WRKY45	6,9	2E-05	1,6	2E-01	2,6	1E-02	1,7	1E-02
AT1G75390 AT1G75388	CPuORF5 (Conserved peptide upstream open reading frame 5)	3,1	3E-06	1,3	1E-01	1,0	8E-01	1,2	1E-01
AT4G34590 AT4G34588	GBF6 (G-BOX BINDING FACTOR 6)	3,7	5E-07	1,9	2E-03	2,6	3E-05	1,4	2E-03
AT3G02550	LBD41 (LOB DOMAIN-CONTAINING PROTEIN 41)	2,2	4E-04	1,5	5E-02	1,6	2E-02	1,2	3E-02

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
RNA		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G10585	transcription factor	9,1	2E-04	0,6	3E-01	1,9	2E-01	0,3	2E-03
DNA									
AT3G13610	oxidoreductase, 2OG-Fe(II) oxygenase family protein	3,8	2E-02	0,3	5E-02	2,9	5E-02	0,4	5E-03
Protein									
AT5G38640 AT2G44070	eukaryotic translation initiation factor 2B family protein	2,4	5E-06	1,4	3E-02	1,7	2E-03	1,7	4E-03
AT4G03320	tic20-IV (TRANSLOCON AT THE INNER ENVELOPE MEMBRANE OF CHLOROPLASTS 20-IV)	3,4	1E-06	1,2	3E-01	1,95	1E-03	1,5	2E-02
AT1G29330	ERD2 (ENDOPLASMIC RETICULUM RETENTION DEFECTIVE 2)	2,7	6E-07	1,1	4E-01	1,96	1E-04	1,0	9E-01
AT1G61370	S-locus lectin protein kinase family protein	2,5	5E-03	1,0	1E+00	1,9	5E-02	1,0	9E-01
AT5G14640	SK13 (SHAGGY-LIKE KINASE 13)	2,2	3E-04	1,1	6E-01	1,2	3E-01	1,2	2E-01
AT5G65600	legume lectin family protein	2,3	2E-04	1,3	1E-01	1,9	2E-03	1,6	5E-02
AT5G03730	CTR1 (CONSTITUTIVE TRIPLE RESPONSE 1)	2,3	8E-05	1,0	1E+00	1,1	7E-01	1,0	9E-01
AT5G60300	lectin protein kinase family protein	2,3	3E-06	2,3	3E-06	1,99	3E-05	2,5	6E-05
AT5G67080	MAPKKK19	7,5	2E-07	3,3	2E-04	4,5	1E-05	2,6	6E-03
AT3G50310	MAPKKK20	6,5	6E-11	5,5	3E-10	5,4	4E-10	7,3	5E-06
AT5G10520	RBK1 (Rop Binding protein Kinases 1)	10,1	1E-08	5,7	9E-07	5,9	7E-07	4,3	1E-05
AT1G72540	protein kinase	2,6	2E-03	1,1	8E-01	1,99	2E-02	1,1	5E-01
AT2G16740	UBC29 (ubiquitin-conjugating enzyme 29)	2,0	2E-03	1,4	1E-01	1,0	9E-01	0,9	4E-01
AT5G27420	zinc finger (C3HC4-type RING finger) family protein	2,4	7E-03	0,9	7E-01	2,1	2E-02	1,2	3E-01
AT1G63840	zinc finger (C3HC4-type RING finger) family protein	2,7	1E-03	0,7	1E-01	1,7	5E-02	0,8	7E-02
AT1G15670	kelch repeat-containing F-box family protein	2,6	3E-02	0,8	6E-01	1,4	4E-01	0,8	1E-01
AT2G27310	F-box family protein	2,1	5E-03	1,0	9E-01	1,4	1E-01	1,0	9E-01
AT1G24140	matrixin family protein	2,3	5E-03	0,7	2E-01	1,7	7E-02	1,0	8E-01
AT1G43910	AAA-type ATPase family protein	3,6	2E-03	0,4	3E-02	1,0	1E+00	0,5	5E-03
AT5G57500	transferase	2,3	7E-06	1,6	5E-03	2,3	7E-06	1,5	1E-03
Minor CHO metabolism									
AT3G57520	AtSIP2 (Arabidopsis thaliana seed imbibition 2)	2,9	1E-05	2,1	6E-04	3,4	2E-06	2,2	6E-04
AT2G37770	aldo/keto reductase family protein	4,1	2E-03	0,4	3E-02	3,5	4E-03	0,5	9E-03
AT2G37760	aldo/keto reductase family protein	4,7	2E-04	0,6	2E-01	2,4	2E-02	0,8	2E-01
Signalling									
AT5G52390	photoassimilate-responsive protein	4,5	1E-02	4,0	2E-02	10,3	3E-04	12,0	5E-06
AT5G25930	leucine-rich repeat family protein	2,1	2E-02	0,8	5E-01	1,6	1E-01	0,9	7E-01
AT4G28490	HAE (HAESA)	2,6	2E-03	0,6	6E-02	1,6	1E-01	0,7	3E-02
AT1G09970	LRR XI-23	3,0	3E-05	0,9	5E-01	1,7	1E-02	1,0	1E+00
AT4G23190	CRK11 (CYSTEINE-RICH RLK11)	2,3	4E-03	1,0	9E-01	1,7	5E-02	1,6	9E-03
AT1G11050	protein kinase family protein	2,1	6E-06	1,4	2E-02	2,1	6E-06	1,6	5E-03
AT1G61380	SD1-29 (S-DOMAIN-1 29)	2,2	2E-04	0,8	2E-01	1,3	2E-01	1,0	1E+00
AT4G21390	B120	3,2	1E-04	1,5	1E-01	2,4	2E-03	1,0	9E-01
AT3G55950	CCR3 (ARABIDOPSIS THALIANA CRINKLY4 RELATED 3)	2,1	8E-03	0,7	2E-01	1,5	1E-01	0,9	6E-01
AT3G09010	protein kinase family protein	2,1	3E-02	0,8	5E-01	1,4	3E-01	0,5	5E-03
AT1G53430	leucine-rich repeat family protein	2,2	2E-03	2,3	9E-04	2,0	3E-03	1,7	7E-03
AT3G24982	protein binding	2,2	6E-03	1,1	8E-01	1,9	2E-02	1,6	3E-02
AT1G07390	AtRLP1	2,3	6E-06	0,9	6E-01	1,1	6E-01	1,1	5E-01
AT3G50770	calmodulin-related protein	2,2	4E-02	1,2	7E-01	2,0	6E-02	1,6	9E-02
AT3G63380	calcium-transporting ATPase	3,3	8E-03	1,0	9E-01	2,5	4E-02	0,9	8E-01
AT2G15760	calmodulin-binding protein	2,1	3E-03	1,3	3E-01	1,4	1E-01	1,2	8E-02
AT4G18430	AtRABA1e (Arabidopsis Rab GTPase homolog A1e)	2,3	3E-02	0,6	2E-01	1,6	2E-01	0,6	3E-02
AT1G73500	MKK9	2,2	4E-03	1,4	2E-01	1,8	2E-02	1,6	2E-03
AT5G58350	WNK4 (WITH NO K (=LYSINE) 4)	2,7	7E-04	1,3	4E-01	2,1	7E-03	1,4	3E-02
AT1G51660	ATMKK4	2,0	1E-04	0,8	2E-01	1,3	6E-02	1,0	8E-01

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Signalling		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G18350	ATMKK7	11,4	2E-12	2,9	1E-06	2,7	3E-06	3,3	9E-05
AT2G33130	RALFL18 (ralf-like 18)	3,1	2E-06	1,6	1E-02	1,6	1E-02	2,1	7E-04
Development									
AT2G26560	PLA2A (PHOSPHOLIPASE A 2A)	2,7	2E-02	1,3	5E-01	2,6	2E-02	0,9	6E-01
AT4G02380	SAG21 (SENESCENCE-ASSOCIATED GENE 21)	3,3	3E-04	0,8	6E-01	1,6	9E-02	0,9	4E-01
AT1G54890	late embryogenesis abundant protein-related	11,6	3E-10	1,8	1E-02	5,3	2E-07	4,4	1E-05
AT1G52890	ANAC019 (Arabidopsis NAC domain containing protein 19)	2,3	5E-02	0,5	9E-02	0,9	8E-01	0,5	6E-02
AT4G22530	embryo-abundant protein-related	2,2	3E-02	0,6	2E-01	1,6	2E-01	0,5	2E-03
AT4G08300	nodulin MtN21 family protein	3,0	1E-02	1,3	6E-01	1,0	1E+00	1,0	9E-01
AT1G69490	NAP (NAC-like, activated by AP3/PI)	5,9	9E-03	1,1	9E-01	2,0	3E-01	1,0	8E-01
AT1G77450	anac032 (Arabidopsis NAC domain containing protein 32)	2,4	5E-03	0,8	3E-01	6,4	1E-06	1,4	2E-01
AT5G39610	ATNAC6 (ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN 6)	4,1	2E-03	1,0	9E-01	1,4	4E-01	0,9	2E-01
AT3G01930	nodulin family protein	2,1	1E-04	1,8	9E-04	2,0	3E-04	2,5	4E-04
AT2G22860	ATPSK2 (PHYTOSULFOKINE 2 PRECURSOR)	4,8	2E-05	0,6	1E-01	1,2	5E-01	0,7	1E-02
AT2G41380	embryo-abundant protein-related	11,8	2E-06	0,8	5E-01	2,2	5E-02	0,9	2E-01
AT5G59450	scarecrow-like transcription factor 11 (SCL11)	2,5	9E-07	1,3	7E-02	1,6	1E-03	1,5	4E-03
AT3G49620	DIN11 (DARK INDUCIBLE 11)	46,3	7E-09	10,3	1E-05	5,3	4E-04	7,6	1E-05
Transport									
AT2G29410	MTPB1 (METAL TOLERANCE PROTEIN B1)	3,7	2E-06	2,3	3E-04	3,1	1E-05	2,6	2E-03
AT3G51860	CAX3 (CATION EXCHANGER 3)	2,1	2E-02	1,1	7E-01	1,7	9E-02	2,1	1E-02
AT1G59740	proton-dependent oligopeptide transport (POT) family protein	2,2	3E-03	1,0	1E+00	1,2	5E-01	1,2	3E-01
AT4G21680	proton-dependent oligopeptide transport (POT) family protein	45,7	1E-11	5,4	6E-06	9,4	1E-07	5,0	5E-05
AT5G13490	AAC2 (ADP/ATP carrier 2)	2,0	2E-02	0,5	3E-02	1,6	1E-01	0,6	2E-02
AT5G47560	TDT (TONOPLAST DICARBOXYLATE TRANSPORTER)	2,4	1E-05	1,4	4E-02	1,96	2E-04	1,7	2E-03
AT2G47800	ATMRP4	3,0	4E-03	0,5	7E-02	1,3	4E-01	0,6	2E-04
AT1G15520	PDR12 (PLEIOTROPIC DRUG RESISTANCE 12)	7,1	5E-04	1,1	8E-01	2,8	4E-02	0,9	7E-01
AT3G62150	PGP21 (P-GLYCOPROTEIN 21)	3,8	3E-04	4,0	2E-04	4,0	2E-04	3,5	2E-04
AT2G36380	PDR6; ATPase, coupled to transmembrane movement of substances	2,5	6E-08	2,4	1E-07	2,5	5E-08	3,4	4E-06
AT5G17860	CAX7 (calcium exchanger 7)	5,1	3E-05	1,1	9E-01	2,5	6E-03	1,2	3E-01
AT1G31820	amino acid permease family protein	2,0	3E-04	0,9	7E-01	1,6	1E-02	1,3	6E-02
AT5G63850	AAP4	2,5	1E-04	1,7	9E-03	1,5	4E-02	1,5	2E-03
AT3G56200	amino acid transporter family protein	2,9	1E-06	1,7	3E-03	2,0	2E-04	1,8	2E-03
AT1G23090	AST91 (SULFATE TRANSPORTER 91)	2,6	2E-06	1,5	1E-02	1,9	2E-04	2,0	3E-03
AT3G15990	SULTR3;4 (SULFATE TRANSPORTER 3;4)	3,6	1E-06	1,2	3E-01	1,95	2E-03	1,4	8E-02
AT1G61800	GPT2; antiporter/ glucose-6-phosphate transmembrane transporter	2,9	3E-02	0,5	1E-01	1,1	9E-01	0,4	1E-02
AT3G48850	mitochondrial phosphate transporter	5,8	3E-05	1,0	9E-01	2,7	6E-03	0,8	4E-01
AT4G27970	SLAH2 (SLAC1 HOMOLOGUE 2)	2,3	2E-06	1,8	9E-05	1,5	3E-03	1,3	3E-02
AT1G71140	MATE efflux family protein	2,5	3E-02	0,7	3E-01	1,8	2E-01	0,4	2E-03
AT2G17500	auxin efflux carrier family protein	2,8	7E-03	0,6	1E-01	1,5	2E-01	0,4	2E-03
AT1G75170	SEC14 cytosolic factor family protein	2,2	4E-03	0,7	9E-02	1,3	3E-01	0,8	1E-01
AT1G33110	MATE efflux family protein	2,2	7E-04	1,0	1E+00	1,4	8E-02	0,7	2E-02
AT3G23550	MATE efflux family protein	13,4	7E-05	5,4	4E-03	5,7	3E-03	3,2	9E-05
AT5G52450	MATE efflux protein-related	2,4	2E-06	1,2	2E-01	1,4	1E-02	1,0	1E+00
Not assigned									
AT3G28270	unknown protein	2,6	5E-02	1,1	9E-01	0,4	7E-02	0,8	9E-02
AT1G65690	harpin-induced protein-related	2,4	3E-02	0,7	3E-01	1,8	1E-01	0,9	7E-01
AT5G58770	dehydrodolichyl diphosphate synthase	3,4	3E-02	0,9	8E-01	1,2	8E-01	1,0	1E+00
AT5G02590	chloroplast lumen common family protein	2,0	2E-02	0,8	5E-01	1,5	1E-01	1,0	9E-01
AT1G63720	hydroxyproline-rich glycoprotein family protein	2,1	2E-02	0,7	2E-01	1,7	6E-02	0,7	2E-02
AT1G67360	rubber elongation factor (REF) family protein	2,1	8E-03	0,7	2E-01	1,0	1E+00	0,8	9E-02

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Not assigned		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT4G01870	tolB protein-related	3,4	3E-03	0,5	6E-02	2,7	1E-02	0,7	2E-01
AT5G27760	hypoxia-responsive family protein	2,1	3E-03	1,2	4E-01	1,8	1E-02	0,9	5E-01
AT2G35730	heavy-metal-associated domain-containing protein	3,2	9E-04	2,3	1E-02	2,0	3E-02	1,4	4E-01
AT3G22160	VQ motif-containing protein	2,7	8E-04	0,7	2E-01	1,7	4E-02	1,0	9E-01
AT5G17780	hydrolase, alpha/beta fold family protein	2,0	6E-04	1,2	3E-01	0,8	1E-01	1,4	5E-02
AT5G22460	esterase/lipase/thioesterase family protein	2,9	4E-04	1,6	8E-02	0,8	3E-01	0,9	5E-01
AT4G19460	glycosyl transferase family 1 protein	3,5	2E-05	1,3	2E-01	2,2	3E-03	1,7	9E-03
AT2G41180	sigA-binding protein-related	2,9	2E-05	2,0	2E-03	2,2	4E-04	2,2	9E-04
AT4G39955	hydrolase, alpha/beta fold family protein	3,1	8E-06	1,1	5E-01	1,6	2E-02	1,0	9E-01
AT4G24340 AT4G24350	phosphorylase family protein	4,3	1E-06	6,9	1E-08	3,7	4E-06	4,8	1E-05
AT4G24350	phosphorylase family protein	5,4	6E-07	8,1	2E-08	3,9	1E-05	4,6	1E-06
AT1G72800	nuM1-related	3,5	9E-09	1,3	5E-02	1,5	5E-03	1,0	8E-01
AT2G15780	glycine-rich protein	5,9	8E-08	1,0	8E-01	4,0	2E-06	4,8	9E-06
AT1G23040	hydroxyproline-rich glycoprotein family protein	2,4	7E-04	0,7	6E-02	1,2	5E-01	0,9	3E-01
AT5G49280	hydroxyproline-rich glycoprotein family protein	2,4	2E-05	1,5	2E-02	2,1	1E-04	1,8	3E-03
AT1G13340	unknown protein	2,2	4E-02	1,1	9E-01	1,9	1E-01	0,8	5E-01
AT1G76960	unknown protein	2,5	3E-02	0,8	6E-01	1,7	2E-01	1,6	1E-01
AT4G19370	unknown protein	2,6	2E-02	1,4	4E-01	1,9	1E-01	0,7	3E-01
AT1G02470	unknown protein	2,5	2E-02	0,5	9E-02	1,2	7E-01	0,5	1E-02
AT5G25260 AT5G25250	unknown protein	2,6	2E-02	0,8	5E-01	3,2	5E-03	1,2	4E-01
AT4G28085	unknown protein	2,4	2E-02	1,3	4E-01	2,1	4E-02	1,4	2E-01
AT3G26440	unknown protein	2,4	1E-02	0,7	3E-01	1,6	1E-01	0,6	2E-02
AT1G79270	ECT8 (evolutionarily conserved C-terminal region 8)	2,0	8E-03	0,7	2E-01	1,1	7E-01	0,7	1E-01
AT1G65500	unknown protein	2,2	8E-03	0,8	5E-01	0,9	7E-01	0,8	2E-01
AT1G49470	unknown protein	2,0	8E-03	1,0	9E-01	1,2	5E-01	0,9	5E-01
AT1G10140	unknown protein	3,0	5E-03	1,3	5E-01	1,8	1E-01	1,1	6E-01
AT3G10320	transferase	2,5	5E-03	1,0	1E+00	1,7	1E-01	0,9	6E-01
AT1G19180	JAZ1	2,3	3E-03	1,1	7E-01	1,4	2E-01	0,9	6E-01
AT1G28190	unknown protein	2,8	3E-03	1,4	3E-01	2,2	1E-02	1,3	2E-01
AT4G18630	unknown protein	2,1	3E-03	0,7	2E-01	1,2	5E-01	1,1	4E-01
AT4G33560	unknown protein	2,5	2E-03	1,1	7E-01	1,7	6E-02	1,3	2E-01
AT3G46110	unknown protein	2,1	1E-03	1,1	7E-01	1,3	2E-01	1,1	6E-01
AT2G25625	unknown protein	2,4	8E-04	1,1	8E-01	1,0	8E-01	0,9	7E-01
AT1G28520	VOZ1 (VASCULAR PLANT ONE ZINC FINGER PROTEIN)	2,0	7E-04	0,9	5E-01	1,1	6E-01	0,9	1E-01
AT4G26950	unknown protein	2,1	4E-04	1,5	4E-02	1,6	1E-02	1,3	3E-02
AT2G25680	MOT1 (molybdate transporter 1)	2,1	2E-04	1,9	1E-03	1,2	4E-01	2,0	6E-05
AT3G18560	unknown protein	2,8	2E-04	1,1	6E-01	2,4	9E-04	1,5	5E-03
AT1G16950	unknown protein	3,0	2E-04	1,0	1E+00	1,9	1E-02	1,7	4E-02
AT5G54970	unknown protein	2,3	2E-04	1,5	3E-02	2,1	5E-04	1,7	3E-03
AT1G19380	unknown protein	2,1	2E-04	1,3	2E-01	1,4	3E-02	1,7	1E-03
AT4G35720	unknown protein	3,1	8E-05	2,8	3E-04	0,5	8E-03	0,5	5E-02
AT4G32460	unknown protein	2,5	6E-05	1,2	4E-01	0,5	1E-03	0,7	2E-01
AT4G21310	unknown protein	2,8	4E-05	1,0	8E-01	0,8	4E-01	0,7	5E-02
AT2G44080	ARL (ARGOS-LIKE)	4,2	3E-05	1,1	7E-01	1,1	7E-01	1,1	1E-01
AT4G16670	phosphoinositide binding	2,6	2E-05	0,8	3E-01	1,1	5E-01	1,0	1E+00
AT4G39675	unknown protein	2,5	7E-06	1,9	3E-04	2,7	2E-06	1,3	2E-01
AT5G54300	unknown protein	3,8	5E-06	2,6	2E-04	1,9	5E-03	2,4	1E-04
AT1G23850	unknown protein	3,6	9E-07	2,0	8E-04	3,0	6E-06	2,5	7E-04
AT3G61930	unknown protein	5,7	9E-07	3,0	3E-04	2,4	2E-03	1,8	4E-02

ACC-induced genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Not assigned		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT2G32240	unknown protein	2,7	3E-07	1,8	2E-04	1,9	7E-05	1,5	2E-03
AT1G53180	unknown protein	3,2	3E-07	1,9	4E-04	1,3	8E-02	1,2	1E-01
AT3G59900	ARGOS (AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE)	13,6	2E-09	1,0	8E-01	0,8	3E-01	0,9	4E-01
AT1G30135	JAZ8	6,3	1E-09	5,3	7E-09	4,2	7E-08	4,7	1E-04
AT2G41230	unknown protein	70,3	3E-13	1,6	7E-02	1,6	7E-02	2,3	3E-03
AT4G10955 AT4G10960	UGE5 (UDP-D-glucose/UDP-D-galactose 4-epimerase 5)	2,3	4E-04	0,7	1E-01	1,1	5E-01	0,7	1E-02
Glycolysis									
AT2G17280	phosphoglycerate/bisphosphoglycerate mutase family protein	2,9	3E-06	1,6	6E-03	1,7	3E-03	1,7	2E-04
AT1G74030	enolase	2,1	4E-06	1,8	9E-05	1,8	9E-05	1,6	4E-04
AT1G54100	ALDH7B4 (Aldehyde Dehydrogenase 7B4)	2,0	7E-03	1,0	1E+00	1,7	4E-02	1,1	6E-01
Fermentation									
AT5G17380	pyruvate decarboxylase family protein	3,3	3E-06	1,5	4E-02	2,5	7E-05	1,5	3E-02
AT5G54960	PDC2 (pyruvate decarboxylase-2)	2,9	8E-07	2,0	2E-04	2,8	2E-06	2,6	5E-04
Gluconeogenese									
AT4G15530	PPDK (pyruvate orthophosphate dikinase)	2,3	1E-04	0,8	2E-01	1,2	4E-01	1,0	8E-01
OPP									
AT2G27510	ATFD3 (ferredoxin 3)	2,1	3E-05	1,3	7E-02	1,7	1E-03	1,3	2E-02
AT4G05390	ATRFNR1 (ROOT FNR 1)	2,2	4E-06	1,2	2E-01	1,6	1E-03	1,2	7E-02
TCA									
AT5G11670	ATNADP-ME2 (NADP-malic enzyme 2)	2,2	1E-04	2,0	5E-04	2,4	8E-05	2,0	2E-05
AT1G23730	BCA3 (BETA CARBONIC ANHYDRASE 4)	3,0	9E-06	1,3	2E-01	1,1	7E-01	1,0	9E-01
Mitochondrial electron transport									
AT4G05020	NDB2 (NAD(P)H dehydrogenase B2)	2,5	4E-03	0,5	2E-02	1,7	6E-02	0,5	2E-03
AT1G32350	AOX1D (alternative oxidase 1D)	3,3	4E-03	0,7	4E-01	1,6	2E-01	0,8	2E-01
AT4G10040	CYTC-2 (cytochrome c-2)	2,8	6E-05	1,0	9E-01	1,5	4E-02	1,1	5E-01

Table S2 Cluster I genes of ACC-induced genes according to MarVis analysis.

Cluster I (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Cell wall		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT5G44480	DUR (DEFECTIVE UGE IN ROOT)	7,1	4E-06	3,1	2E-03	3,7	4E-04	1,8	1E-02
AT5G04310	pectate lyase family protein	2,9	3E-06	3,0	2E-06	2,8	6E-06	2,7	2E-04
AT5G04310	pectate lyase family protein	2,1	1E-06	2,1	1E-06	1,9	2E-05	2,4	3E-05
Lipid metabolism									
AT5G07010	ST2A (SULFOTRANSFERASE 2A)	13,8	2E-06	5,0	5E-04	4,6	8E-04	1,7	2E-03
AT5G07000	ST2B (SULFOTRANSFERASE 2B)	3,6	5E-07	2,3	1E-04	1,9	2E-03	1,2	3E-01
Amino acid metabolism									
AT1G10070	ATBCAT-2	4,1	4E-05	2,2	8E-03	2,9	6E-04	2,1	4E-04
AT4G23600	CORI3 (CORONATINE INDUCED 1)	2,4	1E-02	2,9	3E-03	1,2	6E-01	2,2	4E-03
AT1G08630	THA1 (Threonine Aldolase 1)	9,8	7E-12	3,2	4E-07	4,9	3E-09	3,4	2E-04
Secondary metabolism									
AT1G76470	3-beta-hydroxy-delta5-steroid dehydrogenase	19,5	8E-09	1,8	6E-02	3,2	1E-03	1,9	1E-02
AT5G57220	CYP81F2	3,8	2E-03	2,5	2E-02	4,1	1E-03	2,6	3E-03
AT5G61160	AACT1 (anthocyanin 5-aromatic acyltransferase 1)	36,8	1E-07	12,3	2E-05	6,9	3E-04	7,8	3E-04
AT1G59950	aldo/keto reductase	3,5	9E-05	3,6	7E-05	3,1	4E-04	4,2	1E-02
AT5G63580	FLS2 (FLAVONOL SYNTHASE 2)	2,6	2E-04	2,2	1E-03	1,1	8E-01	1,2	3E-01
Hormone metabolism									
AT1G53700	WAG1 (WAG 1)	3,6	7E-06	2,9	7E-05	1,6	5E-02	2,0	2E-03
AT2G28085	auxin-responsive family protein	3,1	3E-06	3,9	2E-07	6,2	2E-09	4,2	2E-05
AT1G06160	ORA59	6,2	8E-04	4,1	7E-03	6,3	8E-04	4,5	5E-04
AT2G31230	ATERF15	2,8	3E-06	2,3	5E-05	4,3	2E-08	3,1	1E-04
AT5G43410	ethylene-responsive factor	3,7	1E-07	3,5	2E-07	3,7	1E-07	3,8	4E-04
AT3G23240	ERF1	9,6	1E-09	2,5	3E-04	3,0	5E-05	2,9	7E-04
AT1G55020	LOX1; lipoxygenase	3,0	1E-07	2,8	4E-07	1,9	1E-04	2,7	2E-04
AT1G66690 AT1G66700	S-adenosyl-L-methionine:carboxyl methyltransferase family protein	39,5	6E-10	9,1	2E-06	6,3	2E-05	5,6	6E-05
Co-factor and vitamine metabolism									
AT1G67810	SUFE2 (SULFUR E 2)	6,9	1E-05	2,0	5E-02	6,9	2E-05	3,4	2E-04
Stress									
AT2G43580	chitinase	80,2	2E-10	20,1	9E-08	25,2	3E-08	65,2	2E-08
AT3G47540	chitinase	2,1	2E-02	0,8	4E-01	1,5	2E-01	0,6	4E-02
AT3G12500	ATHCHIB	15,5	3E-09	3,8	7E-05	2,7	1E-03	4,1	2E-04
AT4G33720	pathogenesis-related protein	25,3	3E-11	24,8	3E-11	12,5	2E-09	23,7	7E-06
AT1G19670	ATCLH1	2,4	3E-03	4,6	9E-06	1,7	4E-02	2,5	1E-04
AT3G13650	disease resistance response	2,1	3E-02	3,9	6E-04	2,1	3E-02	3,2	2E-06
AT5G44420	PDF1.2	14,4	3E-04	21,7	6E-05	10,9	8E-04	26,7	3E-05
AT2G26020	PDF1.2b	9,5	2E-04	22,6	5E-06	8,3	4E-04	12,0	3E-05
AT4G11650	ATOSM34 (osmotin 34)	14,9	4E-06	7,7	1E-04	4,4	2E-03	6,2	8E-06
Polyamine metabolism									
AT2G16500	ADC1 (ARGININE DECARBOXYLASE 1)	3,2	1E-08	2,2	6E-06	2,6	4E-07	2,1	2E-04
Nucleotide metabolism									
AT4G29700	type I phosphodiesterase	2,4	9E-05	3,9	3E-07	1,0	9E-01	2,1	1E-05
Misc									
AT3G26200	CYP71B22	7,6	2E-08	9,1	7E-09	5,8	2E-07	9,7	3E-06
AT1G64710	alcohol dehydrogenase	2,7	4E-04	3,1	1E-04	2,2	3E-03	2,3	7E-04
AT4G11290	peroxidase	6,0	1E-04	5,4	2E-04	6,0	1E-04	13,8	4E-07

Cluster I (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Misc		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT5G58390	peroxidase	5,8	6E-08	3,2	2E-05	3,5	8E-06	4,7	2E-06
AT4G08770	peroxidase	83,3	3E-10	3,3	5E-03	5,8	1E-04	7,4	5E-07
AT4G08780	peroxidase	88,9	8E-13	3,3	3E-04	7,5	5E-07	12,0	2E-06
AT3G16530	legume lectin family protein	4,2	2E-05	2,5	2E-03	2,7	8E-04	2,1	6E-04
AT1G78850 AT1G78860	lectin family protein	2,2	5E-07	2,1	1E-06	2,9	5E-09	2,2	2E-04
AT1G62760	invertase/pectin methylesterase inhibitor family protein	7,6	1E-06	3,1	9E-04	3,4	4E-04	3,7	1E-03
AT2G44790	UCC2 (UCLACYANIN 2)	12,5	2E-09	2,5	1E-03	3,6	3E-05	3,1	4E-06
AT2G43840	UGT74F1	4,9	6E-10	3,9	9E-09	3,5	3E-08	4,2	2E-05
AT1G07260	UGT71C3	24,4	5E-11	13,9	1E-09	10,0	1E-08	11,1	3E-06
AT2G39030	GCN5-related N-acetyltransferase (GNAT) family protein	14,5	3E-07	5,5	1E-04	6,4	4E-05	6,6	2E-04
AT2G44480	BGLU17 (BETA GLUCOSIDASE 17)	2,2	2E-04	2,4	5E-05	1,97	8E-04	2,2	5E-03
AT5G56870	BGAL4 (beta-galactosidase 4)	4,7	1E-04	2,4	1E-02	2,6	8E-03	2,5	2E-03
AT4G16260	catalytic/ cation binding / hydrolase	17,9	3E-09	2,6	3E-03	2,9	1E-03	2,7	1E-03
AT1G26380	FAD-binding domain-containing protein	5,1	8E-04	2,0	1E-01	3,8	4E-03	2,1	3E-02
AT1G27130	ATGSTU13	2,5	6E-04	2,8	1E-04	2,2	2E-03	2,1	2E-03
RNA									
AT1G66370	MYB113	6,1	7E-08	4,4	1E-06	3,1	5E-05	4,7	2E-04
Protein									
AT5G60300	lectin protein kinase family protein	2,3	3E-06	2,3	3E-06	1,99	3E-05	2,5	6E-05
AT5G67080	MAPKKK19	7,5	2E-07	3,3	2E-04	4,5	1E-05	2,6	6E-03
AT3G50310	MAPKKK20	6,5	6E-11	5,5	3E-10	5,4	4E-10	7,3	5E-06
AT5G10520	RBK1 (Rop Binding protein Kinases 1)	10,1	1E-08	5,7	9E-07	5,9	7E-07	4,3	1E-05
Minor CHO metabolism									
AT3G57520	AtSIP2 (Arabidopsis thaliana seed imbibition 2)	2,9	1E-05	2,1	6E-04	3,4	2E-06	2,2	6E-04
Signalling									
AT5G52390	photoassimilate-responsive protein	4,5	1E-02	4,0	2E-02	10,3	3E-04	12,0	5E-06
AT1G53430	leucine-rich repeat family protein	2,2	2E-03	2,3	9E-04	2,0	3E-03	1,7	7E-03
AT1G18350	ATMKK7	11,4	2E-12	2,9	1E-06	2,7	3E-06	3,3	9E-05
Development									
AT1G54890	late embryogenesis abundant protein-related	11,6	3E-10	1,8	1E-02	5,3	2E-07	4,4	1E-05
AT3G49620	DIN11 (DARK INDUCIBLE 11)	46,3	7E-09	10,3	1E-05	5,3	4E-04	7,6	1E-05
Transport									
AT2G29410	MTPB1 (METAL TOLERANCE PROTEIN B1)	3,7	2E-06	2,3	3E-04	3,1	1E-05	2,6	2E-03
AT4G21680	proton-dependent oligopeptide transport (POT) family protein	45,7	1E-11	5,4	6E-06	9,4	1E-07	5,0	5E-05
AT3G62150	PGP21 (P-GLYCOPROTEIN 21)	3,8	3E-04	4,0	2E-04	4,0	2E-04	3,5	2E-04
AT2G36380	PDR6; ATPase, coupled to transmembrane movement of substances	2,5	6E-08	2,4	1E-07	2,5	5E-08	3,4	4E-06
AT3G23550	MATE efflux family protein	13,4	7E-05	5,4	4E-03	5,7	3E-03	3,2	9E-05
Not assigned									
AT2G35730	heavy-metal-associated domain-containing protein	3,2	9E-04	2,3	1E-02	2,0	3E-02	1,4	4E-01
AT2G41180	sigA-binding protein-related	2,9	2E-05	2,0	2E-03	2,2	4E-04	2,2	9E-04
AT4G24340 AT4G24350	phosphorylase family protein	4,3	1E-06	6,9	1E-08	3,7	4E-06	4,8	1E-05
AT4G24350	phosphorylase family protein	5,4	6E-07	8,1	2E-08	3,9	1E-05	4,6	1E-06
AT4G35720	unknown protein	3,1	8E-05	2,8	3E-04	0,5	8E-03	0,5	5E-02
AT5G54300	unknown protein	3,8	5E-06	2,6	2E-04	1,9	5E-03	2,4	1E-04
AT1G23850	unknown protein	3,6	9E-07	2,0	8E-04	3,0	6E-06	2,5	7E-04
AT3G61930	unknown protein	5,7	9E-07	3,0	3E-04	2,4	2E-03	1,8	4E-02
AT1G30135	JAZ8	6,3	1E-09	5,3	7E-09	4,2	7E-08	4,7	1E-04

Cluster I (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Fermentation		**x-fold ind.**	***p-value***	**x-fold repr.**	***p-value***	**x-fold repr.**	***p-value***	**x-fold repr.**	***p-value***
AT5G54960	**PDC2 (pyruvate decarboxylase-2)**	2,9	8E-07	2,0	2E-04	2,8	2E-06	2,6	5E-04
TCA									
AT5G11670	**ATNADP-ME2 (NADP-malic enzyme 2)**	2,2	1E-04	2,0	5E-04	2,4	8E-05	2,0	2E-05

Table S3 Cluster II genes of ACC-induced genes according to MarVis analysis.

Cluster II (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Photosystem		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT4G18360	(S)-2-hydroxy-acid oxidase	6,2	1E-06	1,8	3E-02	3,2	3E-04	1,1	6E-01
Cell wall									
AT3G29810	COBL2 (COBRA-LIKE PROTEIN 2 PRECURSOR)	2,2	1E-02	0,9	6E-01	1,5	2E-01	1,1	4E-01
AT2G22470	AGP2 (ARABINOGALACTAN PROTEIN 2)	2,8	6E-05	1,5	5E-02	2,1	1E-03	1,7	2E-02
AT5G06860	PGIP1 (POLYGALACTURONASE INHIBITING PROTEIN 1)	3,5	3E-04	1,2	6E-01	3,3	5E-04	1,0	8E-01
AT3G48580	xyloglucosyl transferase	3,4	4E-06	1,6	3E-02	2,1	1E-03	1,1	7E-01
AT3G09410	unknown protein	2,4	4E-03	0,7	3E-01	2,1	1E-02	0,9	8E-01
Lipid metabolism									
AT5G27600	LACS7 (LONG-CHAIN ACYL-COA SYNTHETASE 7)	2,4	1E-03	0,8	4E-01	2,9	3E-04	1,3	2E-01
N-metabolism									
AT5G07440	GDH2 (GLUTAMATE DEHYDROGENASE 2)	2,3	9E-04	1,4	1E-01	2,2	1E-03	1,3	3E-01
Amino acid metabolism									
AT4G34200	EDA9 (embryo sac development arrest 9)	4,2	1E-06	1,7	2E-02	2,7	1E-04	1,3	1E-01
AT4G35630	PSAT; O-phospho-L-serine:2-oxoglutarate aminotransferase	2,6	5E-05	1,4	6E-02	2,2	5E-04	1,2	1E-01
AT3G54640	TSA1 (TRYPTOPHAN SYNTHASE ALPHA CHAIN)	2,5	2E-04	0,9	8E-01	2,0	1E-03	1,0	7E-01
AT5G54810 AT4G27070	TSB1 (TRYPTOPHAN SYNTHASE BETA-SUBUNIT 1)	2,3	3E-06	1,8	2E-04	2,3	2E-06	1,8	5E-03
Secondary metabolism									
AT4G34230	ATCAD5 (CINNAMYL ALCOHOL DEHYDROGENASE 5)	3,6	1E-05	1,9	8E-03	2,4	8E-04	1,6	2E-02
AT5G22300	NIT4 (NITRILASE 4)	5,6	1E-05	1,4	3E-01	3,1	1E-03	1,3	2E-01
AT5G05600	oxidoreductase, 2OG-Fe(II) oxygenase family protein	2,8	2E-03	1,7	1E-01	2,2	1E-02	1,8	3E-02
Hormone metabolism									
AT1G51760 AT1G51780	IAR3 (IAA-ALANINE RESISTANT 3)	4,6	1E-07	1,6	2E-02	2,7	4E-05	1,5	4E-02
AT4G11280	ACS6	3,8	2E-06	1,7	1E-02	3,6	3E-06	1,9	2E-03
AT3G50260	CEJ1	2,1	2E-03	1,5	8E-02	2,1	2E-03	1,6	3E-02
AT3G44860 AT3G44870	FAMT (farnesoic acid carboxyl-O-methyltransferase)	2,5	9E-04	1,2	5E-01	2,4	1E-03	1,4	5E-02
Major CHO metabolism									
AT4G34860	beta-fructofuranosidase	3,0	2E-06	1,4	4E-02	2,3	7E-05	1,9	4E-03
Stress									
AT3G28930	AIG2 (AVRRPT2-INDUCED GENE 2)	2,8	2E-04	1,3	3E-01	2,3	2E-03	1,5	1E-02
AT1G72900	disease resistance protein (TIR-NBS class)	2,8	1E-02	0,7	4E-01	2,5	2E-02	0,7	2E-02
Misc									
AT3G14620	CYP72A8	2,2	1E-03	1,1	7E-01	2,2	8E-04	1,3	3E-02
AT5G67310	CYP81G1	4,0	2E-04	1,6	1E-01	2,2	2E-02	1,4	2E-01
AT2G30770	CYP71A13	11,6	1E-04	0,8	6E-01	4,4	9E-03	0,9	7E-01
AT3G26830	PAD3 (PHYTOALEXIN DEFICIENT 3)	11,5	2E-05	1,4	5E-01	4,0	5E-03	1,4	9E-02
AT4G37530 AT4G37520	peroxidase	4,2	1E-03	0,8	7E-01	3,1	8E-03	1,6	1E-01
AT5G20230	ATBCB (ARABIDOPSIS BLUE-COPPER-BINDING PROTEIN)	4,3	7E-04	0,9	9E-01	2,4	2E-02	1,5	9E-02
AT4G34131 AT4G34135	UGT73B3 (UDP-glucosyl transferase 73B3)	2,2	1E-02	0,9	8E-01	4,0	1E-04	2,1	1E-04
AT4G15490	UGT84A3	2,9	3E-03	1,1	8E-01	2,6	5E-03	1,1	8E-01
AT2G30140	UDP-glucoronosyl/UDP-glucosyl transferase family protein	2,8	8E-04	0,8	4E-01	2,1	8E-03	1,0	7E-01
AT4G13180	short-chain dehydrogenase/reductase (SDR) family protein	2,1	4E-03	1,0	9E-01	24,2	2E-11	13,0	9E-08
AT3G26820 AT3G26840	esterase/lipase/thioesterase family protein	3,5	1E-04	0,9	6E-01	2,1	1E-02	0,9	8E-01
AT1G17170	ATGSTU24	3,3	2E-02	0,5	1E-01	4,0	8E-03	0,3	3E-03

Cluster II (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
RNA		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G27730	STZ (salt tolerance zinc finger)	3,9	9E-06	1,3	3E-01	2,8	3E-04	1,3	8E-02
AT5G04340	ZAT6	5,1	9E-07	2,1	5E-03	4,1	7E-06	1,5	3E-02
AT1G06180	ATMYB13	3,5	5E-05	1,0	9E-01	2,8	5E-04	1,0	9E-01
AT3G15500	ANAC055	3,0	8E-04	1,6	9E-02	2,2	1E-02	1,3	2E-01
AT3G01970	WRKY45	6,9	2E-05	1,6	2E-01	2,6	1E-02	1,7	1E-02
AT4G34590 AT4G34588	GBF6 (G-BOX BINDING FACTOR 6)	3,7	5E-07	1,9	2E-03	2,6	3E-05	1,4	2E-03
DNA									
AT3G13610	oxidoreductase, 2OG-Fe(II) oxygenase family protein	3,8	2E-02	0,3	5E-02	2,9	5E-02	0,4	5E-03
Protein									
AT5G27420	zinc finger (C3HC4-type RING finger) family protein	2,4	7E-03	0,9	7E-01	2,1	2E-02	1,2	3E-01
AT5G57500	transferase	2,3	7E-06	1,6	5E-03	2,3	7E-06	1,5	1E-03
Minor CHO metabolism									
AT2G37770	aldo/keto reductase family protein	4,1	2E-03	0,4	3E-02	3,5	4E-03	0,5	9E-03
AT2G37760	aldo/keto reductase family protein	4,7	2E-04	0,6	2E-01	2,4	2E-02	0,8	2E-01
Signalling									
AT1G11050	protein kinase family protein	2,1	6E-06	1,4	2E-02	2,1	6E-06	1,6	5E-03
AT4G21390	B120	3,2	1E-04	1,5	1E-01	2,4	2E-03	1,0	9E-01
AT3G63380	calcium-transporting ATPase	3,3	8E-03	1,0	9E-01	2,5	4E-02	0,9	8E-01
AT5G58350	WNK4 (WITH NO K (=LYSINE) 4)	2,7	7E-04	1,3	4E-01	2,1	7E-03	1,4	3E-02
Development									
AT2G26560	PLA2A (PHOSPHOLIPASE A 2A)	2,7	2E-02	1,3	5E-01	2,6	2E-02	0,9	6E-01
AT1G77450	anac032 (Arabidopsis NAC domain containing protein 32)	2,4	5E-03	0,8	3E-01	6,4	1E-06	1,4	2E-01
AT2G41380	embryo-abundant protein-related	11,8	2E-06	0,8	5E-01	2,2	5E-02	0,9	2E-01
Transport									
AT1G15520	PDR12 (PLEIOTROPIC DRUG RESISTANCE 12)	7,1	5E-04	1,1	8E-01	2,8	4E-02	0,9	7E-01
AT5G17860	CAX7 (calcium exchanger 7)	5,1	3E-05	1,1	9E-01	2,5	6E-03	1,2	3E-01
AT3G56200	amino acid transporter family protein	2,9	1E-06	1,7	3E-03	2,0	2E-04	1,8	2E-03
AT3G48850	mitochondrial phosphate transporter	5,8	3E-05	1,0	9E-01	2,7	6E-03	0,8	4E-01
Not assigned									
AT4G01870	tolB protein-related	3,4	3E-03	0,5	6E-02	2,7	1E-02	0,7	2E-01
AT4G19460	glycosyl transferase family 1 protein	3,5	2E-05	1,3	2E-01	2,2	3E-03	1,7	9E-03
AT2G15780	glycine-rich protein	5,9	8E-08	1,0	8E-01	4,0	2E-06	4,8	9E-06
AT5G49280	hydroxyproline-rich glycoprotein family protein	2,4	2E-05	1,5	2E-02	2,1	1E-04	1,8	3E-03
AT5G25260 AT5G25250	unknown protein	2,6	2E-02	0,8	5E-01	3,2	5E-03	1,2	4E-01
AT4G28085	unknown protein	2,4	2E-02	1,3	4E-01	2,1	4E-02	1,4	2E-01
AT1G28190	unknown protein	2,8	3E-03	1,4	3E-01	2,2	1E-02	1,3	2E-01
AT3G18560	unknown protein	2,8	2E-04	1,1	6E-01	2,4	9E-04	1,5	5E-03
AT5G54970	unknown protein	2,3	2E-04	1,5	3E-02	2,1	5E-04	1,7	3E-03
AT4G39675	unknown protein	2,5	7E-06	1,9	3E-04	2,7	2E-06	1,3	2E-01
Fermentation									
AT5G17380	pyruvate decarboxylase family protein	3,3	3E-06	1,5	4E-02	2,5	7E-05	1,5	3E-02

Table S4 Cluster III genes of ACC-induced genes not included into MarVis analysis.

Cluster III (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Photosystem		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT2G45290	transketolase	2,1	1E-06	1,5	2E-03	1,6	4E-04	1,5	8E-03
Cell wall									
AT1G30620	MUR4 (MURUS 4); UDP-arabinose 4-epimerase	2,1	1E-03	1,2	4E-01	1,8	7E-03	0,9	7E-01
AT4G24000	ATCSLG2; cellulose synthase	2,1	7E-03	0,8	4E-01	1,3	4E-01	0,7	5E-02
AT3G29810	COBL2 (COBRA-LIKE PROTEIN 2 PRECURSOR)	2,2	1E-02	0,9	6E-01	1,5	2E-01	1,1	4E-01
AT5G12880	proline-rich family protein	2,6	1E-05	1,6	1E-02	1,9	7E-04	1,7	4E-03
AT2G43870	polygalacturonase	2,4	1E-04	1,2	3E-01	0,6	7E-03	1,0	9E-01
AT3G43270	pectinesterase family protein	2,0	3E-02	1,0	1E+00	1,0	1E+00	1,1	7E-01
Lipid metabolism									
AT1G04220	KCS2 (3-KETOACYL-COA SYNTHASE 2)	2,7	7E-03	1,2	5E-01	0,7	3E-01	1,5	2E-01
AT1G27980	DPL1; carboxy-lyase	2,2	4E-04	0,7	8E-02	1,2	3E-01	0,8	7E-02
AT1G48320	thioesterase family protein	2,2	8E-04	0,6	1E-02	1,0	1E+00	0,7	2E-03
Amino acid metabolism									
AT1G50110	branched-chain amino acid aminotransferase 6	3,3	5E-06	1,3	2E-01	1,0	8E-01	1,2	7E-02
AT1G17745	PGDH (3-PHOSPHOGLYCERATE DEHYDROGENASE)	2,6	4E-05	0,8	3E-01	1,7	7E-03	1,1	6E-01
AT3G29200	CM1 (CHORISMATE MUTASE 1)	2,2	2E-06	1,3	3E-02	1,5	5E-03	1,1	4E-01
AT3G44720	ADT4 (arogenate dehydratase 4)	2,1	1E-03	0,8	2E-01	1,9	5E-03	0,9	5E-01
AT5G05730	ASA1 (ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1)	2,1	5E-03	1,2	5E-01	1,9	1E-02	0,9	6E-01
AT1G25155 AT5G57890 AT1G25083 AT1G24909 AT1G25220 AT1G24807	anthranilate synthase beta subunit	2,4	3E-05	1,1	5E-01	1,9	1E-03	1,0	8E-01
AT2G04400	indole-3-glycerol phosphate synthase	2,2	5E-03	0,9	8E-01	1,5	1E-01	0,7	5E-02
AT5G14760	AO (L-ASPARTATE OXIDASE)	2,1	2E-03	0,8	3E-01	1,0	9E-01	0,7	9E-02
AT5G38710	proline oxidase	2,9	4E-02	1,0	1E+00	1,8	3E-01	0,8	3E-01
AT5G46180	delta-OAT; ornithine-oxo-acid transaminase	2,1	8E-04	1,0	1E+00	1,7	2E-02	1,2	5E-01
AT3G08860	alanine--glyoxylate aminotransferase	2,4	7E-03	0,8	5E-01	1,0	1E+00	0,8	5E-01
AT1G21400 AT5G34780	dehydrogenase E1 component family protein	2,1	7E-04	0,7	9E-02	1,4	1E-01	1,0	1E+00
Metal handling									
AT2G24850	TAT3 (TYROSINE AMINOTRANSFERASE 3)	2,6	5E-02	0,4	5E-02	1,8	2E-01	0,5	4E-02
AT3G15352	ATCOX17; copper chaperone	2,2	6E-03	1,1	8E-01	1,7	5E-02	0,9	5E-01
Secondary metabolism									
AT3g50280	transferase family protein	2,1	9E-04	1,2	4E-01	1,3	2E-01	1,0	7E-01
AT5G01210	transferase family protein	6,0	5E-05	0,9	9E-01	1,1	7E-01	0,9	5E-01
AT4G37790	ELI3-2 (ELICITOR-ACTIVATED GENE 3-2)	2,1	4E-03	0,9	7E-01	1,2	4E-01	1,0	9E-01
AT3G51440	strictosidine synthase family protein	2,1	2E-02	0,5	3E-02	1,7	1E-01	0,7	3E-02
AT5G48180	NSP5 (NITRILE SPECIFIER PROTEIN 5)	2,1	3E-03	0,8	3E-01	1,1	8E-01	0,6	1E-02
AT2G39980	transferase family protein	4,4	1E-06	0,6	4E-02	1,1	7E-01	1,0	9E-01
AT5G39050	transferase	2,8	1E-02	0,6	2E-01	1,5	3E-01	0,4	6E-05
AT1G17020	SRG1 (SENESCENCE-RELATED GENE 1)	4,6	4E-04	0,6	1E-01	1,9	9E-02	0,7	2E-01
AT4G39230	isoflavone reductase	2,9	3E-06	1,0	9E-01	1,8	3E-03	1,4	2E-02
AT5G52810	ornithine cyclodeaminase/mu-crystallin family protein	2,1	1E-02	0,5	2E-02	1,6	8E-02	0,6	3E-02
Hormone metabolism									
AT5G59220	protein phosphatase 2C	2,2	4E-02	0,5	4E-02	0,8	6E-01	0,4	8E-03
AT1G60730	aldo/keto reductase family protein	2,3	7E-03	0,7	2E-01	0,5	2E-02	0,7	9E-04

Cluster III (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Hormone metabolism		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT4G34770	auxin-responsive family protein	3,3	2E-04	2,0	2E-02	1,3	3E-01	1,4	2E-02
AT1G74360	leucine-rich repeat transmembrane protein kinase	2,1	9E-03	0,6	5E-02	1,4	2E-01	0,6	2E-03
AT1G62380	ACO2 (ACC OXIDASE 2)	2,8	5E-06	1,3	1E-01	1,0	8E-01	1,1	5E-01
AT5G47220	ATERF-2	2,7	2E-02	1,2	6E-01	2,1	7E-02	1,1	6E-01
AT4G17500	ATERF-1	2,6	8E-03	1,1	8E-01	1,9	5E-02	0,9	5E-01
AT5G44210	ERF9	2,7	1E-05	1,3	2E-01	1,3	1E-01	1,3	4E-02
AT1G04310	ERS2 (ETHYLENE RESPONSE SENSOR 2)	3,3	9E-06	1,1	7E-01	1,0	1E+00	0,9	3E-01
AT1G28370	ERF11	3,1	2E-06	1,3	1E-01	1,9	1E-03	1,9	1E-03
AT3G23150	ETR2 (ethylene response 2)	4,3	7E-07	1,3	2E-01	1,0	9E-01	1,1	5E-01
AT4G37150	MES9 (METHYL ESTERASE 9)	2,4	8E-04	0,8	4E-01	1,2	5E-01	1,3	4E-01
AT3G25780	AOC3 (ALLENE OXIDE CYCLASE 3)	3,0	2E-04	0,9	6E-01	1,7	4E-02	0,9	6E-01
AT5G56300	GAMT2 (GIBBERELLIC ACID METHYLTRANSFERASE 2)	2,2	1E-02	0,8	5E-01	0,7	1E-01	0,9	8E-01
Major CHO metabolism									
AT4G15210	BAM5 (BETA-AMYLASE 5)	6,4	4E-02	4,9	7E-02	0,7	7E-01	2,3	2E-01
Stress									
AT3G47540	chitinase	2,1	2E-02	0,8	4E-01	1,5	2E-01	0,6	4E-02
AT2G43620	chitinase	3,0	7E-03	0,5	5E-02	0,8	6E-01	1,1	6E-01
AT4G19810	glycosyl hydrolase family 18 protein	2,6	3E-03	1,0	9E-01	1,6	1E-01	1,5	1E-01
AT3G04720	PR4 (PATHOGENESIS-RELATED 4)	2,6	7E-05	2,0	2E-03	1,7	1E-02	1,8	4E-03
AT2G15120 AT2G15220	pseudogene, disease-resistance family protein	2,6	4E-05	1,7	1E-02	1,8	4E-03	1,1	4E-01
AT5G47910	RBOHD (RESPIRATORY BURST OXIDASE HOMOLOGUE D)	2,6	1E-06	1,7	9E-04	1,9	2E-04	2,0	3E-04
AT2G32680	AtRLP23	2,3	2E-02	0,8	6E-01	1,8	8E-02	2,0	9E-04
AT4G04220	AtRLP46	2,0	1E-02	0,5	3E-02	0,7	2E-01	0,9	3E-01
AT1G58170	disease resistance-responsive protein-related	2,9	4E-04	1,1	6E-01	1,2	5E-01	1,2	2E-01
AT1G64160	disease resistance-responsive family protein	2,4	5E-05	1,4	4E-02	1,6	1E-02	0,9	6E-01
AT4g13900 At4g13920	AtRLP49	2,4	4E-03	0,5	4E-02	1,4	2E-01	1,0	8E-01
AT1G73260	trypsin and protease inhibitor family protein	3,6	1E-03	1,3	5E-01	1,9	8E-02	1,6	1E-01
AT3G01420	DOX1; lipoxygenase	2,3	3E-02	0,5	8E-02	0,8	6E-01	0,5	2E-02
AT5G52640	ATHSP90.1 (HEAT SHOCK PROTEIN 90.1)	2,4	3E-02	0,5	1E-01	1,6	3E-01	0,7	2E-01
AT3G12580	HSP70 (heat shock protein 70)	3,2	6E-03	1,0	9E-01	2,1	5E-02	1,0	1E+00
AT5G38940 AT5G38930	manganese ion binding / nutrient reservoir	2,4	1E-03	1,5	1E-01	1,7	4E-02	1,2	3E-01
Redox regulation									
AT2G16060	AHB1 (ARABIDOPSIS HEMOGLOBIN 1)	2,5	3E-03	1,1	6E-01	5,5	1E+00	0,9	3E-01
Polyamine metabolism									
AT4G34710	ADC2 (ARGININE DECARBOXYLASE 2)	2,1	7E-04	0,8	4E-01	1,6	2E-02	0,8	2E-01
Nucleotide metabolism									
AT2G19570	CDA1 (CYTIDINE DEAMINASE 1)	2,6	1E-04	0,8	4E-01	1,3	2E-01	0,9	2E-01
Biodegradation of xenobiotics									
AT1G49660	AtCXE5 (Arabidopsis thaliana carboxyesterase 5)	2,3	4E-07	1,2	6E-02	1,1	4E-01	1,3	1E-02
AT1G80160	lactoylglutathione lyase family protein	2,5	7E-03	0,7	3E-01	1,6	2E-01	0,8	5E-01
C1-metabolism									
AT2G21550	bifunctional dihydrofolate reductase-thymidylate synthase	2,0	5E-05	1,6	4E-03	1,8	5E-04	2,0	3E-04
AT5G14780	FDH (FORMATE DEHYDROGENASE)	2,2	2E-03	1,0	1E+00	1,2	4E-01	1,1	1E-01
Misc									
AT2G34500	CYP710A1	3,1	4E-02	0,6	3E-01	1,7	3E-01	0,3	3E-03
AT4G37370	CYP81D8	2,3	4E-02	0,5	1E-01	1,9	1E-01	0,3	1E-03
AT4G37430	CYP91A2	2,1	5E-03	0,7	2E-01	1,0	9E-01	0,9	6E-01

Cluster III (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Misc		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT4G37310	CYP81H1	2,0	4E-05	1,0	1E+00	1,3	4E-02	1,1	6E-01
AT5G36220	CYP81D1	3,0	3E-07	1,3	1E-01	1,1	4E-01	1,3	1E-02
AT5G05340	peroxidase	3,4	5E-04	1,1	9E-01	1,3	3E-01	1,2	5E-01
AT3G49120 AT3G49110	PRXCB (PEROXIDASE CB)	2,0	6E-05	1,0	8E-01	1,6	2E-03	1,5	2E-02
AT5G64120	peroxidase	7,1	5E-06	0,8	5E-01	0,9	8E-01	1,1	7E-01
AT3G17790	PAP17; acid phosphatase/ phosphatase/ protein serine/threonine phosphatase	2,1	5E-04	0,9	5E-01	1,2	3E-01	0,9	3E-01
AT5G18470	lectin family protein	3,4	2E-03	0,9	7E-01	2,0	6E-02	1,6	8E-03
AT5G53870	plastocyanin-like domain-containing protein	2,2	2E-02	0,8	4E-01	1,0	9E-01	1,7	2E-02
AT4G15490	UGT84A3	2,9	3E-03	1,1	8E-01	2,6	5E-03	1,1	8E-01
AT4G15550	IAGLU (INDOLE-3-ACETATE BETA-D-GLUCOSYLTRANSFERASE)	2,6	9E-04	0,9	8E-01	1,8	2E-02	1,7	4E-03
AT5G12890	UDP-glucoronosyl/UDP-glucosyl transferase family protein	2,0	2E-06	1,1	6E-01	1,4	3E-03	1,3	4E-02
AT4G12480	pEARLI 1	3,1	9E-03	0,9	9E-01	1,5	3E-01	1,9	1E-02
AT3G18280	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2,2	6E-04	0,8	2E-01	1,1	6E-01	0,9	6E-01
AT1G62790	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2,4	3E-05	1,0	9E-01	1,6	7E-03	1,0	9E-01
AT3G04000	short-chain dehydrogenase/reductase (SDR) family protein	2,1	3E-03	0,8	4E-01	1,5	6E-02	0,4	3E-03
AT3G60140	DIN2 (DARK INDUCIBLE 2)	2,5	4E-02	1,5	3E-01	1,0	1E+00	0,6	1E-01
AT4G27830	BGLU10 (BETA GLUCOSIDASE 10)	2,4	4E-05	0,8	3E-01	1,8	2E-03	0,9	2E-01
AT3G57240	BG3 (BETA-1,3-GLUCANASE 3)	2,6	7E-03	0,3	4E-03	1,7	1E-01	0,8	7E-02
AT2G27500	glycosyl hydrolase family 17 protein	2,5	2E-03	1,6	7E-02	1,9	2E-02	1,1	6E-01
AT1G12200	flavin-containing monooxygenase family protein	2,0	7E-03	0,7	1E-01	2,0	9E-03	0,8	1E-01
AT4G12290 AT4G12280	copper amine oxidase family protein	2,1	1E-03	0,7	4E-02	1,6	3E-02	1,2	4E-01
AT2G17720	oxidoreductase	2,2	1E-04	0,9	6E-01	1,8	2E-03	1,0	9E-01
AT1G26420	FAD-binding domain-containing protein	2,4	3E-02	0,5	1E-01	1,9	1E-01	0,9	4E-01
AT4G20830	FAD-binding domain-containing protein	2,2	5E-03	0,7	1E-01	1,8	2E-02	1,0	1E+00
AT2G29350	SAG13; alcohol dehydrogenase/ oxidoreductase	6,3	2E-03	1,1	9E-01	2,5	9E-02	1,1	8E-01
AT5G44390	FAD-binding domain-containing protein	2,9	3E-04	1,2	6E-01	1,3	3E-01	1,4	3E-02
AT1G69920	ATGSTU12	2,0	1E-03	1,5	5E-02	1,5	4E-02	1,3	2E-01
AT2G29460	ATGSTU4	7,0	1E-03	0,9	8E-01	2,9	5E-02	0,7	2E-01
AT2G29470	ATGSTU3	9,3	8E-05	1,1	8E-01	1,5	4E-01	0,9	7E-01
AT2G29440	ATGSTU6	3,2	3E-05	1,8	1E-02	1,5	9E-02	1,6	9E-03
RNA									
AT4G16265	NRPB9B	2,1	3E-04	1,6	9E-03	1,7	4E-03	1,6	3E-03
AT5G58620	zinc finger (CCCH-type) family protein	2,1	3E-05	0,9	4E-01	1,9	2E-04	1,5	1E-02
AT5G53980	ATHB52 (ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 52)	2,2	2E-04	1,1	6E-01	0,3	2E-06	1,0	1E+00
AT1G66390	MYB90	3,9	3E-03	0,5	9E-02	0,9	7E-01	0,6	2E-01
AT2G47190	MYB2	3,2	2E-04	1,1	7E-01	1,6	9E-02	1,2	3E-01
AT5G13330	Rap2.6L (related to AP2 6L)	3,1	3E-03	0,6	1E-01	1,7	1E-01	0,5	8E-04
AT3G25730	AP2 domain-containing transcription factor	2,1	3E-05	1,1	7E-01	1,6	4E-03	1,5	5E-03
AT1G44830	AP2 domain-containing transcription factor TINY	4,1	7E-06	1,0	9E-01	0,6	3E-02	0,8	3E-01
AT1G53170	ERF8	2,2	1E-06	1,5	1E-03	1,5	2E-03	1,8	1E-03
AT4G39780	AP2 domain-containing transcription factor	2,3	1E-07	1,6	2E-04	1,7	6E-05	2,1	5E-04
AT4G23810	WRKY53	2,3	4E-02	0,5	6E-02	1,1	8E-01	0,7	2E-02
AT2G38470	WRKY33	2,0	2E-02	0,7	3E-01	1,7	7E-02	0,6	3E-02
AT5G13080	WRKY75	5,5	7E-03	1,1	9E-01	2,1	2E-01	0,9	7E-01
AT1G80840	WRKY40	5,2	4E-04	0,8	5E-01	1,7	2E-01	0,8	3E-01
AT1G75390 AT1G75388	CPuORF5 (Conserved peptide upstream open reading frame 5)	3,1	3E-06	1,3	1E-01	1,0	8E-01	1,2	1E-01

Cluster III (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
RNA		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT3G02550	LBD41 (LOB DOMAIN-CONTAINING PROTEIN 41)	2,2	4E-04	1,5	5E-02	1,6	2E-02	1,2	3E-02
AT1G10585	transcription factor	9,1	2E-04	0,6	3E-01	1,9	2E-01	0,3	2E-03
Protein									
AT5G38640 AT2G44070	eukaryotic translation initiation factor 2B family protein	2,4	5E-06	1,4	3E-02	1,7	2E-03	1,7	4E-03
AT4G03320	tic20-IV (TRANSLOCON AT THE INNER ENVELOPE MEMBRANE OF CHLOROPLASTS 20-IV)	3,4	1E-06	1,2	3E-01	1,95	1E-03	1,5	2E-02
AT1G29330	ERD2 (ENDOPLASMIC RETICULUM RETENTION DEFECTIVE 2)	2,7	6E-07	1,1	4E-01	1,96	1E-04	1,0	9E-01
AT1G61370	S-locus lectin protein kinase family protein	2,5	5E-03	1,0	1E+00	1,9	5E-02	1,0	9E-01
AT5G14640	SK13 (SHAGGY-LIKE KINASE 13)	2,2	3E-04	1,1	6E-01	1,2	3E-01	1,2	2E-01
AT5G65600	legume lectin family protein	2,3	2E-04	1,3	1E-01	1,9	2E-03	1,6	5E-02
AT5G03730	CTR1 (CONSTITUTIVE TRIPLE RESPONSE 1)	2,3	8E-05	1,0	1E+00	1,1	7E-01	1,0	9E-01
AT1G72540	protein kinase	2,6	2E-03	1,1	8E-01	1,99	2E-02	1,1	5E-01
AT2G16740	UBC29 (ubiquitin-conjugating enzyme 29)	2,0	2E-03	1,4	1E-01	1,0	9E-01	0,9	4E-01
AT1G63840	zinc finger (C3HC4-type RING finger) family protein)	2,7	1E-03	0,7	1E-01	1,7	5E-02	0,8	7E-02
AT1G15670	kelch repeat-containing F-box family protein	2,6	3E-02	0,8	6E-01	1,4	4E-01	0,8	1E-01
AT2G27310	F-box family protein	2,1	5E-03	1,0	9E-01	1,4	1E-01	1,0	9E-01
AT1G24140	matrixin family protein	2,3	5E-03	0,7	2E-01	1,7	7E-02	1,0	8E-01
AT1G43910	AAA-type ATPase family protein	3,6	2E-03	0,4	3E-02	1,0	1E+00	0,5	5E-03
Signalling									
AT5G25930	leucine-rich repeat family protein	2,1	2E-02	0,8	5E-01	1,6	1E-01	0,9	7E-01
AT4G28490	HAE (HAESA)	2,6	2E-03	0,6	6E-02	1,6	1E-01	0,7	3E-02
AT1G09970	LRR XI-23	3,0	3E-05	0,9	5E-01	1,7	1E-02	1,0	1E+00
AT4G23190	CRK11 (CYSTEINE-RICH RLK11)	2,3	4E-03	1,0	9E-01	1,7	5E-02	1,6	9E-03
AT1G61380	SD1-29 (S-DOMAIN-1 29)	2,2	2E-04	0,8	2E-01	1,3	2E-01	1,0	1E+00
AT3G55950	CCR3 (ARABIDOPSIS THALIANA CRINKLY4 RELATED 3)	2,1	8E-03	0,7	2E-01	1,5	1E-01	0,9	6E-01
AT3G09010	protein kinase family protein	2,1	3E-02	0,8	5E-01	1,4	3E-01	0,5	5E-03
AT3G24982	protein binding	2,2	6E-03	1,1	8E-01	1,9	2E-02	1,6	3E-02
AT1G07390	AtRLP1	2,3	6E-06	0,9	6E-01	1,1	6E-01	1,1	5E-01
AT3G50770	calmodulin-related protein	2,2	4E-02	1,2	7E-01	2,0	6E-02	1,6	9E-02
AT2G15760	calmodulin-binding protein	2,1	3E-03	1,3	3E-01	1,4	1E-01	1,2	8E-02
AT4G18430	AtRABA1e (Arabidopsis Rab GTPase homolog A1e)	2,3	3E-02	0,6	2E-01	1,6	2E-01	0,6	3E-02
AT1G73500	MKK9	2,2	4E-03	1,4	2E-01	1,8	2E-02	1,6	2E-03
AT1G51660	ATMKK4	2,0	1E-04	0,8	2E-01	1,3	6E-02	1,0	8E-01
AT2G33130	RALFL18 (ralf-like 18)	3,1	2E-06	1,6	1E-02	1,6	1E-02	2,1	7E-04
Development									
AT4G02380	SAG21 (SENESCENCE-ASSOCIATED GENE 21)	3,3	3E-04	0,8	6E-01	1,6	9E-02	0,9	4E-01
AT1G52890	ANAC019 (Arabidopsis NAC domain containing protein 19)	2,3	5E-02	0,5	9E-02	0,9	8E-01	0,5	6E-02
AT4G22530	embryo-abundant protein-related	2,2	3E-02	0,6	2E-01	1,6	2E-01	0,5	2E-03
AT4G08300	nodulin MtN21 family protein	3,0	1E-02	1,3	6E-01	1,0	1E+00	1,0	9E-01
AT1G69490	NAP (NAC-like, activated by AP3/PI)	5,9	9E-03	1,1	9E-01	2,0	3E-01	1,0	8E-01
AT5G39610	ATNAC6 (ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN 6)	4,1	2E-03	1,0	9E-01	1,4	4E-01	0,9	2E-01
AT3G01930	nodulin family protein	2,1	1E-04	1,8	9E-04	2,0	3E-04	2,5	4E-04
AT2G22860	ATPSK2 (PHYTOSULFOKINE 2 PRECURSOR)	4,8	2E-05	0,6	1E-01	1,2	5E-01	0,7	1E-02
AT5G59450	scarecrow-like transcription factor 11 (SCL11)	2,5	9E-07	1,3	7E-02	1,6	1E-03	1,5	4E-03
Transport									
AT3G51860	CAX3 (CATION EXCHANGER 3)	2,1	2E-02	1,1	7E-01	1,7	9E-02	2,1	1E-02
AT1G59740	proton-dependent oligopeptide transport (POT) family protein	2,2	3E-03	1,0	1E+00	1,2	5E-01	1,2	3E-01
AT5G13490	AAC2 (ADP/ATP carrier 2)	2,0	2E-02	0,5	3E-02	1,6	1E-01	0,6	2E-02
AT5G47560	TDT (TONOPLAST DICARBOXYLATE TRANSPORTER)	2,4	1E-05	1,4	4E-02	1,96	2E-04	1,7	2E-03
AT2G47800	ATMRP4	3,0	4E-03	0,5	7E-02	1,3	4E-01	0,6	2E-04

Cluster III (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Transport		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G31820	amino acid permease family protein	2,0	3E-04	0,9	7E-01	1,6	1E-02	1,3	6E-02
AT5G63850	AAP4	2,5	1E-04	1,7	9E-03	1,5	4E-02	1,5	2E-03
AT1G23090	AST91 (SULFATE TRANSPORTER 91)	2,6	2E-06	1,5	1E-02	1,9	2E-04	2,0	3E-03
AT3G15990	SULTR3;4 (SULFATE TRANSPORTER 3;4)	3,6	1E-06	1,2	3E-01	1,95	2E-03	1,4	8E-02
AT1G61800	GPT2; antiporter/ glucose-6-phosphate transmembrane transporter	2,9	3E-02	0,5	1E-01	1,1	9E-01	0,4	1E-02
AT4G27970	SLAH2 (SLAC1 HOMOLOGUE 2)	2,3	2E-06	1,8	9E-05	1,5	3E-03	1,3	3E-02
AT1G71140	MATE efflux family protein	2,5	3E-02	0,7	3E-01	1,8	2E-01	0,4	2E-03
AT2G17500	auxin efflux carrier family protein	2,8	7E-03	0,6	1E-01	1,5	2E-01	0,4	2E-03
AT1G75170	SEC14 cytosolic factor family protein	2,2	4E-03	0,7	9E-02	1,3	3E-01	0,8	1E-01
AT1G33110	MATE efflux family protein	2,2	7E-04	1,0	1E+00	1,4	8E-02	0,7	2E-02
AT5G52450	MATE efflux protein-related	2,4	2E-06	1,2	2E-01	1,4	1E-02	1,0	1E+00
Not assigned									
AT3G28270	unknown protein	2,6	5E-02	1,1	9E-01	0,4	7E-02	0,8	9E-02
AT1G65690	harpin-induced protein-related	2,4	3E-02	0,7	3E-01	1,8	1E-01	0,9	7E-01
AT5G58770	dehydrodolichyl diphosphate synthase	3,4	3E-02	0,9	8E-01	1,2	8E-01	1,0	1E+00
AT5G02590	chloroplast lumen common family protein	2,0	2E-02	0,8	5E-01	1,5	1E-01	1,0	9E-01
AT1G63720	hydroxyproline-rich glycoprotein family protein	2,1	2E-02	0,7	2E-01	1,7	6E-02	0,7	2E-02
AT1G67360	rubber elongation factor (REF) family protein	2,1	8E-03	0,7	2E-01	1,0	1E+00	0,8	9E-02
AT5G27760	hypoxia-responsive family protein	2,1	3E-03	1,2	4E-01	1,8	1E-02	0,9	5E-01
AT3G22160	VQ motif-containing protein	2,7	8E-04	0,7	2E-01	1,7	4E-02	1,0	9E-01
AT5G17780	hydrolase, alpha/beta fold family protein	2,0	6E-04	1,2	3E-01	0,8	1E-01	1,4	5E-02
AT5G22460	esterase/lipase/thioesterase family protein	2,9	4E-04	1,6	8E-02	0,8	3E-01	0,9	5E-01
AT4G39955	hydrolase, alpha/beta fold family protein	3,1	8E-06	1,1	5E-01	1,6	2E-02	1,0	9E-01
AT1G72800	nuM1-related	3,5	9E-09	1,3	5E-02	1,5	5E-03	1,0	8E-01
AT1G23040	hydroxyproline-rich glycoprotein family protein	2,4	7E-04	0,7	6E-02	1,2	5E-01	0,9	3E-01
AT1G13340	unknown protein	2,2	4E-02	1,1	9E-01	1,9	1E-01	0,8	5E-01
AT1G76960	unknown protein	2,5	3E-02	0,8	6E-01	1,7	2E-01	1,6	1E-01
AT4G19370	unknown protein	2,6	2E-02	1,4	4E-01	1,9	1E-01	0,7	3E-01
AT1G02470	unknown protein	2,5	2E-02	0,5	9E-02	1,2	7E-01	0,5	1E-02
AT3G26440	unknown protein	2,4	1E-02	0,7	3E-01	1,6	1E-01	0,6	2E-02
AT1G79270	ECT8 (evolutionarily conserved C-terminal region 8)	2,0	8E-03	0,7	2E-01	1,1	7E-01	0,7	1E-01
AT1G65500	unknown protein	2,2	8E-03	0,8	5E-01	0,9	7E-01	0,8	2E-01
AT1G49470	unknown protein	2,0	8E-03	1,0	9E-01	1,2	5E-01	0,9	5E-01
AT1G10140	unknown protein	3,0	5E-03	1,3	5E-01	1,8	1E-01	1,1	6E-01
AT3G10320	transferase	2,5	5E-03	1,0	1E+00	1,7	1E-01	0,9	6E-01
AT1G19180	JAZ1	2,3	3E-03	1,1	7E-01	1,4	2E-01	0,9	6E-01
AT4G18630	unknown protein	2,1	3E-03	0,7	2E-01	1,2	5E-01	1,1	4E-01
AT4G33560	unknown protein	2,5	2E-03	1,1	7E-01	1,7	6E-02	1,3	2E-01
AT3G46110	unknown protein	2,1	1E-03	1,1	7E-01	1,3	2E-01	1,1	6E-01
AT2G25625	unknown protein	2,4	8E-04	1,1	8E-01	1,0	8E-01	0,9	7E-01
AT1G28520	VOZ1 (VASCULAR PLANT ONE ZINC FINGER PROTEIN)	2,0	7E-04	0,9	5E-01	1,1	6E-01	0,9	1E-01
AT4G26950	unknown protein	2,1	4E-04	1,5	4E-02	1,6	1E-02	1,3	3E-02
AT2G25680	MOT1 (molybdate transporter 1)	2,1	2E-04	1,9	1E-03	1,2	4E-01	2,0	6E-05
AT1G16950	unknown protein	3,0	2E-04	1,0	1E+00	1,9	1E-02	1,7	4E-02
AT1G19380	unknown protein	2,1	2E-04	1,3	2E-01	1,4	3E-02	1,7	1E-03
AT4G32460	unknown protein	2,5	6E-05	1,2	4E-01	0,5	1E-03	0,7	2E-01
AT4G21310	unknown protein	2,8	4E-05	1,0	8E-01	0,8	4E-01	0,7	5E-02
AT2G44080	ARL (ARGOS-LIKE)	4,2	3E-05	1,1	7E-01	1,1	7E-01	1,1	1E-01
AT4G16670	phosphoinositide binding	2,6	2E-05	0,8	3E-01	1,1	5E-01	1,0	1E+00
AT2G32240	unknown protein	2,7	3E-07	1,8	2E-04	1,9	7E-05	1,5	2E-03
AT1G53180	unknown protein	3,2	3E-07	1,9	4E-04	1,3	8E-02	1,2	1E-01

Cluster III (up)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
Not assigned		x-fold ind.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT3G59900	ARGOS (AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE)	13,6	2E-09	1,0	8E-01	0,8	3E-01	0,9	4E-01
AT2G41230	unknown protein	70,3	3E-13	1,6	7E-02	1,6	7E-02	2,3	3E-03
AT4G10955 AT4G10960	UGE5 (UDP-D-glucose/UDP-D-galactose 4-epimerase 5)	2,3	4E-04	0,7	1E-01	1,1	5E-01	0,7	1E-02
Glycolysis									
AT2G17280	phosphoglycerate/bisphosphoglycerate mutase family protein	2,9	3E-06	1,6	6E-03	1,7	3E-03	1,7	2E-04
AT1G74030	enolase	2,1	4E-06	1,8	9E-05	1,8	9E-05	1,6	4E-04
AT1G54100	ALDH7B4 (Aldehyde Dehydrogenase 7B4)	2,0	7E-03	1,0	1E+00	1,7	4E-02	1,1	6E-01
Gluconeogenese									
AT4G15530	PPDK (pyruvate orthophosphate dikinase)	2,3	1E-04	0,8	2E-01	1,2	4E-01	1,0	8E-01
OPP									
AT2G27510	ATFD3 (ferredoxin 3)	2,1	3E-05	1,3	7E-02	1,7	1E-03	1,3	2E-02
AT4G05390	ATRFNR1 (ROOT FNR 1)	2,2	4E-06	1,2	2E-01	1,6	1E-03	1,2	7E-02
TCA									
AT1G23730	BCA3 (BETA CARBONIC ANHYDRASE 4)	3,0	9E-06	1,3	2E-01	1,1	7E-01	1,0	9E-01
Mitochondrial electron transport									
AT4G05020	NDB2 (NAD(P)H dehydrogenase B2)	2,5	4E-03	0,5	2E-02	1,7	6E-02	0,5	2E-03
AT1G32350	AOX1D (alternative oxidase 1D)	3,3	4E-03	0,7	4E-01	1,6	2E-01	0,8	2E-01
AT4G10040	CYTC-2 (cytochrome c-2)	2,8	6E-05	1,0	9E-01	1,5	4E-02	1,1	5E-01

Table S5 List of repressed genes (2-fold, p-value < 0,05) in ACC-treated wild-type plants.

ACC repressed genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
Photosystem									
AT5G45040	Cytochromes c6 (ATC6)	2,4	6E-05	1,3	2E-01	0,7	5E-02	0,9	2E-01
Cell wall									
AT1G23480	ATCSLA03 (CELLULOSE SYNTHASE-LIKE A3)	2,0	6E-04	1,3	1E-01	0,9	5E-01	0,8	3E-02
AT1G03870	FLA9 (FASCICLIN-LIKE ARABINOOGALACTAN 9)	2,5	8E-06	1,2	3E-01	1,0	8E-01	1,0	1E+00
AT5G65390	AGP7	2,2	2E-03	1,2	5E-01	0,5	7E-03	0,6	3E-02
AT1G55330	AGP21	2,1	1E-03	2,3	5E-04	0,5	7E-03	0,5	3E-04
AT3G52840	BGAL2 (beta-galactosidase 2)	2,0	5E-03	1,4	1E-01	0,7	7E-02	1,2	1E-01
AT3G15720	glycoside hydrolase family 28 protein	2,8	1E-03	1,3	3E-01	0,7	1E-01	0,6	6E-02
AT4G23820	glycoside hydrolase family 28 protein	2,3	9E-05	1,7	8E-03	0,8	3E-01	1,0	7E-01
AT3G62110	glycoside hydrolase family 28 protein	2,0	4E-04	1,2	2E-01	0,8	3E-01	1,1	4E-01
AT2G40610	ATEXPA8 (ARABIDOPSIS THALIANA EXPANSIN A8)	2,9	2E-02	1,6	3E-01	0,6	2E-01	0,6	4E-02
AT3G29030	EXPA5 (EXPANSIN A5)	2,7	7E-03	1,9	6E-02	0,6	2E-01	0,8	2E-01
AT4G03210	XTH9 (XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 9)	2,5	8E-05	1,7	9E-03	1,0	9E-01	1,1	3E-01
AT3G45970	ATEXLA1 (ARABIDOPSIS THALIANA EXPANSIN-LIKE A1)	2,3	3E-03	0,9	7E-01	1,2	5E-01	1,3	5E-02
AT1G20190	ATEXPA11 (ARABIDOPSIS THALIANA EXPANSIN 11)	2,2	2E-03	1,6	5E-02	0,4	3E-04	0,5	2E-03
AT5G47500	pectinesterase family protein	3,4	4E-03	1,1	8E-01	0,3	4E-03	0,3	4E-03
Lipid metabolism									
AT4G34250	KCS16 (3-KETOACYL-COA SYNTHASE 16)	2,7	6E-05	1,0	8E-01	0,8	2E-01	1,1	5E-01
AT2G15090	KCS8 (3-KETOACYL-COA SYNTHASE 8)	2,6	2E-03	1,6	1E-01	1,0	9E-01	1,0	9E-01
AT1G75960	AMP-binding protein	2,2	3E-04	1,3	2E-01	0,9	7E-01	1,1	7E-01
AT1G06100	fatty acid desaturase family protein	3,0	1E-04	1,0	9E-01	0,5	9E-03	0,8	3E-01
AT1G06360 AT1G06350	fatty acid desaturase family protein	2,3	9E-04	1,7	3E-02	0,6	2E-02	0,7	4E-03
AT4G00400	GPAT8 (glycerol-3-phosphate acyltransferase 8)	2,3	1E-03	2,2	2E-03	0,8	3E-01	1,1	6E-01
Amino acid metabolism									
AT1G62800	ASP4 (ASPARTATE AMINOTRANSFERASE 4)	2,3	9E-07	1,2	2E-01	0,6	2E-04	0,8	7E-02
AT3G22740	HMT3; homocysteine S-methyltransferase	4,5	1E-05	1,2	4E-01	0,7	1E-01	1,1	3E-01
AT3G19710	BCAT4 (BRANCHED-CHAIN AMINOTRANSFERASE4)	4,3	3E-03	5,3	9E-04	0,6	2E-01	2,1	3E-03
AT1G31180 AT5G14200	3-isopropylmalate dehydrogenase	2,5	8E-03	1,7	1E-01	1,0	9E-01	1,6	2E-02
AT1G69040	ACR4 (ACT REPEAT 4)	2,2	9E-04	1,4	9E-02	0,8	3E-01	1,0	8E-01
Metal handling									
AT5G04950	NAS1 (NICOTIANAMINE SYNTHASE 1)	3,4	3E-03	1,4	4E-01	0,7	4E-01	1,8	2E-02
AT5G50740	metal ion binding	2,5	8E-04	1,6	5E-02	0,5	5E-03	0,5	5E-03
AT5G50740	metal ion binding	2,5	3E-04	1,8	1E-02	0,5	3E-03	0,6	2E-03
Secondary metabolism									
AT1G78970	LUP1 (LUPEOL SYNTHASE 1)	2,7	7E-05	1,1	6E-01	0,2	5E-07	0,3	1E-05
AT1G65860	FMO GS-OX1 (FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 1)	4,8	4E-04	2,3	3E-02	0,5	6E-02	1,1	7E-01
AT1G62560	FMO GS-OX3 (FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 3)	3,5	3E-03	1,6	2E-01	0,6	1E-01	1,4	1E-01
AT4G03060	AOP2 (ALKENYL HYDROXALKYL PRODUCING 2)	8,4	3E-06	1,5	2E-01	0,3	6E-04	0,8	3E-01
AT5G23010	MAM1 (METHYLTHIOALKYLMALATE SYNTHASE 1)	3,8	2E-03	3,8	2E-03	0,6	2E-01	1,7	6E-03
AT3G58990	aconitase C-terminal domain-containing protein	3,4	9E-03	3,1	1E-02	0,7	4E-01	1,7	3E-02
AT2G43100	aconitase C-terminal domain-containing protein	2,2	4E-02	3,0	7E-03	0,7	4E-01	2,0	2E-02
AT1G16410 AT1G16400	CYP79F1	4,5	4E-03	4,5	5E-03	0,7	5E-01	1,9	2E-03
AT4G13770	CYP83A1 (CYTOCHROME P450 83A1)	2,2	8E-03	1,9	3E-02	0,7	2E-01	1,6	7E-03
AT1G74090	SOT18 (DESULFO-GLUCOSINOLATE SULFOTRANSFERASE 18)	2,2	3E-03	1,7	3E-02	0,6	7E-02	1,2	1E-01
AT5G07690	ATMYB29	7,3	2E-07	1,1	7E-01	0,3	2E-04	0,7	4E-02

ACC repressed genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT5G61420	MYB28	2,5	4E-04	1,0	9E-01	0,4	2E-04	0,6	3E-02
AT5G60890	MYB34	2,6	2E-03	1,5	1E-01	0,4	3E-03	0,5	1E-02
AT4G12030	bile acid:sodium symporter family protein	4,2	2E-04	2,2	3E-02	0,5	6E-02	1,2	3E-01
AT2G18560 AT2G18570	UDP-glucoronosyl/UDP-glucosyl transferase family protein	2,2	2E-05	0,9	5E-01	0,8	9E-02	0,8	3E-01
Hormone metabolism									
AT2G45400	BEN1	2,0	5E-04	1,1	6E-01	0,6	2E-02	0,7	7E-03
AT1G29500	auxin-responsive protein	3,4	3E-04	2,0	2E-02	0,5	4E-02	0,6	3E-02
AT2G33830	dormancy/auxin associated family protein	2,9	3E-02	0,9	7E-01	1,0	1E+00	0,9	6E-01
AT3G03840	auxin-responsive protein	2,7	4E-05	1,0	8E-01	0,5	5E-04	0,6	7E-03
AT5G18060	auxin-responsive protein	2,7	8E-04	1,4	2E-01	0,6	7E-02	0,6	2E-03
AT1G29510	SAUR68 (SMALL AUXIN UPREGULATED 68)	2,6	2E-03	2,4	4E-03	0,5	1E-02	0,8	2E-01
AT4G34760	auxin-responsive family protein	2,5	2E-04	1,4	9E-02	0,7	9E-02	0,9	3E-01
AT1G29430 AT5G27780	auxin-responsive family protein	2,3	3E-02	2,5	2E-02	0,7	3E-01	0,9	3E-01
AT5G08330	TCP family transcription factor	2,2	4E-04	1,4	8E-02	0,8	2E-01	0,8	2E-01
AT4G38850	SAUR15 (SMALL AUXIN UPREGULATED 15)	2,2	9E-05	1,5	2E-02	0,8	2E-01	1,0	7E-01
AT1G29440	unknown protein	2,2	3E-03	1,6	6E-02	0,7	8E-02	0,6	8E-03
AT1G29450	auxin-responsive protein	2,1	3E-03	1,5	1E-01	0,6	6E-02	0,7	3E-02
AT1G29460	auxin-responsive protein	2,1	3E-03	1,6	5E-02	0,8	3E-01	1,1	8E-01
AT2G46690	auxin-responsive family protein	2,0	6E-04	1,2	2E-01	0,8	3E-01	1,1	4E-01
AT2G26710	BAS1 (PHYB ACTIVATION TAGGED SUPPRESSOR 1)	2,4	2E-03	1,2	5E-01	0,8	4E-01	0,8	1E-01
AT1G74670	gibberellin-responsive protein	3,9	7E-03	1,8	2E-01	0,6	3E-01	0,8	6E-01
AT1G22690	gibberellin-responsive protein	3,8	3E-06	1,2	5E-01	1,4	1E-01	0,8	3E-01
AT3G16450	jacalin lectin family protein	2,7	2E-04	1,2	3E-01	0,6	3E-02	0,8	5E-02
Tetrapyrrole synthesis									
AT5G54190	PORA; oxidoreductase/ protochlorophyllide reductase	2,5	2E-02	1,3	4E-01	1,0	1E+00	1,2	4E-02
Major CHO metabolism									
AT3G30720	QQS (QUA-QUINE STARCH)	2,1	5E-02	1,1	9E-01	1,0	1E+00	0,8	5E-01
Stress									
AT1G66100	thionin	2,8	1E-03	4,2	3E-05	1,1	6E-01	1,2	4E-01
AT1G70830	MLP28 (MLP-LIKE PROTEIN 28)	2,3	6E-06	1,8	4E-04	0,9	4E-01	1,0	8E-01
AT1G73330	ATDR4	2,1	4E-02	1,4	3E-01	0,9	9E-01	0,9	6E-01
AT1G54050	17.4 kDa class III heat shock protein (HSP17.4-CIII)	2,2	1E-03	1,6	3E-02	0,4	6E-04	0,6	5E-03
AT1G56300	DNAJ heat shock N-terminal domain-containing protein	2,0	2E-02	1,1	7E-01	0,9	7E-01	0,9	2E-01
AT2G42530	COR15B (COLD REGULATED 15B)	3,3	3E-02	2,1	2E-01	0,7	5E-01	1,2	4E-01
AT4G30650	hydrophobic protein	2,1	1E-02	1,5	2E-01	0,8	4E-01	1,0	9E-01
AT5G66590	allergen V5/Tpx-1-related family protein	2,9	2E-05	1,3	2E-01	0,7	1E-01	1,1	3E-01
AT3G50970	LTI30 (LOW TEMPERATURE-INDUCED 30)	2,9	2E-02	0,8	5E-01	1,2	7E-01	0,7	9E-02
AT1G70890	MLP43 (MLP-LIKE PROTEIN 43)	2,5	1E-04	2,1	9E-04	0,7	1E-01	1,3	8E-02
AT1G35260	MLP165 (MLP-LIKE PROTEIN 165)	2,2	1E-06	0,8	8E-02	1,2	9E-02	1,1	4E-01
AT1G69080	universal stress protein (USP) family protein	2,1	6E-05	0,9	7E-01	0,5	9E-05	0,6	5E-04
Redox regulation									
AT3G10520	AHB2 (ARABIDOPSIS HAEMOGLOBIN 2)	2,2	9E-04	1,5	7E-02	1,0	9E-01	1,2	1E-01
AT3G62950	glutaredoxin family protein	2,2	3E-02	1,6	2E-01	1,4	3E-01	1,3	1E-01
Nucleotide metabolism									
AT4G29610	cytidine deaminase	2,2	9E-03	1,1	7E-01	0,8	4E-01	0,9	4E-01
AT1G80050	APT2 (ADENINE PHOSPHORIBOSYL TRANSFERASE 2)	2,2	3E-03	1,2	5E-01	0,8	2E-01	0,8	1E-01
Misc									
AT1G25230	purple acid phosphatase family protein	2,5	3E-02	1,1	8E-01	0,7	4E-01	0,8	2E-01
AT5G63140	ATPAP29 (ARABIDOPSIS THALIANA PURPLE ACID PHOSPHATASE 29)	2,4	1E-02	1,6	2E-01	0,9	6E-01	0,9	3E-01

ACC repressed genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G14700	PAP3 (PURPLE ACID PHOSPHATASE 3)	2,2	4E-04	1,7	8E-03	0,5	4E-04	0,9	7E-01
AT5G45700	NLI interacting factor (NIF) family protein	2,0	3E-03	1,2	4E-01	1,1	7E-01	1,0	1E+00
AT2G39310	JAL22 (JACALIN-RELATED LECTIN 22)	2,9	2E-06	0,9	5E-01	0,5	6E-04	0,6	6E-04
AT5G62360	invertase/pectin methylesterase inhibitor family protein	3,1	2E-02	3,1	2E-02	1,1	9E-01	1,7	5E-02
AT4G31840	plastocyanin-like domain-containing protein	2,1	1E-03	1,1	6E-01	0,8	3E-01	1,0	8E-01
AT5G05960	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	4,0	7E-04	1,1	7E-01	3,6	1E-03	6,1	2E-06
AT2G10940	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	3,2	3E-04	3,8	6E-05	0,5	3E-02	0,5	5E-03
AT5G48490	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	3,2	6E-03	3,8	2E-03	0,6	2E-01	0,9	5E-01
AT1G55260	lipid binding	2,4	2E-02	2,9	6E-03	0,7	3E-01	1,1	6E-01
AT2G45180	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2,0	4E-03	0,9	5E-01	0,7	1E-01	1,0	1E+00
AT4G28780	GDSL-motif lipase/hydrolase family protein	2,8	4E-03	2,4	1E-02	0,6	2E-01	1,0	9E-01
AT5G45950	GDSL-motif lipase/hydrolase family protein	2,4	4E-04	2,0	5E-03	0,6	3E-02	0,8	3E-02
AT1G18650	PDCB3 (PLASMODESMATA CALLOSE-BINDING PROTEIN 3)	2,0	5E-03	2,4	7E-04	0,7	2E-01	1,0	9E-01
AT1G31710	copper amine oxidase	2,3	7E-04	1,7	2E-02	0,8	3E-01	1,1	6E-01
AT2G29310	tropinone reductase	2,3	8E-06	1,4	2E-02	0,6	5E-04	1,1	5E-01
AT3G03190	ATGSTF11	4,9	3E-06	2,2	4E-03	0,5	6E-03	1,2	4E-01
AT1G78370	ATGSTU20	2,8	2E-02	4,4	2E-03	0,6	3E-01	1,4	1E-01
RNA									
AT4G17460	HAT1; DNA binding / transcription factor	2,6	1E-04	1,2	3E-01	0,7	9E-02	0,8	1E-01
AT3G51910	AT-HSFA7A	2,2	6E-04	1,0	9E-01	0,7	6E-02	1,0	9E-01
AT3G46130	MYB111	2,1	3E-03	1,1	7E-01	0,6	2E-02	0,8	4E-01
AT2G21650	MEE3 (MATERNAL EFFECT EMBRYO ARREST 3)	3,6	1E-06	1,1	7E-01	1,2	3E-01	1,0	8E-01
AT1G18330	EPR1 (EARLY-PHYTOCHROME-RESPONSIVE1)	2,4	1E-03	0,8	3E-01	0,9	5E-01	1,0	6E-01
AT1G75250	ATRL6 (ARABIDOPSIS RAD-LIKE 6)	2,1	3E-02	1,4	4E-01	0,9	7E-01	0,8	4E-01
AT1G69690	TCP family transcription factor	2,0	5E-04	1,2	3E-01	0,7	2E-02	0,9	5E-01
AT4G25480	DREB1A (DEHYDRATION RESPONSE ELEMENT B1A)	3,1	1E-02	1,0	9E-01	1,0	1E+00	1,0	8E-01
AT3G58120	BZIP61; DNA binding / transcription activator/ transcription factor	2,7	6E-03	1,6	1E-01	0,6	9E-02	0,4	9E-05
AT1G04240	SHY2 (SHORT HYPOCOTYL 2)	2,9	1E-05	1,3	2E-01	0,6	6E-03	0,7	9E-03
AT1G52830	IAA6 (INDOLE-3-ACETIC ACID 6)	2,1	2E-03	1,0	9E-01	0,7	1E-01	0,8	3E-01
AT3G18960 AT4G01580	transcriptional factor B3 family protein	2,1	2E-04	1,2	2E-01	0,6	9E-03	0,8	8E-03
AT3G48100	ARR5 (ARABIDOPSIS RESPONSE REGULATOR 5)	2,4	9E-04	0,9	5E-01	0,9	5E-01	0,6	4E-03
AT5G39860	PRE1 (PACLOBUTRAZOL RESISTANCE1)	3,0	2E-03	1,3	4E-01	0,5	4E-02	0,5	1E-02
AT2G18300	basic helix-loop-helix (bHLH) family protein	2,9	1E-03	1,0	9E-01	0,6	5E-02	0,8	3E-01
AT5G46690	bHLH071 (beta HLH protein 71)	2,6	2E-03	1,7	7E-02	0,4	6E-03	0,7	7E-02
AT3G05800	transcription factor	2,2	7E-04	1,5	7E-02	0,7	1E-01	0,7	5E-02
AT4G00480	ATMYC1	2,1	2E-06	0,9	3E-01	0,6	5E-04	0,7	3E-03
AT1G68810	basic helix-loop-helix (bHLH) family protein	2,0	4E-04	1,4	4E-02	0,7	4E-02	1,2	3E-01
AT4G23800	high mobility group (HMG1/2) family protein	2,1	5E-04	1,0	9E-01	0,9	6E-01	1,0	8E-01
AT4G04840	ATMSRB6	4,2	2E-06	2,7	2E-04	0,6	6E-02	0,5	2E-04
AT4G04830	ATMSRB5	2,1	2E-03	1,6	4E-02	0,6	3E-02	0,8	3E-01
AT3G61260	DNA-binding family protein / remorin family protein	2,1	2E-02	1,5	2E-01	0,8	4E-01	0,9	6E-01
AT2G42190	unknown protein	2,1	4E-04	0,9	7E-01	0,7	3E-02	0,8	1E-01
AT5G57660	ATCOL5, COL5 | zinc finger (B-box type) family protein	2,8	3E-02	0,8	7E-01	0,9	8E-01	0,7	2E-01
AT5G44560	VPS2.2	2,0	2E-05	1,0	9E-01	0,7	1E-02	0,9	2E-01
AT3G47500	CDF3 (CYCLING DOF FACTOR 3)	2,2	1E-02	1,0	1E+00	0,8	4E-01	0,8	6E-02
AT1G09750	chloroplast nucleoid DNA-binding protein-related	2,4	2E-03	2,0	1E-02	0,9	6E-01	1,0	8E-01
AT4G30410	transcription factor	2,2	9E-03	1,1	8E-01	0,4	6E-03	0,5	7E-03
AT3G48550	unknown protein	2,1	4E-06	1,0	7E-01	0,7	2E-02	0,9	3E-01
AT1G32540	LOL1 (LSD ONE LIKE 1)	2,1	4E-02	1,5	3E-01	0,5	5E-02	0,7	3E-02
AT1G22330	RNA binding / nucleic acid binding / nucleotide binding	2,8	1E-03	1,4	2E-01	0,7	1E-01	0,6	5E-03

ACC repressed genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G22330	RNA binding / nucleic acid binding / nucleotide binding	2,2	1E-02	1,6	1E-01	0,6	1E-01	0,7	3E-02
DNA									
AT5G07460	PMSR2 (PEPTIDEMETHIONINE SULFOXIDE REDUCTASE 2)	2,1	3E-03	1,2	3E-01	0,7	2E-01	1,2	1E-01
AT1G03420	Sadhu4-2 | transposable element gene	2,4	5E-03	1,3	3E-01	0,9	8E-01	1,2	1E-01
AT2G18050	HIS1-3 (HISTONE H1-3)	3,3	9E-03	1,0	9E-01	0,7	4E-01	0,8	6E-01
AT5G44680	methyladenine glycosylase family protein	3,4	4E-05	1,8	2E-02	0,5	5E-03	0,6	6E-03
Protein									
AT3G17170	RFC3 (REGULATOR OF FATTY-ACID COMPOSITION 3)	2,1	3E-03	1,0	8E-01	0,9	5E-01	0,8	2E-01
AT5G52280	protein transport protein-related	2,1	3E-03	1,0	1E+00	0,5	8E-03	0,6	3E-02
AT5G45820	CIPK20 (CBL-INTERACTING PROTEIN KINASE 20)	2,3	5E-02	1,0	1E+00	1,0	1E+00	1,1	3E-01
AT5G57630	CIPK21 (CBL-interacting protein kinase 21)	2,1	3E-02	1,1	7E-01	1,0	1E+00	1,1	6E-01
AT3G23340	ckl10 (Casein Kinase I-like 10)	2,0	1E-05	0,8	2E-01	0,5	2E-05	0,6	4E-03
AT1G56720	protein kinase family protein	2,4	6E-04	1,6	4E-02	0,6	2E-02	0,8	9E-02
AT2G18890	protein kinase family protein	2,1	3E-04	1,0	8E-01	0,6	5E-03	0,7	4E-03
AT5G22920	zinc finger (C3HC4-type RING finger) family protein	3,1	3E-02	1,7	3E-01	1,0	1E+00	0,9	8E-01
AT4G03190	GRH1 (GRR1-LIKE PROTEIN 1)	2,1	4E-03	1,1	7E-01	0,6	7E-02	0,5	2E-03
AT3G23880	F-box family protein	2,1	2E-04	1,9	1E-03	0,8	1E-01	1,5	6E-03
AT4G11320 AT4G11310	cysteine proteinase, putative	4,1	3E-06	1,1	7E-01	0,7	1E-01	0,9	5E-01
AT3G12700	aspartyl protease family protein	2,0	8E-05	1,0	8E-01	0,7	2E-02	0,7	2E-03
AT2G22980	SCPL13	3,2	2E-02	1,5	4E-01	0,5	2E-01	0,7	2E-01
Minor CHO metabolism									
AT1G09350	AtGolS3 (Arabidopsis thaliana galactinol synthase 3)	2,3	4E-02	1,0	1E+00	1,1	8E-01	1,1	2E-01
Signalling									
AT2G43010	PIF4 (phytochrome interacting factor 4)	3,7	1E-02	0,9	8E-01	0,7	5E-01	0,7	9E-02
AT4G29080	PAP2 (PHYTOCHROME-ASSOCIATED PROTEIN 2)	2,1	3E-06	0,8	6E-02	0,7	8E-03	0,8	2E-02
AT3G19850	phototropic-responsive NPH3 family protein	2,1	4E-02	1,0	9E-01	0,6	2E-01	0,9	5E-01
AT1G54820	protein kinase family protein	2,1	1E-03	1,0	9E-01	0,5	6E-03	0,6	1E-03
AT3G49260	iqd21 (IQ-domain 21)	2,4	8E-05	2,1	4E-04	0,6	1E-02	0,8	5E-02
AT4G05520	ATEHD2 (EPS15 HOMOLOGY DOMAIN 2)	2,0	5E-03	1,1	6E-01	0,8	2E-01	0,8	2E-01
AT2G46710	rac GTPase activating protein	2,3	3E-03	1,6	7E-02	0,7	1E-01	0,8	1E-01
AT1G22740	RABG3B; GTP binding	2,1	4E-03	1,5	6E-02	0,7	7E-02	1,0	9E-01
Cell									
AT5G48460	fimbrin-like protein	2,3	4E-05	1,8	2E-03	0,6	8E-03	0,9	4E-01
AT3G50240	KICP-02	2,2	2E-02	1,2	6E-01	0,4	1E-02	0,4	8E-03
AT1G20010	TUB5; structural constituent of cytoskeleton	2,1	3E-04	1,5	2E-02	0,8	2E-01	1,2	2E-01
AT4G33270 AT4G33260	CDC20.1; signal transducer	2,2	1E-02	1,0	1E+00	0,7	2E-01	0,7	8E-02
AT2G44740	CYCP4;1 (cyclin p4;1)	2,1	4E-03	1,1	8E-01	0,7	2E-01	0,9	3E-01
AT2G26760	CYCB1;4 (Cyclin B1;4)	2,0	3E-04	0,9	6E-01	1,0	9E-01	1,1	2E-01
AT1G08560	SYP111 (SYNTAXIN OF PLANTS 111)	2,3	3E-05	1,2	3E-01	0,9	6E-01	1,2	9E-02
Development									
AT5G44020	acid phosphatase class B family protein	3,2	1E-04	2,4	2E-03	0,8	3E-01	1,0	9E-01
AT5G43270	SPL2 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 2)	2,3	2E-02	0,7	3E-01	0,4	4E-03	0,3	6E-03
AT2G33810	SPL3 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3)	2,2	1E-02	1,4	3E-01	0,7	3E-01	0,8	3E-01
AT1G07050	CONSTANS-like protein-related	2,4	4E-06	1,4	3E-02	0,7	3E-02	0,9	6E-01
AT1G44800	nodulin MtN21 family protein	2,2	1E-02	0,7	2E-01	1,3	4E-01	0,6	6E-02
AT3G28130	nodulin MtN21 family protein	2,1	6E-05	1,0	8E-01	0,5	9E-05	0,9	2E-01
AT4G26370	antitermination NusB domain-containing protein	2,1	4E-03	1,7	4E-02	0,9	5E-01	1,1	6E-01
AT3G16690	nodulin MtN3 family protein	2,1	9E-06	1,2	2E-01	1,1	5E-01	1,3	2E-02
Transport									

ACC repressed genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT4G30110	HMA2; cadmium-transporting ATPase	2,5	1E-06	0,9	4E-01	1,2	3E-01	1,2	2E-01
AT1G10970	ZIP4 (ZINC TRANSPORTER 4 PRECURSOR)	2,4	2E-07	0,9	2E-01	0,9	2E-01	1,0	9E-01
AT1G22570	proton-dependent oligopeptide transport (POT) family protein	2,4	7E-03	0,8	6E-01	1,2	5E-01	1,2	1E-01
AT1G01620	PIP1C (PLASMA MEMBRANE INTRINSIC PROTEIN 1C)	2,3	1E-05	1,4	2E-02	0,7	5E-02	1,0	8E-01
AT2G21050	amino acid permease	2,0	1E-03	1,5	5E-02	1,5	5E-02	1,7	3E-03
AT5G10180	AST68	2,0	1E-03	1,1	7E-01	1,0	8E-01	1,0	8E-01
AT2G29650	PHT4;1	2,1	2E-02	1,1	8E-01	0,7	2E-01	0,7	4E-02
Not assigned									
AT3G46490	oxidoreductase	4,6	4E-08	1,4	5E-02	0,3	2E-06	0,4	7E-04
AT3G05900	neurofilament protein-related	3,1	2E-04	2,0	1E-02	0,7	1E-01	0,7	1E-02
AT4G02850	phenazine biosynthesis PhzC/PhzF family protein	3,0	1E-03	1,7	9E-02	0,8	4E-01	0,8	8E-02
AT3G28290 AT3G28300	AT14A	2,8	7E-04	2,7	1E-03	0,6	7E-02	0,8	2E-01
AT1G78450	SOUL heme-binding family protein	2,6	4E-04	1,6	5E-02	0,7	1E-01	1,1	7E-01
AT3G50440	MES10 (METHYL ESTERASE 10)	2,6	2E-06	1,3	1E-01	0,6	3E-03	1,1	2E-01
AT5G04820	OFP13 (ARABIDOPSIS THALIANA OVATE FAMILY PROTEIN 13)	2,5	4E-07	1,1	5E-01	0,7	6E-03	0,8	1E-01
AT1G21440	mutase family protein	2,3	8E-03	2,2	2E-02	0,6	6E-02	0,9	6E-01
AT5G02890	transferase family protein	2,3	5E-03	2,3	4E-03	0,6	3E-02	0,7	4E-03
AT5G51550	EXL3 (EXORDIUM LIKE 3)	2,2	6E-04	1,1	5E-01	0,9	5E-01	1,1	8E-01
AT2G32100	OFP16 (ARABIDOPSIS THALIANA OVATE FAMILY PROTEIN 16)	2,1	5E-02	1,3	5E-01	0,9	8E-01	1,0	9E-01
AT3G53800	armadillo/beta-catenin repeat family protein	2,1	1E-02	1,3	3E-01	0,7	2E-01	0,9	6E-01
AT5G28630	glycine-rich protein	2,7	1E-03	1,0	9E-01	0,9	6E-01	1,2	3E-01
AT5G61660	glycine-rich protein	2,3	1E-03	1,5	8E-02	1,3	2E-01	2,1	2E-04
AT3G02120	hydroxyproline-rich glycoprotein family protein	2,1	3E-03	0,9	8E-01	1,0	8E-01	1,1	7E-01
AT2G24762	AtGDU4 (Arabidopsis thaliana GLUTAMINE DUMPER 4)	4,9	1E-08	0,9	7E-01	1,0	8E-01	1,2	2E-02
AT3G13980	unknown protein	4,4	8E-07	0,9	7E-01	0,7	2E-01	1,1	3E-01
AT1G27030	unknown protein	3,7	2E-06	3,0	2E-05	0,6	4E-02	1,0	7E-01
AT3G45160	unknown protein	3,6	8E-07	1,4	1E-01	0,7	9E-02	1,3	2E-02
AT1G06980	unknown protein	3,3	2E-07	1,0	8E-01	0,7	1E-02	0,8	6E-02
AT5G57785	unknown protein	3,2	3E-05	0,6	2E-02	0,5	5E-03	0,6	1E-02
AT5G16030	unknown protein	3,1	4E-04	1,8	5E-02	0,6	9E-02	0,9	3E-01
AT1G35612	transposable element gene	3,1	2E-02	0,8	7E-01	0,4	7E-02	0,5	2E-02
AT1G78170	unknown protein	3,0	3E-05	1,8	8E-03	0,5	2E-03	0,7	5E-02
AT4G12970	unknown protein	2,9	1E-04	2,5	6E-04	0,6	1E-02	0,8	6E-02
AT1G48330	unknown protein	2,8	6E-05	1,5	5E-02	0,5	3E-03	0,9	2E-01
AT2G16990	tetracycline transporter	2,6	3E-03	1,1	7E-01	0,6	5E-02	0,5	6E-04
AT5G36710 AT5G36800	unknown protein	2,6	2E-04	0,9	6E-01	0,8	4E-01	1,1	5E-01
AT3G50120	unknown protein	2,5	8E-05	0,9	4E-01	0,6	1E-02	0,8	7E-02
AT5G03120	unknown protein	2,3	6E-04	1,5	7E-02	0,6	1E-02	1,0	7E-01
AT5G67390	unknown protein	2,3	5E-04	1,0	8E-01	0,6	1E-02	0,8	7E-02
AT1G65900	unknown protein	2,3	7E-03	1,5	2E-01	0,5	2E-02	0,7	1E-01
AT2G20670	unknown protein	2,3	3E-02	0,8	6E-01	0,8	6E-01	0,7	1E-01
AT1G18620	unknown protein	2,3	3E-02	1,6	2E-01	0,8	6E-01	0,9	5E-01
AT4G23870	unknown protein	2,3	7E-04	1,8	1E-02	1,0	9E-01	1,4	4E-02
AT2G30930	unknown protein	2,2	8E-03	0,9	6E-01	1,0	9E-01	1,0	9E-01
AT4G04330	unknown protein	2,2	7E-04	1,1	8E-01	0,7	6E-02	0,9	3E-01
AT3G02640	unknown protein	2,2	8E-04	1,0	9E-01	0,9	6E-01	1,1	6E-01
AT5G01015	unknown protein	2,2	6E-03	2,1	9E-03	0,5	2E-02	0,8	4E-01
AT1G12080	unknown protein	2,2	7E-04	1,7	2E-02	0,6	1E-02	0,6	6E-04
AT5G01075	beta-galactosidase	2,2	1E-02	1,7	8E-02	0,5	2E-02	0,9	2E-01
AT1G70420	unknown protein	2,2	7E-03	1,2	6E-01	0,7	2E-01	0,9	4E-01

ACC repressed genes in WT		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT5G06270	unknown protein	2,2	1E-03	2,0	3E-03	0,6	5E-02	1,1	2E-01
AT1G12845	unknown protein	2,1	1E-02	2,3	7E-03	0,6	1E-01	1,1	7E-01
AT4G23496	SP1L5 (SPIRAL1-LIKE5)	2,1	5E-03	1,0	9E-01	1,0	9E-01	0,9	5E-01
AT5G62550	unknown protein	2,1	6E-04	0,9	8E-01	0,9	6E-01	0,9	3E-01
AT4G16515	unknown protein	2,1	5E-03	1,5	1E-01	0,6	8E-02	0,7	9E-02
AT2G34510	unknown protein	2,1	3E-03	1,5	6E-02	0,8	3E-01	1,4	3E-03
AT5G35490	MRU1	2,1	7E-05	1,0	9E-01	1,1	5E-01	1,7	1E-02
AT4G15830	unknown protein	2,1	5E-03	1,1	7E-01	1,1	8E-01	1,1	3E-01
AT1G75190	unknown protein	2,1	3E-03	1,2	4E-01	1,0	9E-01	0,9	7E-01
AT3G26960	unknown protein	2,1	3E-02	1,5	2E-01	0,8	4E-01	0,8	2E-01
AT2G42870	PAR1 (PHY RAPIDLY REGULATED 1)	2,1	5E-02	1,2	5E-01	1,1	8E-01	1,1	4E-01
AT4G31730	GDU1 (GLUTAMINE DUMPER 1)	2,0	3E-05	0,9	6E-01	1,0	8E-01	1,0	8E-01
AT5G60400	unknown protein	2,0	9E-04	1,1	5E-01	0,9	4E-01	1,1	7E-01
AT4G22560	unknown protein	2,0	5E-04	1,1	7E-01	0,7	6E-02	1,0	8E-01
AT2G44230	unknown protein	2,0	7E-03	1,9	1E-02	0,8	4E-01	1,0	1E+00
AT5G53020	unknown protein	2,0	3E-03	1,1	6E-01	0,5	4E-03	0,7	1E-01

Table S6 Cluster I genes of ACC-repressed genes according to MarVis analysis.

Cluster I (down)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
Lipid metabolism									
AT4G00400	GPAT8 (glycerol-3-phosphate acyltransferase 8)	2,3	1E-03	2,2	2E-03	0,8	3E-01	1,1	6E-01
Amino acid metabolism									
AT3G19710	BCAT4 (BRANCHED-CHAIN AMINOTRANSFERASE4)	4,3	3E-03	5,3	9E-04	0,6	2E-01	2,1	3E-03
Secondary metabolism									
AT1G65860	FMO GS-OX1 (FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 1)	4,8	4E-04	2,3	3E-02	0,5	6E-02	1,1	7E-01
AT4G03060	AOP2 (ALKENYL HYDROXALKYL PRODUCING 2)	8,4	3E-06	1,5	2E-01	0,3	6E-04	0,8	3E-01
AT5G23010	MAM1 (METHYLTHIOALKYLMALATE SYNTHASE 1)	3,8	2E-03	3,8	2E-03	0,6	2E-01	1,7	6E-03
AT3G58990	aconitase C-terminal domain-containing protein	3,4	9E-03	3,1	1E-02	0,7	4E-01	1,7	3E-02
AT2G43100	aconitase C-terminal domain-containing protein	2,2	4E-02	3,0	7E-03	0,7	4E-01	2,0	2E-02
AT1G16410 AT1G16400	CYP79F1	4,5	4E-03	4,5	5E-03	0,7	5E-01	1,9	2E-03
AT4G12030	bile acid:sodium symporter family protein	4,2	2E-04	2,2	3E-02	0,5	6E-02	1,2	3E-01
Hormone metabolism									
AT1G29510	SAUR68 (SMALL AUXIN UPREGULATED 68)	2,6	2E-03	2,4	4E-03	0,5	1E-02	0,8	2E-01
AT1G29430 AT5G27780	auxin-responsive family protein	2,3	3E-02	2,5	2E-02	0,7	3E-01	0,9	3E-01
Stress									
AT1G66100	thionin	2,8	1E-03	4,2	3E-05	1,1	6E-01	1,2	4E-01
AT1G70890	MLP43 (MLP-LIKE PROTEIN 43)	2,5	1E-04	2,1	9E-04	0,7	1E-01	1,3	8E-02
Misc									
AT5G62360	invertase/pectin methylesterase inhibitor family protein	3,1	2E-02	3,1	2E-02	1,1	9E-01	1,7	5E-02
AT5G48490	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	3,2	6E-03	3,8	2E-03	0,6	2E-01	0,9	5E-01
AT1G55260	lipid binding	2,4	2E-02	2,9	6E-03	0,7	3E-01	1,1	6E-01
AT4G28780	GDSL-motif lipase/hydrolase family protein	2,8	4E-03	2,4	1E-02	0,6	2E-01	1,0	9E-01
AT1G18650	PDCB3 (PLASMODESMATA CALLOSE-BINDING PROTEIN 3)	2,0	5E-03	2,4	7E-04	0,7	2E-01	1,0	9E-01
AT3G03190	ATGSTF11	4,9	3E-06	2,2	4E-03	0,5	6E-03	1,2	4E-01
AT1G78370	ATGSTU20	2,8	2E-02	4,4	2E-03	0,6	3E-01	1,4	1E-01
Signalling									
AT3G49260	iqd21 (IQ-domain 21)	2,4	8E-05	2,1	4E-04	0,6	1E-02	0,8	5E-02
Development									
AT5G44020	acid phosphatase class B family protein	3,2	1E-04	2,4	2E-03	0,8	3E-01	1,0	9E-01
Not assigned									
AT1G21440	mutase family protein	2,3	8E-03	2,2	2E-02	0,6	6E-02	0,9	6E-01
AT5G02890	transferase family protein	2,3	5E-03	2,3	4E-03	0,6	3E-02	0,7	4E-03
AT1G27030	unknown protein	3,7	2E-06	3,0	2E-05	0,6	4E-02	1,0	7E-01
AT4G12970	unknown protein	2,9	1E-04	2,5	6E-04	0,6	1E-02	0,8	6E-02
AT5G01015	unknown protein	2,2	6E-03	2,1	9E-03	0,5	2E-02	0,8	4E-01
AT5G06270	unknown protein	2,2	1E-03	2,0	3E-03	0,6	5E-02	1,1	2E-01
AT1G12845	unknown protein	2,1	1E-02	2,3	7E-03	0,6	1E-01	1,1	7E-01

Table S7 Cluster II genes of ACC-repressed genes according to MarVis analysis.

Cluster II (down)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
Cell wall									
AT1G55330	AGP21	2,1	1E-03	2,3	5E-04	0,5	7E-03	0,5	3E-04
AT1G20190	ATEXPA11 (ARABIDOPSIS THALIANA EXPANSIN 11)	2,2	2E-03	1,6	5E-02	0,4	3E-04	0,5	2E-03
AT5G47500	pectinesterase family protein	3,4	4E-03	1,1	8E-01	0,3	4E-03	0,3	4E-03
Metal handling									
AT5G50740	metal ion binding	2,5	8E-04	1,6	5E-02	0,5	5E-03	0,5	5E-03
Secondary metabolism									
AT5G07690	ATMYB29	7,3	2E-07	1,1	7E-01	0,3	2E-04	0,7	4E-02
AT5G61420	MYB28	2,5	4E-04	1,0	9E-01	0,4	2E-04	0,6	3E-02
AT5G60890	MYB34	2,6	2E-03	1,5	1E-01	0,4	3E-03	0,5	1E-02
Hormone metabolism									
AT3G03840	auxin-responsive protein	2,7	4E-05	1,0	8E-01	0,5	5E-04	0,6	7E-03
AT2G46690	auxin-responsive family protein	2,0	6E-04	1,2	2E-01	0,8	3E-01	1,1	4E-01
Stress									
AT1G54050	17.4 kDa class III heat shock protein (HSP17.4-CIII)	2,2	1E-03	1,6	3E-02	0,4	6E-04	0,6	5E-03
AT1G69080	universal stress protein (USP) family protein	2,1	6E-05	0,9	7E-01	0,5	9E-05	0,6	5E-04
Misc									
AT1G14700	PAP3 (PURPLE ACID PHOSPHATASE 3)	2,2	4E-04	1,7	8E-03	0,5	4E-04	0,9	7E-01
AT2G10940	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	3,2	3E-04	3,8	6E-05	0,5	3E-02	0,5	5E-03
RNA									
AT3G58120	BZIP61; DNA binding / transcription activator/ transcription factor	2,7	6E-03	1,6	1E-01	0,6	9E-02	0,4	9E-05
AT5G39860	PRE1 (PACLOBUTRAZOL RESISTANCE1)	3,0	2E-03	1,3	4E-01	0,5	4E-02	0,5	1E-02
AT4G04840	ATMSRB6	4,2	2E-06	2,7	2E-04	0,6	6E-02	0,5	2E-04
AT4G30410	transcription factor	2,2	9E-03	1,1	8E-01	0,4	6E-03	0,5	7E-03
AT3G48550	unknown protein	2,1	4E-06	1,0	7E-01	0,7	2E-02	0,9	3E-01
AT1G32540	LOL1 (LSD ONE LIKE 1)	2,1	4E-02	1,5	3E-01	0,5	5E-02	0,7	3E-02
DNA									
AT5G44680	methyladenine glycosylase family protein	3,4	4E-05	1,8	2E-02	0,5	5E-03	0,6	6E-03
Cell									
AT3G50240	KICP-02	2,2	2E-02	1,2	6E-01	0,4	1E-02	0,4	8E-03
Development									
AT5G43270	SPL2 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 2)	2,3	2E-02	0,7	3E-01	0,4	4E-03	0,3	6E-03
AT3G28130	nodulin MtN21 family protein	2,1	6E-05	1,0	8E-01	0,5	9E-05	0,9	2E-01
Not assigned									
AT3G46490	oxidoreductase	4,6	4E-08	1,4	5E-02	0,3	2E-06	0,4	7E-04
AT1G78170	unknown protein	3,0	3E-05	1,8	8E-03	0,5	2E-03	0,7	5E-02
AT1G48330	unknown protein	2,8	6E-05	1,5	5E-02	0,5	3E-03	0,9	2E-01
AT5G01075	beta-galactosidase	2,2	1E-02	1,7	8E-02	0,5	2E-02	0,9	2E-01

Table S8 Cluster III genes of ACC-repressed genes not included into MarVis analysis.

Cluster III (down)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
Photosystem									
AT5G45040	Cytochromes c6 (ATC6)	2,4	6E-05	1,3	2E-01	0,7	5E-02	0,9	2E-01
Cell wall									
AT1G23480	ATCSLA03 (CELLULOSE SYNTHASE-LIKE A3)	2,0	6E-04	1,3	1E-01	0,9	5E-01	0,8	3E-02
AT1G03870	FLA9 (FASCICLIN-LIKE ARABINOOGALACTAN 9)	2,5	8E-06	1,2	3E-01	1,0	8E-01	1,0	1E+00
AT5G65390	AGP7	2,2	2E-03	1,2	5E-01	0,5	7E-03	0,6	3E-02
AT3G52840	BGAL2 (beta-galactosidase 2)	2,0	5E-03	1,4	1E-01	0,7	7E-02	1,2	1E-01
AT3G15720	glycoside hydrolase family 28 protein	2,8	1E-03	1,3	3E-01	0,7	1E-01	0,6	6E-02
AT4G23820	glycoside hydrolase family 28 protein	2,3	9E-05	1,7	8E-03	0,8	3E-01	1,0	7E-01
AT3G62110	glycoside hydrolase family 28 protein	2,0	4E-04	1,2	2E-01	0,8	3E-01	1,1	4E-01
AT2G40610	ATEXPA8 (ARABIDOPSIS THALIANA EXPANSIN A8)	2,9	2E-02	1,6	3E-01	0,6	2E-01	0,6	4E-02
AT3G29030	EXPA5 (EXPANSIN A5)	2,7	7E-03	1,9	6E-02	0,6	2E-01	0,8	2E-01
AT4G03210	XTH9 (XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 9)	2,5	8E-05	1,7	9E-03	1,0	9E-01	1,1	3E-01
AT3G45970	ATEXLA1 (ARABIDOPSIS THALIANA EXPANSIN-LIKE A1)	2,3	3E-03	0,9	7E-01	1,2	5E-01	1,3	5E-02
Lipid metabolism									
AT4G34250	KCS16 (3-KETOACYL-COA SYNTHASE 16)	2,7	6E-05	1,0	8E-01	0,8	2E-01	1,1	5E-01
AT2G15090	KCS8 (3-KETOACYL-COA SYNTHASE 8)	2,6	2E-03	1,6	1E-01	1,0	9E-01	1,0	9E-01
AT1G75960	AMP-binding protein	2,2	3E-04	1,3	2E-01	0,9	7E-01	1,1	7E-01
AT1G06100	fatty acid desaturase family protein	3,0	1E-04	1,0	9E-01	0,5	9E-03	0,8	3E-01
AT1G06360 AT1G06350	fatty acid desaturase family protein	2,3	9E-04	1,7	3E-02	0,6	2E-02	0,7	4E-03
Amino acid metabolism									
AT1G62800	ASP4 (ASPARTATE AMINOTRANSFERASE 4)	2,3	9E-07	1,2	2E-01	0,6	2E-04	0,8	7E-02
AT3G22740	HMT3; homocysteine S-methyltransferase	4,5	1E-05	1,2	4E-01	0,7	1E-01	1,1	3E-01
AT1G31180 AT5G14200	3-isopropylmalate dehydrogenase	2,5	8E-03	1,7	1E-01	1,0	9E-01	1,6	2E-02
AT1G69040	ACR4 (ACT REPEAT 4)	2,2	9E-04	1,4	9E-02	0,8	3E-01	1,0	8E-01
Metal handling									
AT5G04950	NAS1 (NICOTIANAMINE SYNTHASE 1)	3,4	3E-03	1,4	4E-01	0,7	4E-01	1,8	2E-02
AT5G50740	metal ion binding	2,5	3E-04	1,8	1E-02	0,5	3E-03	0,6	2E-03
Secondary metabolism									
AT1G78970	LUP1 (LUPEOL SYNTHASE 1)	2,7	7E-05	1,1	6E-01	0,2	5E-07	0,3	1E-05
AT1G62560	FMO GS-OX3 (FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 3)	3,5	3E-03	1,6	2E-01	0,6	1E-01	1,4	1E-01
AT4G13770	CYP83A1 (CYTOCHROME P450 83A1)	2,2	8E-03	1,9	3E-02	0,7	2E-01	1,6	7E-03
AT1G74090	SOT18 (DESULFO-GLUCOSINOLATE SULFOTRANSFERASE 18)	2,2	3E-03	1,7	3E-02	0,6	7E-02	1,2	1E-01
AT2G18560 AT2G18570	UDP-glucoronosyl/UDP-glucosyl transferase family protein	2,2	2E-05	0,9	5E-01	0,8	9E-02	0,8	3E-01
Hormone metabolism									
AT2G45400	BEN1	2,0	5E-04	1,1	6E-01	0,6	2E-02	0,7	7E-03
AT1G29500	auxin-responsive protein	3,4	3E-04	2,0	2E-02	0,5	4E-02	0,6	3E-02
AT2G33830	dormancy/auxin associated family protein	2,9	3E-02	0,9	7E-01	1,0	1E+00	0,9	6E-01
AT5G18060	auxin-responsive protein	2,7	8E-04	1,4	2E-01	0,6	7E-02	0,6	2E-03
AT4G34760	auxin-responsive family protein	2,5	2E-04	1,4	9E-02	0,7	9E-02	0,9	3E-01
AT5G08330	TCP family transcription factor	2,2	4E-04	1,4	8E-02	0,8	2E-01	0,8	2E-01
AT4G38850	SAUR15 (SMALL AUXIN UPREGULATED 15)	2,2	9E-05	1,5	2E-02	0,8	2E-01	1,0	7E-01
AT1G29440	unknown protein	2,2	3E-03	1,6	6E-02	0,7	8E-02	0,6	8E-03
AT1G29450	auxin-responsive protein	2,1	3E-03	1,5	1E-01	0,6	6E-02	0,7	3E-02
AT1G29460	auxin-responsive protein	2,1	3E-03	1,6	5E-02	0,8	3E-01	1,1	8E-01
AT2G26710	BAS1 (PHYB ACTIVATION TAGGED SUPPRESSOR 1)	2,4	2E-03	1,2	5E-01	0,8	4E-01	0,8	1E-01

Cluster III (down)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT1G74670	gibberellin-responsive protein	3,9	7E-03	1,8	2E-01	0,6	3E-01	0,8	6E-01
AT1G22690	gibberellin-responsive protein	3,8	3E-06	1,2	5E-01	1,4	1E-01	0,8	3E-01
AT3G16450	jacalin lectin family protein	2,7	2E-04	1,2	3E-01	0,6	3E-02	0,8	5E-02
Tetrapyrrole synthesis									
AT5G54190	PORA; oxidoreductase/ protochlorophyllide reductase	2,5	2E-02	1,3	4E-01	1,0	1E+00	1,2	4E-02
Major CHO metabolism									
AT3G30720	QQS (QUA-QUINE STARCH)	2,1	5E-02	1,1	9E-01	1,0	1E+00	0,8	5E-01
Stress									
AT1G70830	MLP28 (MLP-LIKE PROTEIN 28)	2,3	6E-06	1,8	4E-04	0,9	4E-01	1,0	8E-01
AT1G73330	ATDR4	2,1	4E-02	1,4	3E-01	0,9	9E-01	0,9	6E-01
AT1G56300	DNAJ heat shock N-terminal domain-containing protein	2,0	2E-02	1,1	7E-01	0,9	7E-01	0,9	2E-01
AT2G42530	COR15B (COLD REGULATED 15B)	3,3	3E-02	2,1	2E-01	0,7	5E-01	1,2	4E-01
AT4G30650	hydrophobic protein	2,1	1E-02	1,5	2E-01	0,8	4E-01	1,0	9E-01
AT5G66590	allergen V5/Tpx-1-related family protein	2,9	2E-05	1,3	2E-01	0,7	1E-01	1,1	3E-01
AT3G50970	LTI30 (LOW TEMPERATURE-INDUCED 30)	2,9	2E-02	0,8	5E-01	1,2	7E-01	0,7	9E-02
AT1G35260	MLP165 (MLP-LIKE PROTEIN 165)	2,2	1E-06	0,8	8E-02	1,2	9E-02	1,1	4E-01
Redox regulation									
AT3G10520	AHB2 (ARABIDOPSIS HAEMOGLOBIN 2)	2,2	9E-04	1,5	7E-02	1,0	9E-01	1,2	1E-01
AT3G62950	glutaredoxin family protein	2,2	3E-02	1,6	2E-01	1,4	3E-01	1,3	1E-01
Nucleotide metabolism									
AT4G29610	cytidine deaminase	2,2	9E-03	1,1	7E-01	0,8	4E-01	0,9	4E-01
AT1G80050	APT2 (ADENINE PHOSPHORIBOSYL TRANSFERASE 2)	2,2	3E-03	1,2	5E-01	0,8	2E-01	0,8	1E-01
Misc									
AT1G25230	purple acid phosphatase family protein	2,5	3E-02	1,1	8E-01	0,7	4E-01	0,8	2E-01
AT5G63140	ATPAP29 (ARABIDOPSIS THALIANA PURPLE ACID PHOSPHATASE 29)	2,4	1E-02	1,6	2E-01	0,9	6E-01	0,9	3E-01
AT5G45700	NLI interacting factor (NIF) family protein	2,0	3E-03	1,2	4E-01	1,1	7E-01	1,0	1E+00
AT2G39310	JAL22 (JACALIN-RELATED LECTIN 22)	2,9	2E-06	0,9	5E-01	0,5	6E-04	0,6	6E-04
AT4G31840	plastocyanin-like domain-containing protein	2,1	1E-03	1,1	6E-01	0,8	3E-01	1,0	8E-01
AT5G05960	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	4,0	7E-04	1,1	7E-01	3,6	1E-03	6,1	2E-06
AT2G45180	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2,0	4E-03	0,9	5E-01	0,7	1E-01	1,0	1E+00
AT5G45950	GDSL-motif lipase/hydrolase family protein	2,4	4E-04	2,0	5E-03	0,6	3E-02	0,8	3E-02
AT1G31710	copper amine oxidase	2,3	7E-04	1,7	2E-02	0,8	3E-01	1,1	6E-01
AT2G29310	tropinone reductase	2,3	8E-06	1,4	2E-02	0,6	5E-04	1,1	5E-01
RNA									
AT4G17460	HAT1; DNA binding / transcription factor	2,6	1E-04	1,2	3E-01	0,7	9E-02	0,8	1E-01
AT3G51910	AT-HSFA7A	2,2	6E-04	1,0	9E-01	0,7	6E-02	1,0	9E-01
AT3G46130	MYB111	2,1	3E-03	1,1	7E-01	0,6	2E-02	0,8	4E-01
AT2G21650	MEE3 (MATERNAL EFFECT EMBRYO ARREST 3)	3,6	1E-06	1,1	7E-01	1,2	3E-01	1,0	8E-01
AT1G18330	EPR1 (EARLY-PHYTOCHROME-RESPONSIVE1)	2,4	1E-03	0,8	3E-01	0,9	5E-01	1,0	6E-01
AT1G75250	ATRL6 (ARABIDOPSIS RAD-LIKE 6)	2,1	3E-02	1,4	4E-01	0,9	7E-01	0,8	4E-01
AT1G69690	TCP family transcription factor	2,0	5E-04	1,2	3E-01	0,7	2E-02	0,9	5E-01
AT4G25480	DREB1A (DEHYDRATION RESPONSE ELEMENT B1A)	3,1	1E-02	1,0	9E-01	1,0	1E+00	1,0	8E-01
AT1G04240	SHY2 (SHORT HYPOCOTYL 2)	2,9	1E-05	1,3	2E-01	0,6	6E-03	0,7	9E-03
AT1G52830	IAA6 (INDOLE-3-ACETIC ACID 6)	2,1	2E-03	1,0	9E-01	0,7	1E-01	0,8	3E-01
AT3G18960 AT4G01580	transcriptional factor B3 family protein	2,1	2E-04	1,2	2E-01	0,6	9E-03	0,8	8E-03
AT3G48100	ARR5 (ARABIDOPSIS RESPONSE REGULATOR 5)	2,4	9E-04	0,9	5E-01	0,9	5E-01	0,6	4E-03
AT2G18300	basic helix-loop-helix (bHLH) family protein	2,9	1E-03	1,0	9E-01	0,6	5E-02	0,8	3E-01
AT5G46690	bHLH071 (beta HLH protein 71)	2,6	2E-03	1,7	7E-02	0,4	6E-03	0,7	7E-02
AT3G05800	transcription factor	2,2	7E-04	1,5	7E-02	0,7	1E-01	0,7	5E-02

Cluster III (down)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
AT4G00480	ATMYC1	2,1	2E-06	0,9	3E-01	0,6	5E-04	0,7	3E-03
AT1G68810	basic helix-loop-helix (bHLH) family protein	2,0	4E-04	1,4	4E-02	0,7	4E-02	1,2	3E-01
AT4G23800	high mobility group (HMG1/2) family protein	2,1	5E-04	1,0	9E-01	0,9	6E-01	1,0	8E-01
AT4G04830	ATMSRB5	2,1	2E-03	1,6	4E-02	0,6	3E-02	0,8	3E-01
AT3G61260	DNA-binding family protein / remorin family protein	2,1	2E-02	1,5	2E-01	0,8	4E-01	0,9	6E-01
AT2G42190	unknown protein	2,1	4E-04	0,9	7E-01	0,7	3E-02	0,8	1E-01
AT5G57660	ATCOL5, COL5 | zinc finger (B-box type) family protein	2,8	3E-02	0,8	7E-01	0,9	8E-01	0,7	2E-01
AT5G44560	VPS2.2	2,0	2E-05	1,0	9E-01	0,7	1E-02	0,9	2E-01
AT3G47500	CDF3 (CYCLING DOF FACTOR 3)	2,2	1E-02	1,0	1E+00	0,8	4E-01	0,8	6E-02
AT1G09750	chloroplast nucleoid DNA-binding protein-related	2,4	2E-03	2,0	1E-02	0,9	6E-01	1,0	8E-01
AT3G48550	unknown protein	2,1	4E-06	1,0	7E-01	0,7	2E-02	0,9	3E-01
AT1G22330	RNA binding / nucleic acid binding / nucleotide binding	2,8	1E-03	1,4	2E-01	0,7	1E-01	0,6	5E-03
AT1G22330	RNA binding / nucleic acid binding / nucleotide binding	2,2	1E-02	1,6	1E-01	0,6	1E-01	0,7	3E-02
DNA									
AT5G07460	PMSR2 (PEPTIDEMETHIONINE SULFOXIDE REDUCTASE 2)	2,1	3E-03	1,2	3E-01	0,7	2E-01	1,2	1E-01
AT1G03420	Sadhu4-2 | transposable element gene	2,4	5E-03	1,3	3E-01	0,9	8E-01	1,2	1E-01
AT2G18050	HIS1-3 (HISTONE H1-3)	3,3	9E-03	1,0	9E-01	0,7	4E-01	0,8	6E-01
Protein									
AT3G17170	RFC3 (REGULATOR OF FATTY-ACID COMPOSITION 3)	2,1	3E-03	1,0	8E-01	0,9	5E-01	0,8	2E-01
AT5G52280	protein transport protein-related	2,1	3E-03	1,0	1E+00	0,5	8E-03	0,6	3E-02
AT5G45820	CIPK20 (CBL-INTERACTING PROTEIN KINASE 20)	2,3	5E-02	1,0	1E+00	1,0	1E+00	1,1	3E-01
AT5G57630	CIPK21 (CBL-interacting protein kinase 21)	2,1	3E-02	1,1	7E-01	1,0	1E+00	1,1	6E-01
AT3G23340	ckl10 (Casein Kinase I-like 10)	2,0	1E-05	0,8	2E-01	0,5	2E-05	0,6	4E-03
AT1G56720	protein kinase family protein	2,4	6E-04	1,6	4E-02	0,6	2E-02	0,8	9E-02
AT2G18890	protein kinase family protein	2,1	3E-04	1,0	8E-01	0,6	5E-03	0,7	4E-03
AT5G22920	zinc finger (C3HC4-type RING finger) family protein	3,1	3E-02	1,7	3E-01	1,0	1E+00	0,9	8E-01
AT4G03190	GRH1 (GRR1-LIKE PROTEIN 1)	2,1	4E-03	1,1	7E-01	0,6	7E-02	0,5	2E-03
AT3G23880	F-box family protein	2,1	2E-04	1,9	1E-03	0,8	1E-01	1,5	6E-03
AT4G11320 AT4G11310	cysteine proteinase, putative	4,1	3E-06	1,1	7E-01	0,7	1E-01	0,9	5E-01
AT3G12700	aspartyl protease family protein	2,0	8E-05	1,0	8E-01	0,7	2E-02	0,7	2E-03
AT2G22980	SCPL13	3,2	2E-02	1,5	4E-01	0,5	2E-01	0,7	2E-01
Minor CHO metabolism									
AT1G09350	AtGolS3 (Arabidopsis thaliana galactinol synthase 3)	2,3	4E-02	1,0	1E+00	1,1	8E-01	1,1	2E-01
Signalling									
AT2G43010	PIF4 (phytochrome interacting factor 4)	3,7	1E-02	0,9	8E-01	0,7	5E-01	0,7	9E-02
AT4G29080	PAP2 (PHYTOCHROME-ASSOCIATED PROTEIN 2)	2,1	3E-06	0,8	6E-02	0,7	8E-03	0,8	2E-02
AT3G19850	phototropic-responsive NPH3 family protein	2,1	4E-02	1,0	9E-01	0,6	2E-01	0,9	5E-01
AT1G54820	protein kinase family protein	2,1	1E-03	1,0	9E-01	0,5	6E-03	0,6	1E-03
AT4G05520	ATEHD2 (EPS15 HOMOLOGY DOMAIN 2)	2,0	5E-03	1,1	6E-01	0,8	2E-01	0,8	2E-01
AT2G46710	rac GTPase activating protein	2,3	3E-03	1,6	7E-02	0,7	1E-01	0,8	1E-01
AT1G22740	RABG3B; GTP binding	2,1	4E-03	1,5	6E-02	0,7	7E-02	1,0	9E-01
Cell									
AT5G48460	fimbrin-like protein	2,3	4E-05	1,8	2E-03	0,6	8E-03	0,9	4E-01
AT1G20010	TUB5; structural constituent of cytoskeleton	2,1	3E-04	1,5	2E-02	0,8	2E-01	1,2	2E-01
AT4G33270 AT4G33260	CDC20.1; signal transducer	2,2	1E-02	1,0	1E+00	0,7	2E-01	0,7	8E-02
AT2G44740	CYCP4;1 (cyclin p4;1)	2,1	4E-03	1,1	8E-01	0,7	2E-01	0,9	3E-01
AT2G26760	CYCB1;4 (Cyclin B1;4)	2,0	3E-04	0,9	6E-01	1,0	9E-01	1,1	2E-01
AT1G08560	SYP111 (SYNTAXIN OF PLANTS 111)	2,3	3E-05	1,2	3E-01	0,9	6E-01	1,2	9E-02
AT2G33180	SPL38 (SQUAMOSA PORMOTOR BINDING PROTEIN-LIKE 3)	2,2	1E-02	1,4	3E-01	0,7	3E-01	0,8	3E-01

Cluster III (down)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
Development									
AT1G07050	CONSTANS-like protein-related	2,4	4E-06	1,4	3E-02	0,7	3E-02	0,9	6E-01
AT1G44800	nodulin MtN21 family protein	2,2	1E-02	0,7	2E-01	1,3	4E-01	0,6	6E-02
AT4G26370	antitermination NusB domain-containing protein	2,1	4E-03	1,7	4E-02	0,9	5E-01	1,1	6E-01
AT3G16690	nodulin MtN3 family protein	2,1	9E-06	1,2	2E-01	1,1	5E-01	1,3	2E-02
Transport									
AT4G30110	HMA2; cadmium-transporting ATPase	2,5	1E-06	0,9	4E-01	1,2	3E-01	1,2	2E-01
AT1G10970	ZIP4 (ZINC TRANSPORTER 4 PRECURSOR)	2,4	2E-07	0,9	2E-01	0,9	2E-01	1,0	9E-01
AT1G22570	proton-dependent oligopeptide transport (POT) family protein	2,4	7E-03	0,8	6E-01	1,2	5E-01	1,2	1E-01
AT1G01620	PIP1C (PLASMA MEMBRANE INTRINSIC PROTEIN 1C)	2,3	1E-05	1,4	2E-02	0,7	5E-02	1,0	8E-01
AT2G21050	amino acid permease	2,0	1E-03	1,5	5E-02	1,5	5E-02	1,7	3E-03
AT5G10180	AST68	2,0	1E-03	1,1	7E-01	1,0	8E-01	1,0	8E-01
AT2G29650	PHT4;1	2,1	2E-02	1,1	8E-01	0,7	2E-01	0,7	4E-02
Not assigned									
AT3G05900	neurofilament protein-related	3,1	2E-04	2,0	1E-02	0,7	1E-01	0,7	1E-02
AT4G02850	phenazine biosynthesis PhzC/PhzF family protein	3,0	1E-03	1,7	9E-02	0,8	4E-01	0,8	8E-02
AT1G78450	SOUL heme-binding family protein	2,6	4E-04	1,6	5E-02	0,7	1E-01	1,1	7E-01
AT3G50440	MES10 (METHYL ESTERASE 10)	2,6	2E-06	1,3	1E-01	0,6	3E-03	1,1	2E-01
AT5G04820	OFP13 (ARABIDOPSIS THALIANA OVATE FAMILY PROTEIN 13)	2,5	4E-07	1,1	5E-01	0,7	6E-03	0,8	1E-01
AT5G51550	EXL3 (EXORDIUM LIKE 3)	2,2	6E-04	1,1	5E-01	0,9	5E-01	1,1	8E-01
AT2G32100	OFP16 (ARABIDOPSIS THALIANA OVATE FAMILY PROTEIN 16)	2,1	5E-02	1,3	5E-01	0,9	8E-01	1,0	9E-01
AT3G53800	armadillo/beta-catenin repeat family protein	2,1	1E-02	1,3	3E-01	0,7	2E-01	0,9	6E-01
AT5G28630	glycine-rich protein	2,7	1E-03	1,0	9E-01	0,9	6E-01	1,2	3E-01
AT5G61660	glycine-rich protein	2,3	1E-03	1,5	8E-02	1,3	2E-01	2,1	2E-04
AT3G02120	hydroxyproline-rich glycoprotein family protein	2,1	3E-03	0,9	8E-01	1,0	8E-01	1,1	7E-01
AT2G24762	AtGDU4 (Arabidopsis thaliana GLUTAMINE DUMPER 4)	4,9	1E-08	0,9	7E-01	1,0	8E-01	1,2	2E-02
AT3G13980	unknown protein	4,4	8E-07	0,9	7E-01	0,7	2E-01	1,1	3E-01
AT3G45160	unknown protein	3,6	8E-07	1,4	1E-01	0,7	9E-02	1,3	2E-02
AT1G06980	unknown protein	3,3	2E-07	1,0	8E-01	0,7	1E-02	0,8	6E-02
AT5G57785	unknown protein	3,2	3E-05	0,6	2E-02	0,5	5E-03	0,6	1E-02
AT5G16030	unknown protein	3,1	4E-04	1,8	5E-02	0,6	9E-02	0,9	3E-01
AT1G35612	transposable element gene	3,1	2E-02	0,8	7E-01	0,4	7E-02	0,5	2E-02
AT2G16990	tetracycline transporter	2,6	3E-03	1,1	7E-01	0,6	5E-02	0,5	6E-04
AT5G36710 AT5G36800	unknown protein	2,6	2E-04	0,9	6E-01	0,8	4E-01	1,1	5E-01
AT3G50120	unknown protein	2,5	8E-05	0,9	4E-01	0,6	1E-02	0,8	7E-02
AT5G03120	unknown protein	2,3	6E-04	1,5	7E-02	0,6	1E-02	1,0	7E-01
AT5G67390	unknown protein	2,3	5E-04	1,0	8E-01	0,6	1E-02	0,8	7E-02
AT1G65900	unknown protein	2,3	7E-03	1,5	2E-01	0,5	2E-02	0,7	1E-01
AT2G20670	unknown protein	2,3	3E-02	0,8	6E-01	0,8	6E-01	0,7	1E-01
AT1G18620	unknown protein	2,3	3E-02	1,6	2E-01	0,8	6E-01	0,9	5E-01
AT4G23870	unknown protein	2,3	7E-04	1,8	1E-02	1,0	9E-01	1,4	4E-02
AT2G30930	unknown protein	2,2	8E-03	0,9	6E-01	1,0	9E-01	1,0	9E-01
AT4G04330	unknown protein	2,2	7E-04	1,1	8E-01	0,7	6E-02	0,9	3E-01
AT3G02640	unknown protein	2,2	8E-04	1,0	9E-01	0,9	6E-01	1,1	6E-01
AT1G12080	unknown protein	2,2	7E-04	1,7	2E-02	0,6	1E-02	0,6	6E-04
AT1G70420	unknown protein	2,2	7E-03	1,2	6E-01	0,7	2E-01	0,9	4E-01
AT4G23496	SP1L5 (SPIRAL1-LIKE5)	2,1	5E-03	1,0	9E-01	1,0	9E-01	0,9	5E-01
AT5G62550	unknown protein	2,1	6E-04	0,9	8E-01	0,9	6E-01	0,9	3E-01
AT4G16515	unknown protein	2,1	5E-03	1,5	1E-01	0,6	8E-02	0,7	9E-02
AT2G34510	unknown protein	2,1	3E-03	1,5	6E-02	0,8	3E-01	1,4	3E-03

Cluster III (down)		WT mock vs WT ACC		WT ACC vs WT SA/ACC		WT ACC vs *tga256 ACC*		WT ACC vs *tga256 SA/ACC*	
		x-fold rep.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*	x-fold repr.	*p-value*
Not assigned									
AT5G35490	MRU1	2,1	7E-05	1,0	9E-01	1,1	5E-01	1,7	1E-02
AT4G15830	unknown protein	2,1	5E-03	1,1	7E-01	1,1	8E-01	1,1	3E-01
AT1G75190	unknown protein	2,1	3E-03	1,2	4E-01	1,0	9E-01	0,9	7E-01
AT3G26960	unknown protein	2,1	3E-02	1,5	2E-01	0,8	4E-01	0,8	2E-01
AT2G42870	PAR1 (PHY RAPIDLY REGULATED 1)	2,1	5E-02	1,2	5E-01	1,1	8E-01	1,1	4E-01
AT4G31730	GDU1 (GLUTAMINE DUMPER 1)	2,0	3E-05	0,9	6E-01	1,0	8E-01	1,0	8E-01
AT5G60400	unknown protein	2,0	9E-04	1,1	5E-01	0,9	4E-01	1,1	7E-01
AT4G22560	unknown protein	2,0	5E-04	1,1	7E-01	0,7	6E-02	1,0	8E-01
AT2G44230	unknown protein	2,0	7E-03	1,9	1E-02	0,8	4E-01	1,0	1E+00
AT5G53020	unknown protein	2,0	3E-03	1,1	6E-01	0,5	4E-03	0,7	1E-01

Table S9 List of primers used for genotyping, cloning and real-time RT-PCR Analysis

Abbr.	Description	Sequence
P1	TGA25 fwd.	GTC AAT CCG GTT TCA TAT TCT CCT C
P2	TGA25 rev.	CCG CAT AAA CAA TAA ACC AAG AGA G
P3	*tga25* rev.	GAG CGA CAA CTC CTT TCA ACT CAT C
P4	TGA6 fwd.	TTC TCA CTT TGT GAT TTG CCT TTG G
P5	TGA6 rev.	TGG GCA ATC TTG CTA TGA TTT CAA G
P6	ORA59-Pro. fwd.	GGG GACA AGT TTG TAC AAA AAA GCA GGC TCC GGA TTG GTT GCA GGT TAC GAT G
P7	ORA59-Pro.rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC GCA TTT TCG ATC TTT TTT TTT TCT TCT TG
P8	pDONR fwd.	TCG CGT TAA CGC TAG CAT GGA TCT C
P9	ORA59 TGACG overlap rev.	GGA CAA GAC CAG GTT GAG TGT AAA AAA TAC GGC GGC GTA TTC CCG AC
P10	pDONR rev.	GTA ACA TCA GAG ATT TTG AGA CAC
P11	ORA59 TGACG overlap fwd.	GTA TTT TTT ACA CTC AAC CTG GTC TTG TCC

P12	ORA59 G-Box overlap rev.	CAC AGA AGT GGG TGA AAT GTT CAA TAT GGG ACA AGA CCA GGT TGA GTG TAT G
P13	ORA59 G-Box overlap fwd	CAT ATT GAA CAT TTC ACC CAC TTC TGT G
P14	ORA59 EIN3 del. overlap rev.	ATG CAG TTC TTA TAT AAA CGA TTT AGC TAT CAG CGG TTT AGG ATT ATC ACT CTA
P15	ORA59 EIN3 del. overlap fwd.	ATA GCT AAA TCG TTT ATA TAA GAA CTG CAT
P16	EIN3 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GAT GTT TAA TGA GAT GGG AAT GTG
P17	EIN3 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTT AGA ACC ATA TGG ATA CAT CTT GCT G
P18	Actin8 ChIP fwd.	GGT TTT CCC CAG TGT TGT TG
P19	Actin ChIP rev.	CTC CAT GTC ATC CCA GTT GC
P20	GES ChIP fwd.	AAG CAT CTA CAT TCA TGA GAT AAC C
P21	GES ChIP rev.	ATC TAT TGG GAA GTT CTT ACA TGA G
P22	ORA59 ChIP fwd.	CGA GAG AGT ATA TGA AGA GGC CAA
P23	ORA59 ChIP rev.	GGA CAA GAC CAG GTT GAG TG
P24	PDF1.2 RT fwd.	CTT GTT CTC TTT GCT GCT TTC
P25	PDF1.2 RT rev.	CAT GTT TGG CTC CTT CAA G
P26	UBQ5 RT fwd.	GAC GCT TCA TCT CGT CC
P27	UBQ5 RT rev.	GTA AAC GTA GGT GAG TCC A

Chapter IV

CC-type glutaredoxins interact promiscuously with TGA factors and suppress expression of the JA/ET pathway through the C-terminal A(L/I)W(L/V) motif

Mark Zander[1], Shuxia Chen[2], Julia Imkampe[1], Corinna Thurow[1] and Christiane Gatz[1]

[1]Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Georg-August-Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany

[2]College of Horticulture Science, Northwest A&G University, 3-Taicheng Road, Yangling, Shaanxi, 712100, China

Abstract

Glutaredoxins are small heat stable oxidoreductases that transfer electrons from glutathione (GSH) to oxidized Cys residues thereby contributing to protein integrity and regulation under conditions of oxidative stress. In higher plants, floral glutaredoxins ROXY1 and ROXY2 and pathogen-induced ROXY19/GRX480 interact with bZIP transcription factors of the TGACG (TGA) motif-binding family. Whereas ROXY1 and ROXY2 and the TGA factors PERIANTHIA, TGA9 and TGA10 play essential roles in floral development, ROXY19/GRX480 and TGA factors TGA2 and TGA5 mediate the cross-communication between two competing defense pathways. Here we show that ectopic expression of *ROXY19/GRX480*, which is normally induced by elevated levels of the plant defense hormone salicylic acid (SA), suppresses the promoter of the upstream regulator of the jasmonic acid (JA)/ethylene (ET)-induced defense program, *ORA59*. We found that the capacity to interact with TGA factors is a common feature of the 21 members of the land plant-specific CC-type clade of glutaredoxins in *Arabidopsis*. Ten CC-type glutaredoxins can suppress *ORA59* promoter activity, a feature that depends on their conserved C-terminal A(L/I)W(L/V) motif. ROXY19/GRX480-mediated suppression was compromised by mutation of the conserved Gly residue in the putative GSH binding site, although interaction with TGA2 was unaffected in a yeast two hybrid assay. Collectively, our data indicate that the interaction with TGA factors constitutes an ancient feature of CC-type glutaredoxins which is not sufficient for their potential function as modulators of the JA/ET pathway. We postulate that the critical conserved A(L/)IW(L/V) motif and the GSH binding site are important for the redox regulation of a yet unknown target protein.

Introduction

Post-translational modification of proteins constitutes a key mechanism for regulating cellular functions in response to developmental and environmental cues. In plants, many biotic or abiotic stress conditions ultimately lead to oxidative damage since compromising metabolic or cellular processes disturbs the channelling of excited electrons of the photosynthetic apparatus into low energy electrons at reduced carbon atoms. To minimize this damage, plants have evolved highly sophisticated anti-oxidative mechanisms including redox modifications at critical Cys residues of regulatory proteins (Foyer and Noctor, 2009).

The sulphur within Cys residues can either occur as a reduced thiol or in oxidized disulfide bridges, glutathione (GSH)-mixed disulfids or as a sulfenic acid. Reduction is catalyzed by proteins of the thioredoxin/glutaredoxin family, which are small heat stable oxidoreductases found in all organisms from prokaryotes to higher eukaryotes. In contrast to thioredoxins, glutaredoxins recruit electrons from glutathione (GSH) and are involved in the reversible glutathionylation of proteins (Biswas et al., 2006).

The specific demand of land plants for an efficient oxidoreductase system is reflected by the relatively high number of glutaredoxin genes. Whereas the genomes of *Arabidopsis thaliana* and *Oryza sativa* encode 31 and 28 glutaredoxins, respectively, only five are found in the genomes of yeast and humans (Ziemann et al., 2009). Based on the amino acid sequence of the active site motif, glutaredoxins are divided into three classes, namely the CPYC, CGFS, and CC-type classes. CPYC and CGFS-type glutaredoxins are found in all organisms including plant species, whereas the CC-type is specific for land plants and is responsible for the expansion of this protein family. This class might be involved in plant-specific processes that go beyond the evolutionary ancient functions of the CPYC and CGFS-type proteins which facilitate protection from superoxide, synthesis and assembly of iron/sulphur clusters and synthesis of desoxyribonucleotides (Holmgren, 1989).

The specific functions of CC-type glutaredoxins are still relatively unexplored but they seem to be involved in such diverse processes as floral development and defense against microorganisms (Xing et al., 2006; Ndamukong et al., 2007; Murmu et al., 2010). In both cases, they biochemically and genetically interact with the TGA class of bZIP transcription factors which comprises 10 family members with overlapping functions. A yeast two hybrid screen with TGA2 as a bait yielded ROXY19/GRX480 (Ndamukong et al., 2007), and several different members of the TGA family were

identified when ROXY1 served as bait (Li et al., 2009). The importance of the ROXY1-TGA (PERIANTHIA, TGA9, TGA10) interaction is supported by genetic data: *roxy1*, *pan* and the *tga9 tga10* double mutant have defects in floral development (Chuang et al., 1999; Li et al., 2009; Murmu et al., 2010) which correlates with the expression domains of these proteins. The interaction between ROXY19/GRX480, whose transcription is induced after pathogen attack, and the redundant class II TGA factors TGA2 and TGA5 is suggested to be involved in the negative cross-talk between the salicylic acid (SA)-induced defense program and the jasmonic acid (JA)/ethylene (ET)-dependent defense pathways (Ndamukong et al., 2007). This cross-talk seems to be particularly important when plants are attacked by pathogens with different life styles (Spoel et al., 2007). Biotrophic pathogens exploit resources from living pathways and are combated by SA-induced defense processes whereas necrotrophic pathogens kill plants and feed on the remains. Their attack results in the activation of JA/ET-induced defense responses (Glazebrook, 2005). In *Arabidopsis*, simultaneous activation of both pathways is restricted by the strong negative effect of SA on the JA/ET pathway (Spoel and Dong, 2008; Pieterse et al., 2009). Since ectopic expression of the SA-inducible *ROXY19/GRX480* suppresses expression of marker genes of the JA/ET response only in the presence of class II TGA factors it has been postulated that this suppression is also operational after SA induction in the wild-type situation (Ndamukong et al., 2007). However, whereas the importance of TGA factors for the cross-talk between the SA and the JA/ET pathway has been demonstrated using the *tga2 tga5 tga6* mutant, the *roxy19/grx480* mutant showed wild-type like responses to SA and JA/ET (Ndamukong et al., 2007).

Here we aimed to identify those glutaredoxins that might be functionally redundant with ROXY19/GRX480. We found that all tested 17 CC-type glutaredoxins interact with TGA2 in a yeast two hybrid assay. Ten glutaredoxins were functional with respect to the suppression of the promoter of the key regulator of the JA/ET pathway, the AP2/ERF transcription factors ORA59 (Pre et al., 2008). These ten glutaredoxins are characterized by the A(L/I)W(L/V) motif at the very C-terminus that was previously identified as being important for complementing the floral phenotype of *roxy1* mutants (Li et al., 2009). This finding supports the notion that floral and defense-associated regulatory processes are conserved.

Results

Promiscuous interaction between TGA2 and CC-type glutaredoxins

Yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays have shown that TGA2 interacts with ROXY1 and ROXY19/GRX480 but not with the CPYC type glutaredoxin GRX370 (Ndamukong et al., 2007; Li et al., 2009). To assess the potential of other glutaredoxins to act functionally redundant to ROXY19/GRX480, 17 out of the 21 CC-type glutaredoxins were fused to the GAL4 activation domain and tested with respect to their capacity to interact with TGA2 using the yeast two hybrid assay (Fig.1). Since the coding regions of ROXY11 to ROXY15 (At4g15660, At4g15670, At4g15680, At4g15690 and At4g15700) differ by maximal 14 amino acids, only one of them (At4g15690) was chosen as a representative of this group. Whereas expression of TGA2 in the bait vector led to background ß-galactosidase activities, a clear increase was observed upon co-expression of ROXY19/GRX480 and all other tested CC-type glutaredoxins. As described before GRX370 had no effect (Ndamukong *et al.*, 2007). This result indicates that all Arabidopsis CC-type glutaredoxins interact with TGA2.

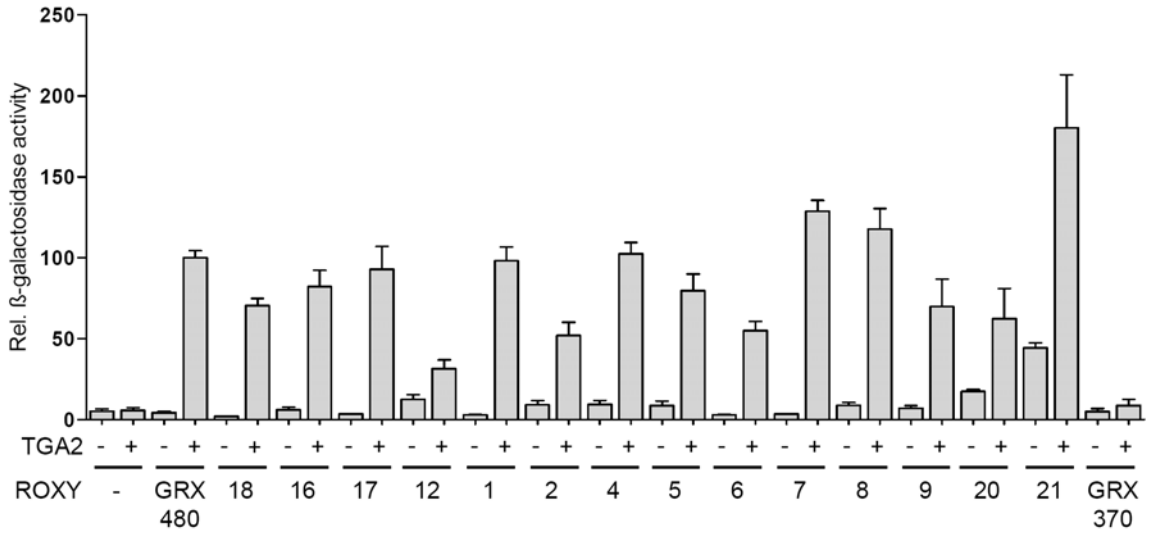

Figure 1. CC-type glutaredoxins promiscuously interact with TGA2 in yeast two hybrid assays

Prey plasmids encode TGA2 fused to the GAL4 binding domain; bait plasmids encode the indicated CC-type glutaredoxins (ROXYs) and CPYC-type GRX370 fused to the GAL4 activation domain; ß-galactosidase expression was measured in yeast strain PJ69-4A, which contains the *lacZ* reporter gene under the control of the GAL7 promoter. Values obtained from the known interaction between TGA2 and ROXY19/GRX480 were set to 100 %. The mean value (±SE) from six independent yeast transformants is shown. Hyphens indicate that transformants contained empty bait or prey vectors, respectively.

Development of a transient functional assay for ROXY19/GRX480 activity

Our finding that all 17 CC-type glutaredoxins interact with TGA2 prompted us to develop a transient assay system to test for their ability to suppress expression of JA/ET-responsive genes. Our previous analysis had shown that ectopic expression of *ROXY19/GRX480* suppresses *PDF1.2* expression (Ndamukong et al., 2007), which is a target gene of the key upstream regulator of the AP2/ERF transcription factor ORA59 (Pre et al., 2008). This promoter integrates the JA and the ET signal and is a target for the SA antagonism at least in ET-treated plants (Leon-Reyes et al., 2009). Consistent with the hypothesis that the SA antagonism is mediated through SA-induced *ROXY19/GRX480*, ACC-induced *ORA59* and *PDF1.2* expression was reduced in transgenic plants expressing *ROXY19/GRX480* under the control of the *CaMV 35S* promoter (Fig.2a,b).

Having established that the *ORA59* promoter is a potential direct target of the SA-ROXY19/GRX480-TGA-mediated suppression, we tested whether it could be used in transient assays to monitor the suppressive effect of ROXY19/GRX480. To this end, a 975 base pair fragment located upstream of the annotated transcriptional start site of the *ORA59* gene was combined with the firefly luciferase gene and transfected into *Arabidopsis* mesophyll protoplasts. As previously shown for other ethylene-responsive genes (Konishi and Yanagisawa, 2008), *ORA59* promoter activity was increased upon co-transfection with the central transcriptional activator of the ET response, EIN3. Importantly, this activation was reduced in the presence of ROXY19/GRX480 but not in the presence of GRX370 (Fig.2c), which does not interact with TGA factors and which does not suppress the JA/ET pathway in the transgenic situation (Ndamukong *et al.*, 2007). Suppression by ROXY19/GRX480 depended on class II TGA factors as indicated by transfection experiments into *tga256* protoplasts. Thus, a functional assay for testing the ability of glutaredoxins to suppress *ORA59* promoter activity was established.

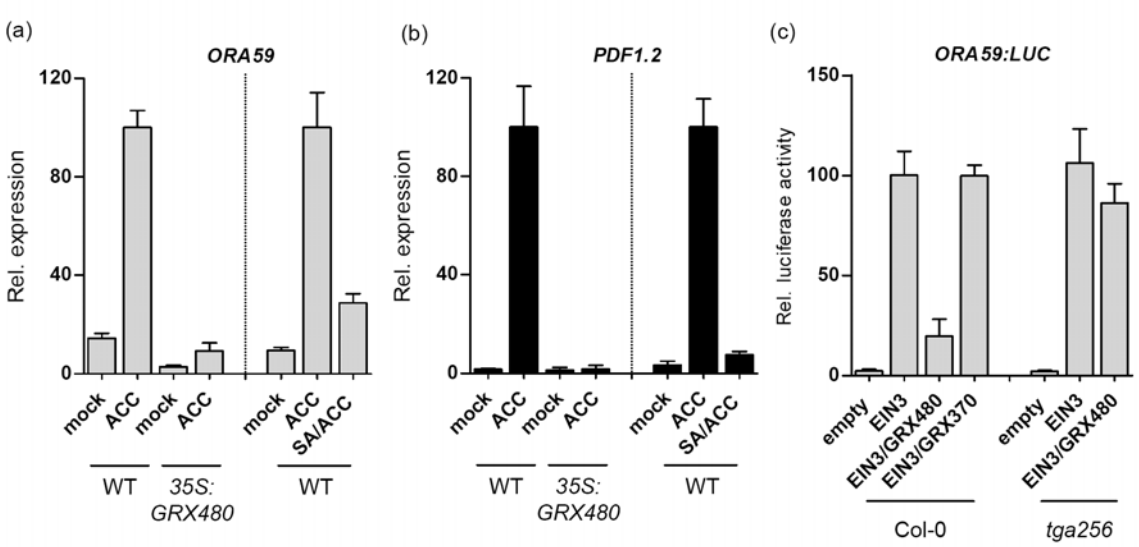

Figure 2. ROXY19/GRX480 suppresses expression from the *ORA59* promoter in transient assays and transgenic plants

(a,b) Four-week-old wild-type and transgenic *35S:GRX480* plants grown on soil were treated for 24 hours with 1 mM of the ET precursor ACC. Control experiments with wild-type plants treated with either 1 mM alone or with 1 mM ACC /1 mM SA was performed in an independent experiment but under the same conditions. Relative *ORA59* (a) and *PDF1.2* (b) transcript levels were determined by quantitative real-time RT-PCR analysis. Values from ACC-treated wild-type plants were set to 100%. The mean values (±SE) obtained from six individual wild-type and six individual *35S:GRX480* mutant plants are shown.
(c) *ORA59* promoter sequences from base pair position -1 to -975 were fused to the firefly luciferase gene. Expression was analyzed in mesophyll protoplasts derived from either wild-type or *tga256* plants in the presence of effector plasmids encoding EIN3, ROXY19/GRX480 and GRX370 under the control of the *CaMV 35S* promoter. Relative LUC activities are expressed in arbitrary luminescence units, normalized to Renilla LUC activity. *ORA59* promoter activity in the presence of EIN3 and the absence ROXYs was set to 100%. Values are means of three replicates (±SE).

Essential function of the C-terminal A(L/I)W(L/V) motif to suppress EIN3-activated *ORA59* promoter activity

Next, all 17 CC-type glutaredoxins were subjected to the functional assay for glutaredoxin mediated repression of *ORA59* promoter activity (Fig.3a). Ten glutaredoxins were able to suppress the EIN3-activated *ORA59* promoter activity. In contrast, seven glutaredoxins had no influence although western blot analysis provided evidence that they were expressed (Fig.3b). As it had been pointed out before that the A(L/)IW(L/V) motif at the C terminus is important for ROXY1 function (Li et al., 2009) we aligned the C terminal sequences of the 17 glutaredoxins and found a strong correlation between the presence of the A(L/I)W(L/V) motif and the capacity to suppress the *ORA59* promoter (Fig.3c). Intriguingly, related motifs like ALWA or AIWI were found in glutaredoxins that were not able to suppress *ORA59* promoter activity. To substantiate the essential role of the C-terminal Leu residue within the ALWL motif,

it was mutated into an Ala residue within the protein sequence of ROXY19/GRX480. Conversely, the Ala residue of the ALWA motif of ROXY20 was converted to a Leu residue. Strikingly, these changes rendered ROXY19/GRX480 less efficient with respect to suppressing *ORA59* promoter activity, whereas ROXY20 gained this capacity (Fig.3d).

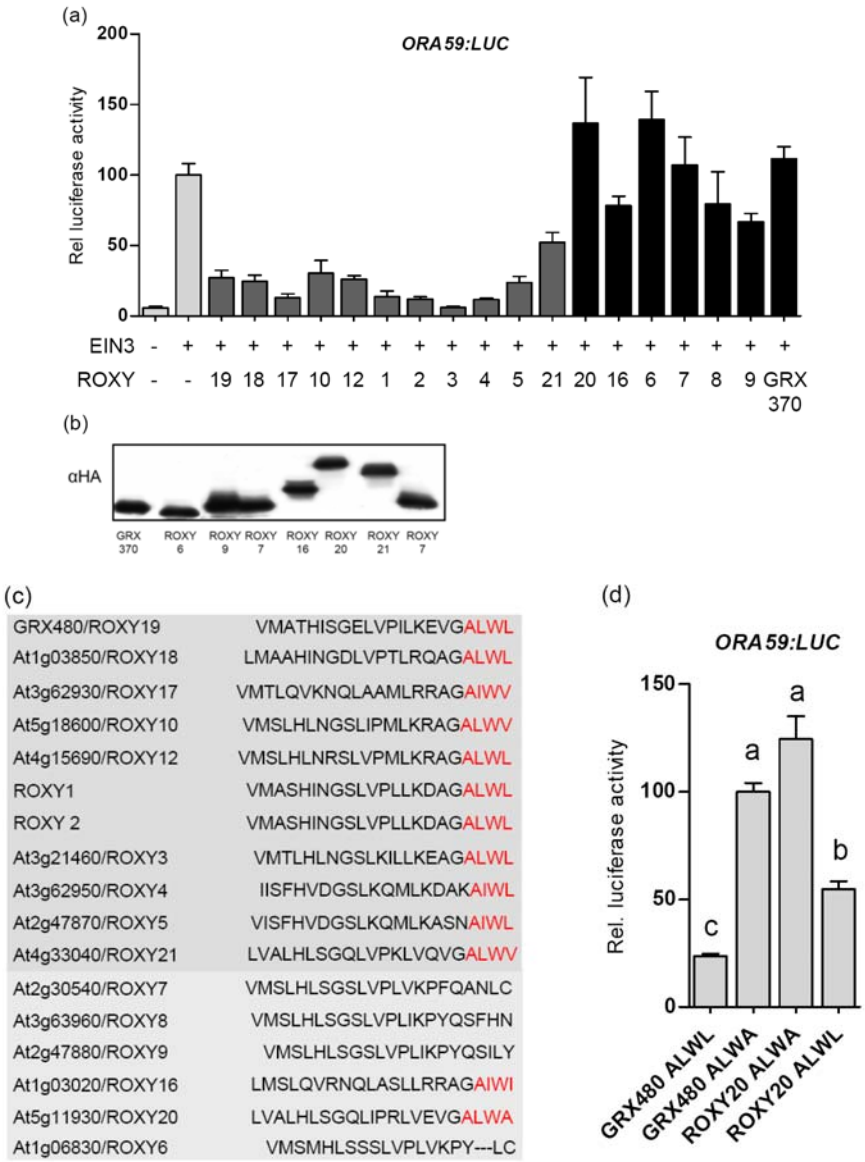

Figure 3. Ten out of 17 tested CC-type glutaredoxins suppress EIN3-activated *ORA59* promoter activity in transiently transformed mesophyll protoplasts

(a) Expression of the *ORA59* promoter fused to the firefly luciferase gene was analyzed in transiently transformed mesophyll protoplasts in the presence of effector plasmids encoding EIN3, different CC-type glutaredoxins (ROXYs) and CPYC-type glutaredoxin GRX370 under the control of the *CaMV 35S* promoter. Relative LUC activities are expressed in arbitrary luminescence units, normalized to Renilla LUC activity. *ORA59* promoter activity in the presence of EIN3 and in the absence of glutaredoxins was set to 100%. Values are means of six replicates (±SE).

(b) Western blot analysis of the expression of non-suppressing glutaredoxins in protoplasts using a HA-antibody.
(c) Alignment of the C terminal sequences of CC-type glutaredoxins. The C-terminal four amino acids A(L/I)W(L/V) shown in red are conserved for all tested CC-type GRXs that suppress *ORA59* promoter activity in transient assays. These are highlighted by a dark grey box.
(d) Expression of the *ORA59* promoter fused to the firefly luciferase gene was analyzed in transiently transformed mesophyll protoplasts in the presence of effector plasmids encoding EIN3, and wild-type and mutant variants of ROXY19/GRX480 and ROXY20 under the control of the *CaMV 35S* promoter. The sequence of the four C terminal amino acid residues is indicated. Relative LUC activities are expressed in arbitrary luminescence units, normalized to Renilla LUC activity. *ORA59* promoter activity in the presence of EIN3 and in the presence of ROXY19/GRX480 ALWA was set to 100%. Values are means of six replicates (±SE). Different letters in indicate significant differences among treatments (Student's t-test, $P < 0.05$).

Analysis of the predicted glutathione binding site of ROXY/19GRX480

Glutaredoxins catalyze the reduction of target proteins through a redox-active site that contains one or two Cys residues. After the transfer of electrons, these are reduced by glutathione which is bound to glutaredoxin at a conserved binding pocket. Mutation of a critical Gly residue in this binding pocket of ROXY1 prevented the complementation of the abnormal flower phenotype in *roxy1* mutants (Xing and Zachgo, 2008). In order to test whether the corresponding mutation within ROXY19/GRX480 affected TGA interaction and/or *ORA59* expression, we mutated the conserved Gly residue at amino acid position 111 into an Ala residue. This mutant protein still interacted with TGA2 in the yeast two hybrid system (Fig.4a). Next, transgenic lines over-expressing this *GRX480m(G111A)* derivative under the control of the *CaMV 35S* promoter were generated and a homozygous transgenic line expressing nearly the same amount of protein compared to the *35S:GRX480* control plants were selected for further analysis (Fig.4b). *ORA59* and *PDF1.2* expression was not affected by the GRX480m(G111A) protein in ET treated plants (Fig.4c,d). Consistently, this protein was not functional in the transient expression system (Fig.4e) again documenting the specificity of this assay. It is concluded that in addition to the capacity of glutaredoxins to interact with TGA factors and the presence of the A(L/I)W(L/V) motif, glutathione binding is required for function.

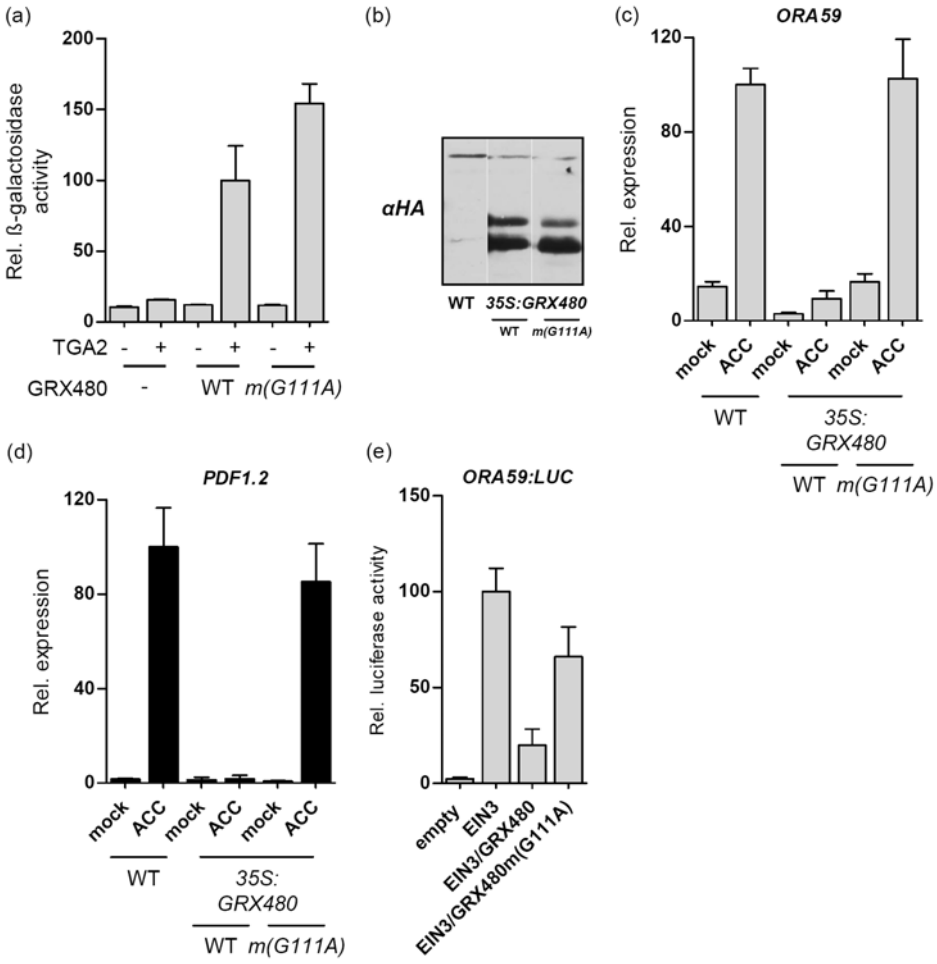

Figure 4. The conserved Gly residue in the glutathione binding site of ROXY19/GRX480 is not important for the interaction with TGA2 but is important for repressing *ORA59* promoter activity

(a) Yeast two hybrid assay. Prey plasmids encode TGA2 fused to the GAL4 binding domain; bait plasmids encode ROXY19/GRX480 and ROXY19/GRX480m(G111A) fused to the GAL4 activation domain; ß-galactosidase expression was measured in yeast strain PJ69-4A, which contains the *lacZ* reporter gene under the control of the GAL7 promoter. Values obtained from the interaction between TGA2 and ROXY19/GRX480 were set to 100 %. The mean value (±SE) from six independent yeast transformants is shown. Hyphens indicate that transformants contained empty bait or prey vectors, respectively.

(b) Western blot analysis of HA-tagged glutaredoxins in homozygous transgenic lines tested in C.

(c,d) *ORA59* and *PDF1.2* expression in transgenic plants expressing *ROXY19/GRX480* and *ROXY19/GRX480m(G111A*), respectively. Four-week-old wild-type and transgenic *35S:GRX480* plants grown on soil were treated for 24 hours with 1 mM of the ET precursor ACC. Relative *ORA59* (c) and *PDF1.2* (d) transcript levels were determined by quantitative real-time RT-PCR analysis. Values from ACC-treated wild-type plants were set to 100%. The mean values (±SE) obtained from six individual plants are shown.

(e) Suppressive effect of *ROXY19/GRX480* and *ROXY19/GRX480m(G111A*) on expression of the *ORA59* promoter in transient assays. Expression of the *ORA59* promoter fused to the firefly luciferase gene was analyzed in transiently transformed mesophyll protoplasts in the presence of effector plasmids encoding EIN3, and wild-type variant of ROXY19/GRX480 and the mutant variant ROXY19/GRX480m(G111A) under the control of the *CaMV 35S* promoter. *ORA59* promoter activity in the presence of EIN3 and in the

absence of glutaredoxins was set to 100%. Values are means of three replicates (±SE). Different letters in indicate significant differences among treatments (Student's t-test, P < 0.05).

Discussion

We show here that 17 CC-type glutaredoxins interact with bZIP transcription factor TGA2 in a yeast two hybrid system. Given the fact that four further glutaredoxins (ROXY11, 13, 14, 15) are very similar to ROXY12 (At4g15680) we assume that all 21 CC-type glutaredoxins are able to interact with TGA2 in yeast. Ten glutaredoxins that are characterized by an A(L/I)W(L/V) motif at the very C-terminus are able to suppress EIN3-mediated transcriptional activation of the *ORA59* promoter and are thus candidates for facilitating the TGA-dependent negative effect of SA on the ET-induced defense pathway.

The interaction between different ROXY proteins and TGA factors has been addressed before (Li et al., 2009). Deletion of the eight C-terminal amino acids including the very C-terminal A(L/I)W(L/V) motif abolished the interaction between ROXY1 and TGA factors PAN, TGA3 and TGA7 in a yeast two hybrid system indicating that these amino acids are important for the interaction. Expression of different glutaredoxins under the control of the *ROXY1* promoter complemented the *roxy1* phenotype only if the glutaredoxins encoded the C-terminal A(L/I)W(L/V) motif. Collectively, these data implicated that the A(L/I)W(L/V) motif is important for the interaction with TGA factors and therefore for their function *in planta*. Here we demonstrate that the A(L/I)W(L/V) motif is not important for the interaction of glutaredoxins with TGA factors but that it is important for their capacity to suppress *ORA59* promoter activity. Our findings can be reconciled with previous data by assuming that truncation of glutaredoxins by 8 C-terminal amino acids disrupt the interaction with TGA factors although the specificity might be determined at another site. An obvious difference between TGA-interacting CC-type glutaredoxins and CPYC-type GRX370 is of course the active site motif itself which is exposed to the surface of the protein (Feng et al., 2006).

Our data are consistent with previously published results that the A(L/I)W(L/V) motif is important for glutaredoxin-mediated functions (Li et al., 2009). Within this motif, the last amino acid has to be a Leu or Val. The closest homolog to ROXY16, which cannot suppress *ORA59* promoter activity and which encodes a C-terminal AIWI motif, is ROXY17, which is functional in this aspect and which encodes a C-terminal AIWL motif. Likewise, mutation of the last amino acid to an Ala residue abolished the suppressive capacity of ROXY19/GRX480 whereas changing an Ala residue of ROXY20 into a Leu residue established this function.

Based on the findings that changing a Cys residue in the activation domain of the floral TGA factor PAN to a Ser residue interfered with PAN function, it has been speculated that glutaredoxins directly target the redox state of PAN (Li et al., 2009). Though this is an attractive model, evidence that this Cys residue is redox-modulated and that this redox modification is mediated by glutaredoxins is missing. The same holds for TGA9 and TGA10. SA-mediated alterations of the redox state has been shown to occur at critical cysteines in TGA1 modulating its in vitro reaction towards the regulatory protein NPR1 (Despres et al., 2003). However, the functional significance of a potential TGA1/NPR1 interaction was never shown. Moreover, evidence that the redox modulation of TGA1 is mediated through glutaredoxins is still lacking. TGA2 does not encode the critical Cys residues that are redox modulated in TGA1 and which is crucial for PAN function. We like to raise the alternative explanation that TGA factors serve to recruit glutaredoxins to their target promoters where they modulate other regulatory factors within the enhanceosome. The A(L/I)W(L/V) motif might serve to mediate the interaction with those targets. This model is consistent with our findings that the interaction of glutaredoxins with TGA factors is not sufficient for their function.

The ability of at least ten glutaredoxins to suppress the *ORA59* promoter suggests a high functional redundancy. ROXY19/GRX480 and its closest homolog ROXY18 are most strongly induced by SA (https://www.genevestigator.ethz.ch/) and are thus the major candidates for mediating the negative cross-talk on the JA/ET pathway. Another candidate is ROXY13, which is weakly induced by SA. The closely related glutaredoxins ROXY14 and ROXY11 which are not represented on microarrays but contain the ALWL motif might contribute to the cross-talk. SA treatment leads to an increase of the total amounts of glutathione and also to a higher ratio of reduced GSH over the oxidized GSSG (Koornneef et al., 2008). It can thus be envisioned that this change in reducing power might activate already pre-existing glutaredoxins to redox modify a yet unknown target protein leading to the inactivation of JA/ET-induced promoters.

The ability of all CC-type glutaredoxins to interact with TGA factors indicates that their ancestors might have already interacted. After gene duplication they diversified to take over distinct functions, with those encoding the A(L/I)W(L/V) motif being able to regulate the expression of genes related to flower development and defense responses. Whether all functions of CC-type glutaredoxins depend on their interaction with TGA factors remains to be shown.

Material and methods

Plant material, growth conditions, chemical treatments and plant transformation

Arabidopsis thaliana (accession Columbia [Col-0]) was used. The *tga256* triple mutant (Zhang et al., 2003) was obtained from Y. Zhang (University of British Columbia, Vancouver, Canada). Plants were grown on soil under controlled environmental conditions (21/19°C, 16-h-light/8-h-dark cycle, 60% relative humidity). For ET induction, four-week old soil-grown plants were sprayed with 1mM of the ethylene precursor 1-aminocyclopropane-carboxylic acid (ACC) (Sigma-Aldrich, St. Louis, Mo, USA). For the SA/ET treatment, 1mM SA (Merck, Darmstadt, Germany) was applied together with the ACC. Water spraying serves as the mock control. Protoplasts assay was carried out as already described (Yoo et al., 2007) using *Arabidopsis thaliana* wild-type plants and *tga256* mutant plants. 7,5µg of each effector plasmid and 5µg of the reporter plasmid pBGWL7:ORA59$_{Pro.-975}$ were transfected. For normalization 1µg of the p70S plasmid containing the *Renilla* LUC gene was co-transfected. Accurate expression of the different effectors was checked on the protein level using a HA-antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, USA).

LUC assays

After 16 hours of incubation the protoplasts were harvested. To measure the LUC activities the dual LUC reporter system from Promega was used with the subsequent analyses in the TD20/20 luminometer from TurnerBiosystems.

Binary vectors and plant transformation

Gateway technology (Invitrogen, Karlsruhe, Germany) was used to generate plasmids for the protoplast assay and yeast-two-hybrid assay. For the protoplast reporter plasmid the promoter region from position -975 to -1 relative to the predicted transcriptional start site of the *ORA59* (At1g06160) gene was amplified (already described in Chapter 3). The promoter fragment was recombined into the binary vector pBGWFL7 (http://www.psb.ugent.be/gateway/) upstream of a luciferase reporter gene. The glutaredoxins and EIN3 were amplified from cDNA using primers which add GATEWAY recombination sites (for primer sequences see table S1) and subsequently recombined into pDONR207 (Invitrogen, Karlsruhe, Germany). The resulting pDONR207 derivatives were recombined into the binary vector pB2GW7-HA for protoplast assays and into pDEST-GBKT7 for yeast-two-hybrid analyses. pDONR207/GRX370, pDONR207/GRX480 and pDONR201/TGA2 were already

described in Ndamukong et al., 2007. pDONR201/TGA2 was recombined into the yeast plasmid pDEST-GAD-HA. The amino acid mutation in GRX480 and ROXY20 were done using primers which introduce the mutation during amplification from cDNA (for primer sequences see table S1). The GRX480 m(G111A) mutation was achieved via site-directed mutagenesis using primers P44/45 and pDONR2077GRX480 as a template. The resulting pDONR/GRX480m(G111A) was recombined into pB2GW7-HA. To generate new transgenic plants the binary plasmids pB2GW7-HA-GRX480 and pB2GW7-HA-GRX480m(G111A) were electroporated (GenePulser II, Bio-Rad) into *Agrobacterium tumefaciens* strain GV3101 (pMP90). These Agrobacteria were used to transform Col-0 plants.

Yeast two hybrid assay

The interaction analysis was conducted as already described in Ndamukong et al., 2007. The yeast strain PJ69-4A was used for the analyses.

Quantitative real-time RT-PCR analysis

RNA extraction and real-time RT PCR analysis was performed as described (Fode et al., 2008b). Calculations were done according to the $2^{-\Delta C_T}$ method (Livak and Schmittgen, 2001). *UBQ5* served as a reference (Kesarwani et al., 2007). Primers used to amplify and quantify the cDNA are indicated in Table S1 (*PDF1.2* (At5g44420) *UBQ5* (At3g62250)). QuantiTect primers were used to amplify mRNA for *ORA59* (At1g06160).

Acknowledgements

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Supplementary material

Table S1: List of primers used for cloning and real-time RT-PCR Analysis

Abbr.	Description	Sequence
P1	ROXY1 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GCA ATA CCA GAC AGA ATC GTG
P2	ROXY1 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC GGA AGG GAT CAG AGC CAG AG
P3	ROXY2 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GCA ATA CAA AAC AGA AAC TCG AG
P4	ROXY2 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC AGC ACT CAT CAG AGC CAA AGA G
P5	ROXY3 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA TGT GGT AGC AAG ATT AGC
P6	ROXY3 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CTA AAG CCA CAA AGC ACC AGC
P7	ROXY4 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA GAG AAT AAG AGA TTT GTC GTC
P8	ROXY4 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CGG ACG CTA TAA CCA AAT GG
P9	ROXY5 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA ACG AGT AAG AGA TTT GGC
P10	ROXY5 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TCA CAA CCA TAT GGC GTT AGA G
P11	ROXY6fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA CAA AGT TAT GAG AAT GTC GTC
P12	ROXY6 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC CCT TCG TTG TTG TTT AAC ATA AAT ATG G
P14	ROXY7 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA CAA AGT TGT GAG AAT GTC G
P15	ROXY7 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC ACA CCT TTT TAA CAT AGA TTG GCT TG

P16	ROXY8 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA CAA GGT TAT GAG AAT GTC ATC
P17	ROXY8 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTC TTA AGC ATC GAT ATT GTT TTT CTA G
P18	ROXY9 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA CAA AGT GAT GAG AAT GTC TTC
P19	ROXY9 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTG CTA GTA AAG GAT GGA CTG ATA GG
P20	ROXY10 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA TAT GAT AAC GAA GAT GGT GAT G
P21	ROXY10 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC AAA ACA TGA TAA GTC AAA CCC ACA ATG
P22	ROXY12 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA GAA CCT ACA GAA GAT GAT C
P23	ROXY12 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TGA AGT TAA AGC CAT AAT GCT CC
P24	ROXY16 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA GAA GAT ATC AAA TTT GTT AGA AGA C
P25	ROXY16 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA AAT CCA TAT GGC TCC AGC TC
P26	ROXY17 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GGA AAG CGT TAG AAG TTT AG
P27	ROXY17 rev.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CTT ACA CCC ATA TGG CTC CG
P28	ROXY18 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GCA AAA AGC AAT TCG ACC
P29	ROXY18 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA GGA GGA TTA AAA ATC AAA GCC
P30	ROXY19/GRX480 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GCA AGG AAC GAT TTC TTG TG
P32	ROXY20 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC

		CAT GAA GAC GAT GCG AGG TTT AC
P33	ROXY20 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC ACA ATT ATG CCC ATA AGG CTC C
P34	ROXY21 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GAT GCA AGA ATT AGG CTT ACA AC
P35	ROXY21 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC AAG ACG ATG AAA ATT ACA ATC ATA CC
P36	ROXY19 /GR480 ALWA rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TCA TGC CCA CAG AGC CCC AAC TTC C
P37	ROXY20 ALWL fwd.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTA CAA CCA TAA GGC TCC AAC CTC G
P38	PDF1.2 RT fwd.	CTT GTT CTC TTT GCT GCT TTC
P39	PDF1.2 RT rev.	CAT GTT TGG CTC CTT CAA G
P40	UBQ5 RT fwd.	GAC GCT TCA TCT CGT CC
P41	UBQ5 RT rev.	GTA AAC GTA GGT GAG TCC A
P42	EIN3 fwd.	GGG GAC AAG TTT GTA CAA AAA AGC AGG CTC CAT GAT GTT TAA TGA GAT GGG AAT GTG
P43	EIN3 rev.	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTC TTT AGA ACC ATA TGG ATA CAT CTT GCT G
P44	ROXY19/GRX480 m(G111A) fwd	GGA GGG AGG TTG TTT GCA GGG TTA GAT AGG G
P45	ROXY19/GRX480 m(G111A) rev	GGG TAT CTA ACC CTG CAA ACA ACC TCC CTC C

Chapter V

JA-induced GRX480 is responsible for the negative effect of MYC2 on the JA/ET pathway

Mark Zander[1], Shuxia Chen[2], Martin Muthreich[1], Julia Imkampe[1], Corinna Thurow[1] and Christiane Gatz[1]

[1]Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Georg-August-Universität Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany

[2]College of Horticulture Science, Northwest A&G University, 3-Taicheng Road, Yangling, Shaanxi, 712100, China

Abstract

In plants, the phytohormone jasmonic acid (JA) regulates defense responses after wounding or herbivore attack. In addition to activating the JA pathway, transcription factor MYC2, negatively regulates a different set of defense genes that is under the control of JA and ethylene (ET). We have shown previously that MYC2 exerts its negative effect by antagonizing the positive action of bZIP transcription factors of the TGA family and that ectopic expression of the TGA-interacting glutaredoxin GRX480 suppresses the JA/ET pathway. Here we show that *GRX480* expression is induced by JA-activated MYC2 suggesting that the negative effect of MYC2 on the JA/ET pathway is realized through GRX480 and/or related glutaredoxins. As a consequence of the JA/MYC2/GRX480-mediated down-regulation of the ET pathway, the reciprocal negative effect of ET on the JA pathway is compromised, thus establishing a GRX480-driven feed forward loop that positively affects MYC2 expression. This mechanism ensures a balanced activation of both pathways depending on the type of stress encountered by the plant.

Introduction

JA is the key signalling compound coordinating defense responses upon wounding, insect attack or infection with necrotrophic pathogens (Thomma et al., 1999; Zhang and Turner, 2008.). Central components of the JA signalling framework are the F-box protein COI1 (CORONATINE INSENSITIVE1), the JAZ repressors (JASMONATE ZIM-domain) and MYC2 (Feys et al.,1994; Xie et al., 1998; Lorenzo et al., 2004; Thines et al., 2007; Chini et al., 2007). JAZ repressors interact with the transcription factor MYC2 to inhibit its function (Chini et al., 2007). MYC2 activity is released when the JAZ repressors are targeted by COI1 whose function as JAZ-repressor-specific E3 ligase is activated by its ligand JA-isoleucine (Thines et al., 2007; Chini et al., 2007; Sheard et al., 2010). After ligand perception, JAZ repressors are ubiquitinylated and subsequently degraded by the 26S proteasome (Thines et al., 2007; Chini et al., 2007). Liberated MYC2 activates its own gene and known JA-responsive genes like *VSP2* (Lorenzo et al., 2004; Dombrecht et al., 2007).

The combination of JA and ET synergistically activates a yet different set of defense genes like *PDF1.2* and *ORA59* (Penninckx et al., 1998; Pre et al., 2008). These genes can be activated by either ET or JA but require central components of the ET pathway and COI1, irrespective of the treatment. MYC2 negatively regulates this pathway as revealed by hyper-induction of *PDF1.2* and *ORA59* in the JA-induced *jin1-1* mutant (Lorenzo et al., 2004; Zander et al., 2010, Chapter 2). Since MYC2 is not expressed if only ET is applied, it does not effect ET-induced expression of *ORA59* or *PDF1.2*. In contrast, subclass II TGA transcription factors are required if the pathway is induced by ET (Chapter 2,3), but not if it is induced by JA. While JA and ET act synergistically within the JA/ET pathway, ET acts antagonistically on the JA pathway. This becomes apparent in JA-treated or wounded ethylene-insensitive mutants where a hyper-stimulated JA response is observed (Rojo et al., 1999).

SA antagonizes both the JA pathway and the JA/ET pathway (Spoel et al., 2003). Loss-of-function analyses revealed that NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1) and subclass II TGA transcription factors are essential regulators for the suppression of the JA/ET pathway if this is induced by JA (Spoel et al., 2003; Ndamukong et al., 2007). In the presence of ethylene, the cross-talk becomes NPR1-independent (Leon-Reyes et al., 2009), but remains TGA-dependent (Zander et al., 2010; Chapter 2).

Here we show that the TGA-interacting glutaredoxin GRX480, which suppresses the JA/ET pathway, is activated by JA-induced MYC2 suggesting that the negative effect of MYC2 is mediated by GRX480 which in turns interferes with the positive function of

TGA factors within the JA/ET pathway. As the consequence of the down-regulation of the JA/ET pathway its inhibitory effect on the JA pathway is decreased prioritizing thereby the JA response.

Results

Genetic evidence suggests that MYC2-activated *GRX480* negatively regulates TGA factors at the *ORA59* promoter

Based on the observation, that MYC2 and GRX480 are both negative regulators of the JA/ET-pathway, we questioned whether they act within one cascade. Therefore, we monitored *GRX480* transcript levels in wild-type and *jin1-1* mutant plants (Berger et al., 1996) after JA treatment. In wild-type plants, the expression of *GRX480* rose already after 1h of induction and increased after 4h and 8h, whereas nearly no induction was detectable in *jin1-1* mutant plants (Fig.1a). Reduced *GRX480* expression in the *jin1-1* mutant correlates with a hyper-induction of *ORA59* (Fig.1a,b). Since ectopically expressed *GRX480* represses *ORA59* transcription (Chapter 4), reduced *GRX480* expression as found in the *jin1-1* mutant might be the reason for enhanced *ORA59* expression. GRX480-mediated suppression of *ORA59* promoter activity depends on TGA factors (Chapter 4). Likewise, hyper-induction of *ORA59* expression in the *jin1-1* mutant depends on TGA factors as indicated by the reduced JA inducibility of *ORA59* in the *tga256 jin1-1* quadruple mutant as compared to the *jin1-1* mutant (Fig.1c). These data collectively suggest that MYC2-activated GRX480 interferes with TGA factor activity at the *ORA59* promoter. At the same time, these data explain, why the induction of the *ORA59* promoter after ET treatment depends on TGA factors, whereas induction after JA treatment does not (Fig.1d): In the presence of ET, MYC2 is not activated. As a consequence, *GRX480* is not expressed so that TGA factors can contribute to promoter activity. In the presence of JA, MYC2 is activated leading to the expression of *GRX480* which inactivates TGA function at the promoter. Due to the JA-induced degradation of JAZ repressors, activation of the *ORA59* promoter can occur even without effective TGA factors, whereas they are required for the induction by ET-induced stabilization of EIN3.

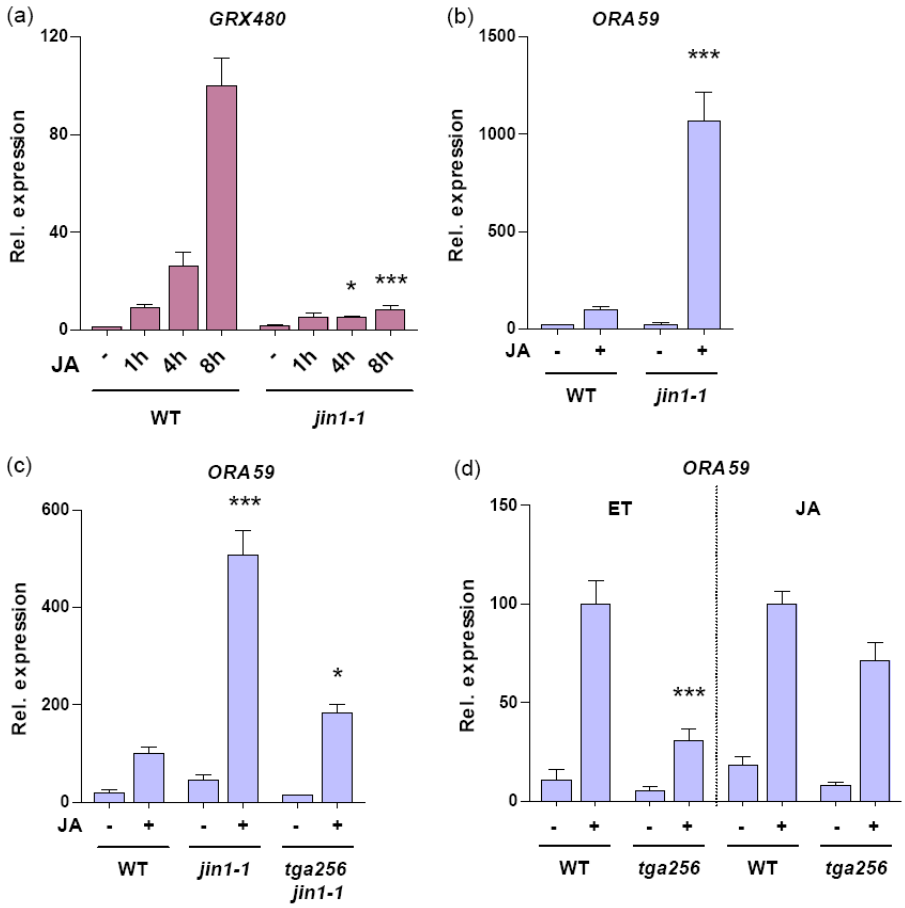


Figure 1. Relationship between *GRX480* and *ORA59* expression

(a) Time-course of *GRX480* expression after JA-treatment in *jin1-1* mutant plants. Four-week old soil-grown wild-type and *jin1-1* plants were induced with methyl jasmonate (MeJA) via the gaseous phase in glass containers for the indicated hours. The mock value is from plants which were for 8 hours in a glass container without MeJA. The relative *GRX480* transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in wild-type plants after 8 hours were set to 100%. The mean values (±SE) from five individual plants per genotype are shown.

(b) The relative *ORA59* transcript levels were determined by quantitative real-time RT-PCR analysis using cDNA from the 8 hours time-point from Fig.1a.

(c) Four-week old soil-grown wild-type, *jin1-1* and *tga256 jin1-1* mutants were induced with methyl jasmonate (MeJA) via the gaseous phase for 24 hours. The mock value is from plants which were for 24 hours in a glass container without MeJA. The relative *ORA59* transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in wild-type plants were set to 100%. The mean values (±SE) from five individual plants per genotype are shown.

(d) Four-week old soil-grown wild-type and *tga256* mutants were or spray-induced with 1 mM ACC or induced with methyl jasmonate (MeJA) via the gaseous phase for 8 hours. The ACC-control is from plants which were sprayed for 24 hours with water. For the MeJA control plants were for 8 hours in a glass container without MeJA. The relative *ORA59* transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in wild-type plants were set to 100%. The mean values (±SE) from five individual plants per genotype are shown. Asterisks represent significant differences between wild-type and the indicated mutants (two-way ANOVA, **P* < 0.05, ****P* < 0.001).

Negative regulation of the JA/ET pathway by GRX480 correlates with hyper-induction of the JA pathway

In a previous study, we have shown that JA-induced expression of *PDF1.2* is suppressed in *35S:GRX480* transgenic plants (Ndamukong et al., 2007). To investigate whether indeed a major part of the JA/ET defense pathway is compromised, symptom development after infection with the necrotrophic fungus *Botrytis cinerea* was monitored in *35S:GRX480* plants. To this end, wild-type and transgenic *35S:GRX480* plants were drop-inoculated with *Botrytis cinerea* and the diameters of the resulting lesions were quantified. The lesion diameters were nearly two-fold higher in *35S:GRX480* mutant plants as compared to wild-type plants showing widespread necrotic lesions (Fig.2).

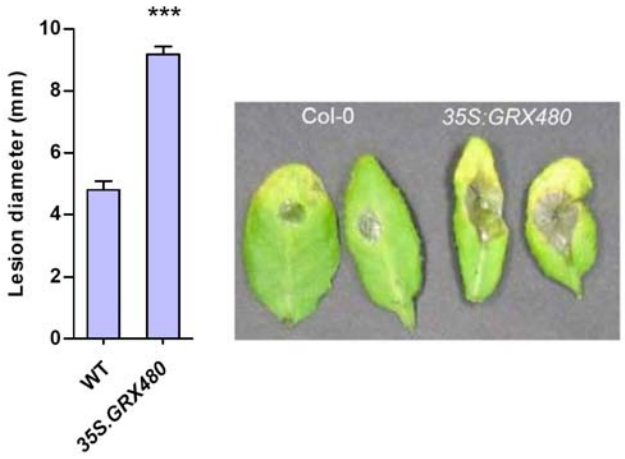
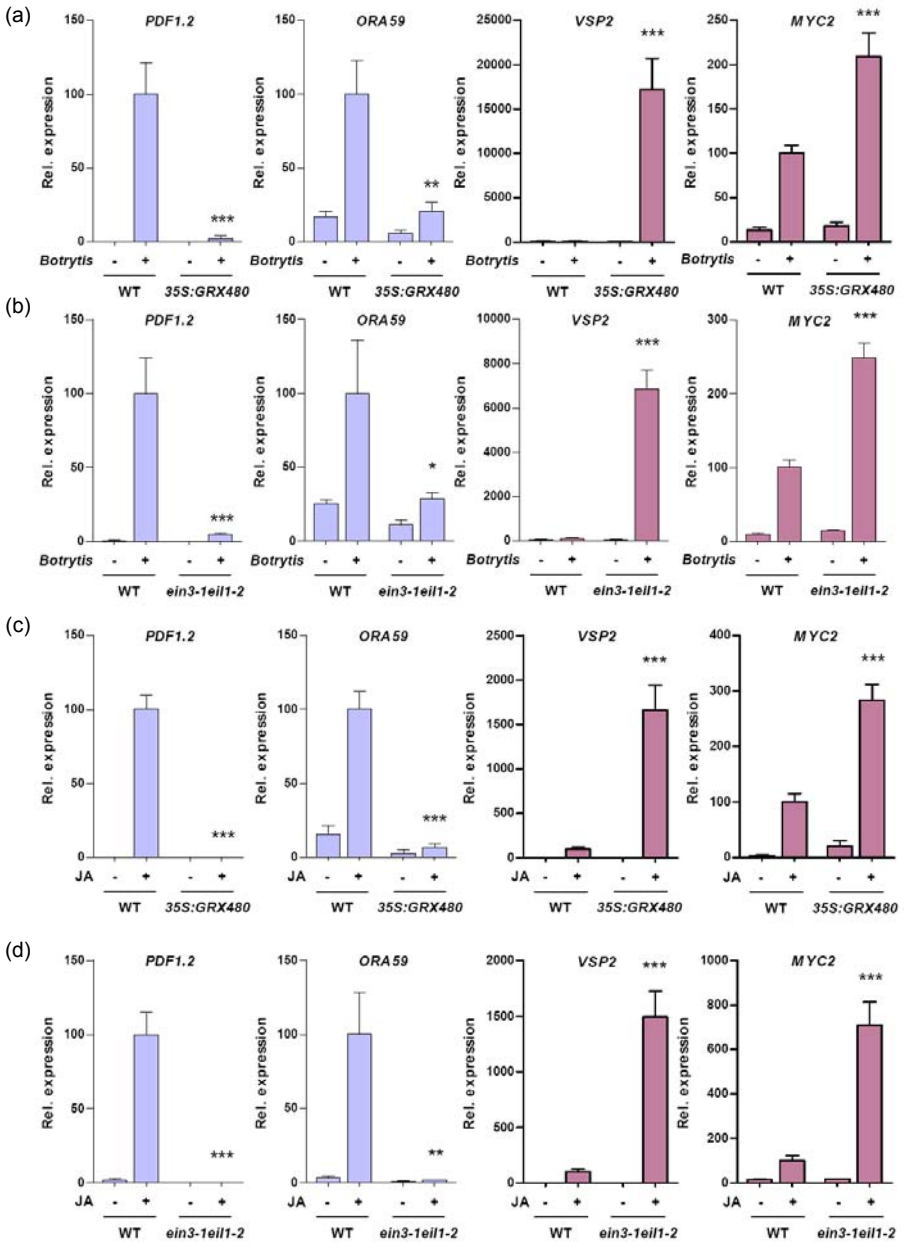


Figure 2. The defense response against *Botrytis cinerea* is compromised in *35S:GRX480* mutant plants

Lesion sizes of wild-type and *35S:GRX480* mutant plants after 3 days of infection with *Botrytis cinerea*. 4-week-old soil grown plants were drop-inoculated with a *Botrytis cinerea* spore solution (5 x 10^4 spores ml^{-1}) or with quarter-strength potato dextrose broth (mock). The diameters of at least 40 lesions per experiment were measured. The mean lesion diameters (±SE) of two independent experiments are shown.

Consistently, expression of the key regulator of the JA/ET pathway, *ORA59* and its target gene *PDF1.2* were reduced (Fig.3a). In contrast to this, a 170-fold stronger induction of *VSP2* and a two-fold stronger induction of *MYC2* were detected when comparing wild-type with transgenic *35S:GRX480* plants (Fig.3a). This reciprocal regulation was reminiscent of expression data from the ethylene insensitive mutant *etr1-3*. The JA response after wounding was hyper-induced, indicating that the ET pathway negatively regulates the JA pathway (Rojo et al., 1999). To test whether this mechanism is also operational after *Botrytis cinerea* infection, we analyzed defense gene expression in *Botrytis cinerea*-infected ethylene insensitive *ein3-1eil1-2* mutant

plants. As observed in *35S:GRX480* plants, *ORA59* and *PDF1.2* expression was down-regulated whereas *MYC2* and *VSP2* were up-regulated (Fig.3b). Since GRX480 suppresses the JA/ET pathway, we conclude that the prioritization of the JA pathway in *35S:GRX480* plants is a consequence of the GRX480-mediated down-regulated of the EIN3-dependent JA/ET pathway. This reciprocal regulation was also observed after JA treatment (Fig.3c,d) and wounding experiments (Fig.3e). In *35S:GRX480* and *ein3-1eil1-2* mutants, the JA/ET pathway is suppressed leading to the hyper-induction of the JA pathway.



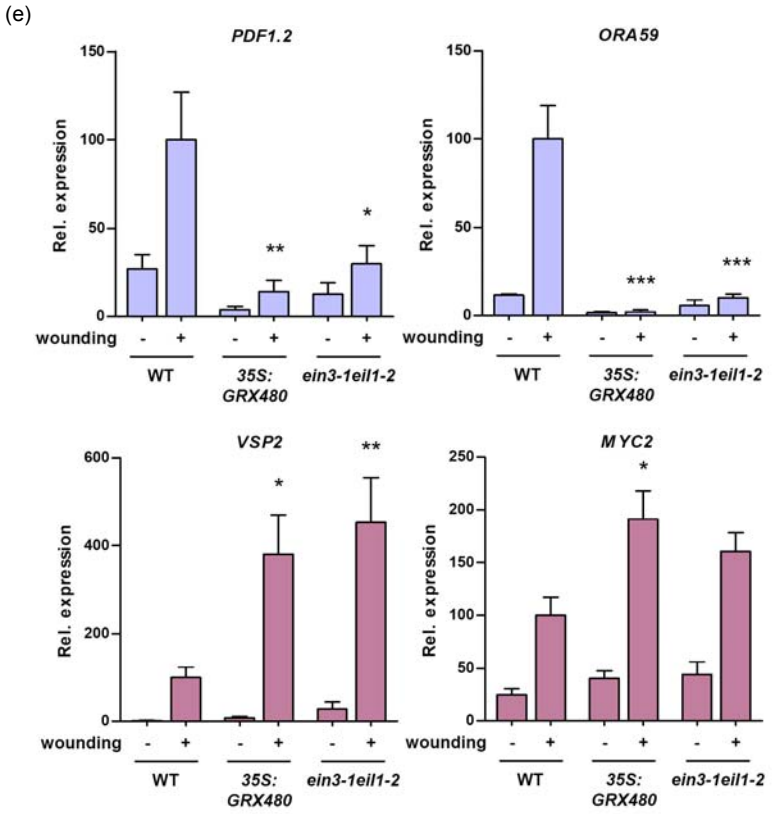


Figure 3. Gene response comparison after *Botrytis cinerea* infection, JA treatment and wounding between *35S:GRX480* and *ein3-1eil1-2* mutants

Quantitative real-time RT-PCR analysis of relative *PDF1.2*, *ORA59*, *VSP2* and *MYC2* transcript levels in wild-type, *35S:GRX480* (a) and *ein3-1eil1-2* (b) mutants after 3 days of drop-inoculation with *Botrytis cinerea*. The average of the relative respective transcript levels in 5 infected wild-type plants was set to 100%. The mean values obtained (±SE) from 5 individual plants per genotype are shown. Asterisks represent significant differences between wild-type and *35S:GRX480* mutant within a treatment (two-way ANOVA: ***, $P < 0.001$).

Quantitative real-time RT-PCR analysis of relative *PDF1.2*, *ORA59*, *VSP2* and *MYC2* transcript levels in wild-type, *35S:GRX480* (c) and *ein3-1eil1-2* (d) mutants after 24 hours of JA treatment. JA-treatment was carried out as described in Fig.1. The relative expression values in wild-type plants were set to 100%. The mean values (±SE) from five individual plants per genotype are shown.

(e) Four-week old soil-grown wild-type, *35S:GRX480 and ein3-1eil1-2* mutants were wounded with a forceps three times on each leaf half and four leaves per plant. The relative *PDF1.2*, *ORA59*, *VSP2* and transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in wild-type plants were set to 100%. The mean values (±SE) from 10 individual plants per genotype are shown. Asterisks represent significant differences between wild-type and *35S:GRX480* mutants *or ein3-1eil1-2* mutants (two-way ANOVA, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

Root growth of transgenic *35S:GRX480* and *ein3-1eil1-2* plants is hyper-sensitive to JA

Mutants with an altered response to JA have been screened in an ethylene-insensitive mutant background to increase the sensitivity of the assay (Lorenzo et al., 2004). To further substantiate our notion that 35S:*GRX480* plants mimic ethylene-insensitive mutants with regard to their reduced repression of the ET signalling cascade, root growth inhibition assays were conducted. Wild-type plants grown on agar plates supplemented with 50µM MeJA exhibited the expected root growth inhibition response which was partially alleviated in *jin1-1* mutant plants. In contrast, the response was more pronounced in *35S:GRX480-HA* and *ein3-1 eil1-2* plants (Fig 4).

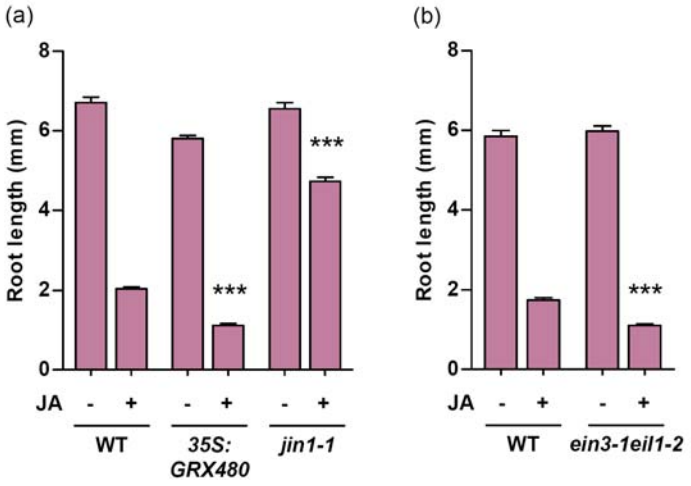


Figure 4. JA-induced root growth inhibition in *35:GRX480*, *jin1-1* and *ein3-1eil1-2* mutants

Quantification of the JA-induced root growth inhibition response of eight-day old wild-type *35:GRX480* (a), *jin1-1*(a) and *ein3-1eil1-2* (b) seedlings grown on MS plates with or without 50 µM MeJA. Bars represent the average root length of 20 seedlings (±SE). Asterisks represent significant differences in the inhibition strength between wild-type and the indicated mutant seedlings (two-way ANOVA ***$P < 0.001$).

The glutathione binding site of GRX480 is required for its capacity to modulate JA responses

Glutaredoxins catalyze the reduction of target proteins through a redox-active site that contains one or two cysteine residues. Oxidized cysteine residues are reduced by glutathione which is bound to glutaredoxin at a conserved binding pocket. Mutation of a critical glycine in this binding pocket prevents the suppressive effect of GRX480 on the ET-induced *ORA59* promoter (Chapter 4). In order to test, how whether this protein was also non-function under conditions of elevated JA levels, transgenic *35S:GRX480m(G111A)* plants were infected with *Botrytis cinerea* or treated with JA. *ORA59* and *MYC2* were expressed to the same level as in wild-type plants indicating

that their imbalanced expression can only be triggered by a redox-active GRX480 (Fig.5a,b).

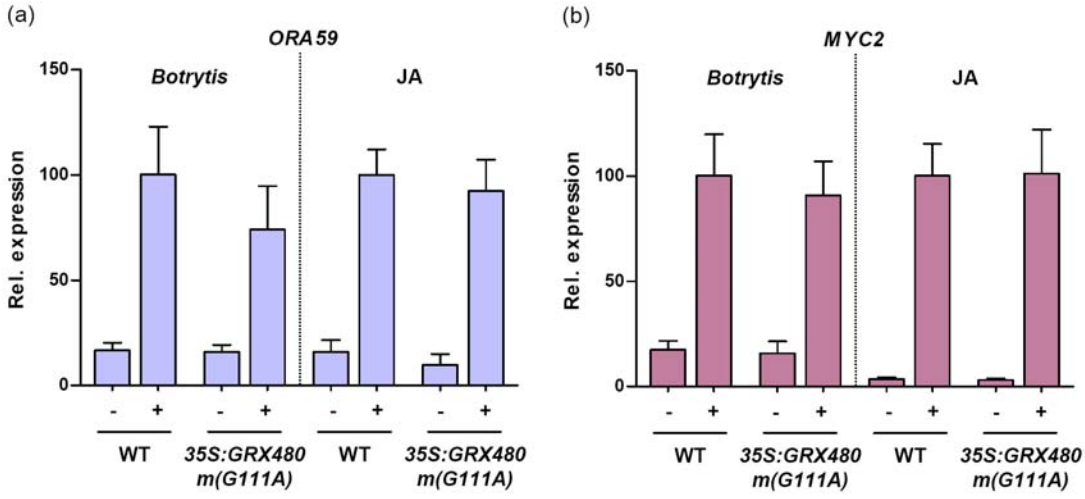


Figure 5. *ORA59* and *MYC2* expression after JA-treatment and *Botrytis cinerea* infection in *35S:GRX480m(G111A)* mutant plants

Four-week old soil-grown wild-type and transgenic *35S:GRX480m(G111A)* plants were induced with methyl jasmonate (MeJA) or drop-inoculated with *Botrytis cinerea* as described in Fig.2. Relative *ORA59* (a) and *MYC2* (b) transcript levels were determined by quantitative real-time RT-PCR analysis. Relative expression values found in induced wild-type plants were set to 100%. The mean values (±SE) from five individual plants are shown. No significant differences between wild-type and *35S:GRX480m(G111A)* plants were detectable.

Discussion

Plant immunity is regulated by a complex network of cross communicating signalling pathways. It has been described before that the JA pathway which is effective against insect attack antagonizes the JA/ET pathway which is efficient against necrotrophic pathogens (Glazebrook, 2005). Here we provide circumstantial evidence that JA-induced expression of *GRX480* mediates the negative effect that JA exerts on the JA/ET pathway. As a consequence of suppression of the JA/ET pathway through ectopically expressed *GRX480* the JA pathway is hyper-induced (Fig 3). Thus GRX480, whose transcription and reducing activity is enhanced by SA (Ndamukong et al., 2007), provides a link between the three pathways. Based on the finding that GRX480 interacts with TGA factors and that TGA factors control *ORA59* promoter activity, we suggest that GRX480 regulates an activating protein at the *ORA59* promoter. Based on the finding that that GRX480 requires a conserved glycine residue in the glutathione binding domain (Fig.5) we speculate that GRX480-mediated

suppression of the JA-induced *ORA59* promoter works through the redox-regulation of a yet unknown target protein.

Less is known how the ET-mediated suppression of the JA-pathway is mechanistically achieved. MYC2 expression in *ein2-5* mutants after JA-application is increased compared to wild-type plants (Lorenzo et al., 2004) which confirms our results with JA-treated *ein3-1eil1-2* mutants. *ERF1* over-expression causes a suppression of JA-induced *VSP2* expression without affecting *MYC2* expression (Lorenzo et al., 2004). This suggests that the negative cross-talk can be exhibited by AP2/ERF transcription factors. This is supported by our results that JA-treated *ora59rnai* mutant plants show a significantly increased *MYC2* and *VSP2* expression (Fig.S1). The more pronounced effect in *ein3-1eil1-2* mutants suggests that additional EIN3-activated processes are involved in the repression of the JA pathway. This is supported by the finding that JA-induced hyper-stimulated *VSP2* expression in *35S:GRX480* mutant plants is suppressed by an additional ACC-treatment (Fig.S2). It is possible that EIN3 directly represses *MYC2* transcription. For example the *SID2* (*SALICYLIC ACID-DEFICIENT2*) promoter is directly repressed by EIN3 (Chen et al., 2009).

Ndamukong and colleagues propose GRX480 as a mediator of the SA/JA cross-talk because it is induced by SA, functionally linked to TGA factors which are indispensable for the SA/JA cross-talk (Ndamukong et al., 2007). It is therefore plausible that SA and JA use the same mechanism to suppress JA/ET signalling. However, obvious differences are observable. Despite the JA-induced negative action of GRX480 on *ORA59* expression, still a strong *ORA59* activation becomes apparent due to an unknown JA-inducible MYC2-independent activator. In contrast an additional SA treatment strongly antagonizes JA-induced *ORA59* expression. Perhaps explainable due to the SA-triggered increase of glutathione levels (Koornneef et al., 2008) serving as a substrate for glutaredoxin function. The experiments with *35S:GRX480m(G111A)* mutants showing no suppression of the JA/ET pathway support the idea that reductive capacity is required. The strong phenotype of *35S:GRX480* mutants without SA accumulation suggests that high amounts of GRX480 increase the chance to get reduced by glutathione. Another difference between *35S:GRX480* plants and SA treatment is the prioritization of the JA-pathway. It is stimulated by GRX480 however after SA-treatment the JA-pathway is suppressed like the JA/ET pathway (Spoel et al., 2003). Hence another second cross-talk mechanism has to be proposed.

JA activates MYC2 to trigger its own transcription (Dombrecht et al., 2007) but simultaneously several feedback-loops are initiated. Beside the JA-induced JAZ repressors also the JA-activated MKK3-MPK6 cascade serves to attenuate *MYC2* transcription avoiding an undamped JA response (Takahashi et al., 2007). Here we

reported the existence of a further regulatory step involving a GRX480-mediated feed-forward loop that triggers MYC2 expression via the down-regulation of the ethylene pathway

Material and method

Plant material, growth conditions, and chemical treatments

Arabidopsis thaliana (accession Columbia [Col-0]) was used. The *tga256* triple mutant (Zhang et al*.,* 2003) was obtained from Y. Zhang (University of British Columbia, Vancouver, Canada)the *jin1-1* mutant from S. Berger (Julius-Maximilians University, Würzburg, Germany). *ein3-1eil1-2* mutants were kindly provided from Richard Vierstra (University of Wisconsin, USA). *ora59rnai*-lines (Pre et al*.,* 2008) were obtained from J. Memelink (University of Leiden, The Netherlands). Generation of *tga256 jin1-1* quadruple mutants were previously described (Zander et al., 2010). Transgenic plants carrying the *35S:GRX480* or *35S:GRx480m(G111A* construct were generated as described in Chapter IV. Plants were grown on soil under controlled environmental conditions (21/19°C, 16-h-light/8-h-dark cycle, 60% relative humidity). For ET induction, four-week old soil-grown plants were sprayed with 1mM of the ethylene precursor 1-aminocyclopropane-carboxylic acid (ACC) (Sigma-Aldrich, St. Louis, Mo, USA). Water spraying serves as the mock control. JA induction of four-week old soil-grown plants was performed in closed glass boxes, where 4.5 µM MeJA (Sigma-Aldrich, St. Louis, Mo, USA) was applied via the gaseous phase. For JA-induced root growth inhibition seedlings were grown on Murashige and Skoog (MS) medium supplemented with or without 50 µM MeJA.

Pathogen infections

For *B. cinerea* infection experiments, five-week old soil-grown wild-type, *35S:GRX480*, *35S:GRx480m(G111A)* and *ein3-1eil1-2* mutants were grown on a under controlled environmental conditions (20-22/16-18°C h 12-h-light/12-h-dark cycle, 60–70% relative humidity). *Botrytis cinerea* strain (isolate 2100, Spanish Type Culture Collection) described in Wang et al., 2008. Droplets of 5 µl of spore suspension (5 x 10^4 spores ml^{-1}) in Vogel buffer were deposited on leaves of five-week-old plants. For assessment of symptom development the diameter of the lesions was measured after 3 days. Vogel buffer served as mock for drop-inoculated plants.

Quantitative real-time RT-PCR analysis

RNA extraction and real-time RT PCR analysis was performed as described (Fode et al., 2008b). Calculations were done according to the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001). *UBQ5* served as a reference (Kesarwani et al*.,* 2007)**.** Primers used to amplify and quantify the cDNA are indicated in Table S1 (*PDF1.2* (At5g44420) P24-25, *UBQ5* (At3g62250) P26-27). QuantiTect primers to amplify mRNA for *ORA59* (At1g06160), *MYC2* (At1g32640) and *GRX480* (At1g28480) were obtained from Quiagen, Düsseldorf, Germany.

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Supplementary material

Figure S1. Expression of *VSP2* and *MYC2* in wild-type and *ora59rnai* mutant plants after treatment with JA

Figure S2. Expression of *VSP2* in wild-type and *35S:GRX480* mutant plants after a combined treatment with JA and ACC

Table S1. List of primers used for and real-time RT-PCR analysis

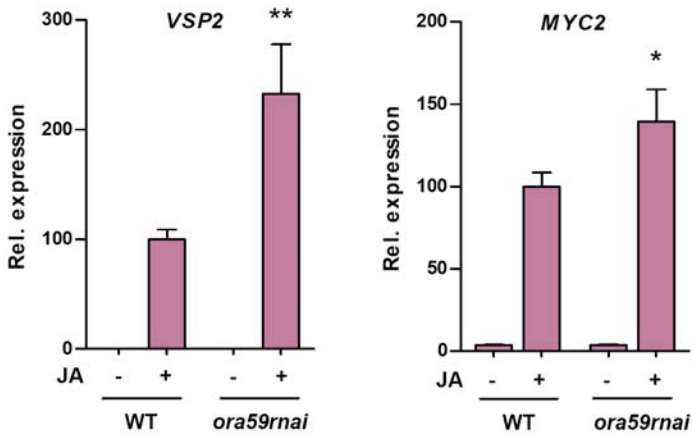

Figure S1. Expression of *VSP2* and *MYC2* in wild-type and *ora59rnai* mutant plants after treatment with JA

Quantitative real-time RT-PCR analysis of relative *VSP2* and *MYC2* transcript levels in wild-type and *ora59rnai* mutants after 24 hours of JA treatment. JA-treatment was carried out as described in Fig.1. The relative expression values in wild-type plants were set to 100%. The mean values (±SE) from five individual plants per genotype are shown (two-way ANOVA, $*P < 0.05$, $**P < 0.01$)

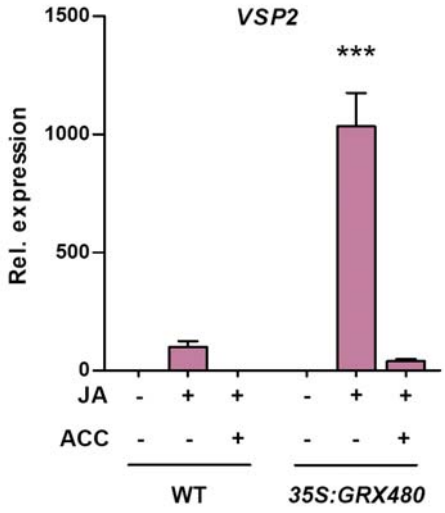

Figure S2. Expression of *VSP2* in wild-type and *35S:GRX480* mutant plants after a combined treatment with JA and ACC

Four-week old soil-grown wild-type and *35:GRX480* mutants were induced with methyl jasmonate (MeJA) via the gaseous phase for 24 hours in a glass container alone or in combination with a spray-induction of 1 mM ACC. Control plants were sprayed for 24 hours with water in a glass container without MeJA. The relative *VSP2* transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in wild-type plants were set to 100%. The mean values (±SE) from five individual plants per genotype are shown. Asterisks represent significant differences between wild-type and the indicated mutants (two-way ANOVA, ***$P < 0.001$).

Table S1: List of primers used real-time RT-PCR analysis

Abbr.	Description	Sequence
P1	PDF1.2 RT fwd.	CTT GTT CTC TTT GCT GCT TTC
P2	PDF1.2 RT rev.	CAT GTT TGG CTC CTT CAA G
P3	UBQ5 RT fwd.	GAC GCT TCA TCT CGT CC
P4	UBQ5 RT rev.	GTA AAC GTA GGT GAG TCC A
P5	VSP2 RT fwd.	CAA ACT AAA CAA TAA ACC ATA CCA TAA
P6	VSP2 ET rev.	GCC AAG AGC AAG AGA AGT GA

Chapter VI

General discussion

We have discovered that subclass II TGA transcription factors are positive regulators of the ET defense response. The interaction of TGA factors with glutaredoxins enables SA and JA to negatively manipulate the ET pathway. We have integrated our findings in one working model (Fig.1). Central point in our model is the promoter of the *ORA59* gene which encodes the master regulator of the JA/ET pathway (Pre et al., 2008). Within a stretch 975 base pairs, at least three hormone stimuli are integrated: SA, JA and ET.

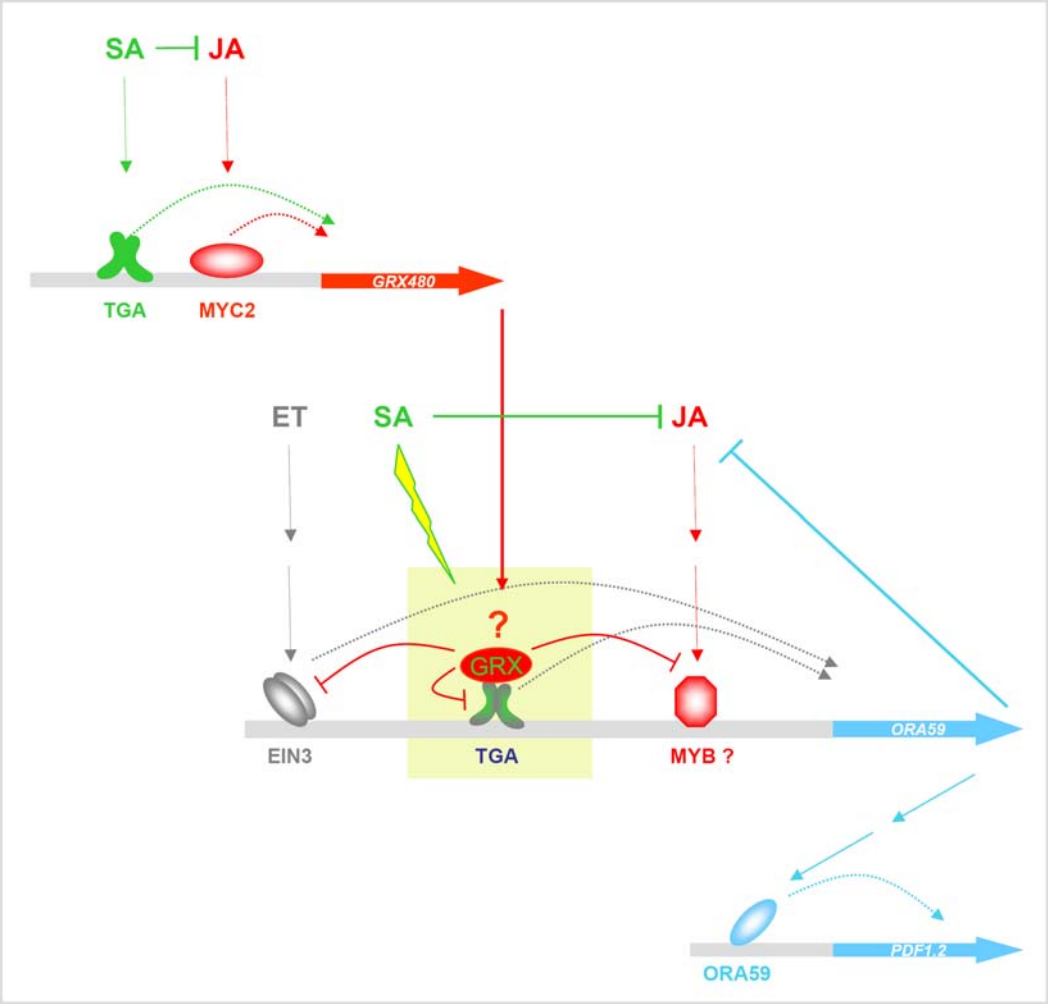

Figure 1. Working model describing the modulation of the JA/ET-induced *ORA59* promoter by JA and SA through TGA-interacting glutaredoxins (for further details, see text).

The *ORA59* promoter can be activated by ET (Fig.1 marked in grey) and by JA (Fig.1 marked in red), but both pathways have to be intact even if only one of the hormones is applied. The ET-induced activation mechanism requires EIN3 to initiate and TGA factors to maintain *ORA59* transcription (Chapter 3). Moreover, the JA-Ile receptor

COI1 is necessary for induction indicating that a basal turn-over of JAZ repressors is required (Pre et al., 2008). Basal and JA-induced activities of the JA pathway are most likely integrated at the critical G-box between base pair positions –333 and –328 (Chapter 3). However, JA does not only induce the promoter, but at the same time it reduces its activity. This becomes apparent in the *jin1-1* mutant where the promoter is hyper-induced after JA but not after ET induction (Chapter 2, 5). In contrast to the JA induction in wild-type plants, hyper-induction in the *jin1-1* mutant is TGA-dependent, as revealed by the reversion of expression levels to wild-type levels in *tga256 jin1-1* quadruple mutants (Chapter 2, 5). A key regulator of this JA-mediated antagonism on the JA/ET pathway is the TGA-interacting glutaredoxin GRX480. Its expression is driven by JA-activated MYC2 and it can suppress JA-induced *ORA59* transcription in a TGA-dependent manner. As a consequence of the GRX480-mediated down-regulation of the JA/ET pathway, the inhibitory effect of the JA/ET pathway on the JA pathway is compromised which in turn promotes activation of the JA pathway.

Since SA (Fig.1 marked in green) induces *GRX480* transcription we speculate that SA antagonizes the JA/ET pathway using the same TGA/GRX repression module as JA. Remarkably, the suppressive effect of SA on the JA/ET pathway is much more pronounced probably due to the transient increase of glutathione (Koornneef et al., 2008). Under these conditions, the reductive capacities of glutaredoxins are likely to be enhanced (marked with the yellow flash). In contrast to the JA-induced GRX-mediated suppression of the JA/ET pathway, which results in a promotion of the JA pathway, SA suppresses the JA pathway (Spoel et al., 2003) via an unknown mechanism.

Since this working model is based on expression analyses in different mutants and transgenic plants with altered expression of the key players, questions regarding mechanistic aspects have to be asked. Which mechanism is responsible for the ET-mediated recruitment of TGA factors to the *ORA59* promoter? How is the essential COI1 function integrated in the promoter and does it change depending of whether the promoter is induced by ET or by JA? The mechanistic understanding of the activation mechanism is crucial for the final question which protein is targeted by the glutaredoxins to suppress *ORA59* promoter activity.

How do TGA factors function at the *ORA59* promoter?

The *tga256* triple mutant fails to maintain ACC-induced *ORA59* expression over a period of 24 hours which is most likely required to induce secondary target genes (Chapter 3). ChIP experiments using an antiserum against TGA2,5 and analyses of $ORA59_{Pro}$*:GUS* plants revealed a direct binding of TGA factors to the TGACG motif in

the *ORA59* promoter. The binding is further increased under elevated levels of ethylene. The molecular mechanism explaining the ACC-induced TGA recruitment remains to be elucidated. One hypothesis is that the ACC-induced activity of EIN3 at the promoter facilitates binding of TGA factors. However, preliminary ChIP results using chromatin of *ein3-1eil1-2* mutants demonstrate constitutively elevated TGA binding (data not shown) suggesting rather a negative effect of EIN3 on TGA binding. We tentatively suggest that the initial stabilization of EIN3 leads to the primary essential direct activation of the *ORA59* promoter. EIN3 presumably activates transcription by association with EER4 (ENHANCED ETHYLENE RESPONSIVENESS 4), a TFIID-like transcription factor which serves as a bridge between EIN3 and the basal transcription machinery (Robles et al., 2007). As soon as EIN3 levels decrease because the upstream signalling components revert to their basal activities, TGA factors might get access to the *ORA59* promoter to maintain transcription. ChIP analyses using EIN3 and TGA25 antibodies at different time points after ET induction provide a means to elucidate the kinetics of promoter occupation by EIN3 and TGA factors.

How does JA activate the *ORA59* promoter?

In contrast to classical JA-responsive genes like *VSP2,* which are positively regulated by MYC2 and COI1 (Lorenzo et al., 2004), JA-induced *ORA59* expression is negatively regulated by MYC2 albeit its induction still relies on a functional COI1 protein (Pre et al., 2008). The high levels of JA-induced *ORA59* transcription in the *jin1-1* mutant indicate the existance of a MYC2-independent COI1-dependent pathway. Since the MYC2-relaed proteins MYC3 and MYC4 are not important for *PDF1.2* expression (Fernandez-Calvo et al., 2011), other transcriptional activators that interact with JAZ repressors are likely to regulate *ORA59* promoter activity most likely at the G-box in the *ORA59* promoter which is necessary for JA-induction (Fig.S1). Candidates are R2R3-MYB transcription factors like MYB21 and MYB24 which can interact with JAZ-repressors (Song et al., 2011). Interestingly we have identified in our microarray analysis (Chapter 3) two R2R3-MYB transcription factors (MYB133 and MYB90) which are induced after ACC-treatment. These are also induced by JA and might support *ORA59* transcription either after JA or after ACC treatment. A feature of the MYC2-dependent signalling cascade is that not only MYC2 is induced by JA, but also that JAZ repressors are induced. As their expression depends on MYC2 (Chini et al., 2007) we have to take into account that the strong activation of the *ORA59* promoter in the *jin1-1* mutant occurs in the absence of most JAZ proteins. Still, if the same regulatory principle holds for the regulatory events at the *ORA59* promoter, ACC and JA-induced JAZ repressors might regulate MYB transcription factors. Indeed, JAZ8 and JAZ1 are

ACC-and JA-inducible. Basal COI1-dependent turnover of JAZ8 or JAZ1 upon ET induction might facilitate MYB133/MYB90 activity at the EIN3/TGA-activated promoter. Upon induction by JA, increased degradation of JAZ8 or JAZ1 would lead to a strong activation of the promoter in the presence of basal EIN3 activities and TGA factors. At least the positive contribution of the TGA factors would be abolished by glutaredoxins being induced by JA-activated MYC2.

Loss of function evidences are still missing regarding glutaredoxin functions

Our postulated scenario where either SA-driven or JA-driven *GRX480* transcription leads to subsequent repression of the JA/ET pathway is mainly based on gain of-function analyses with transgenic plants ectopically expressing GRX480. However, the single *grx480* knockout mutant does not show hyper-activation of *PDF1.2* after JA treatment. Moreover, *PDF1.2* induction is still sensitive to SA (Ndamukong, 2007). Our data provide evidence for a large functional redundancy within the plant-specific glutaredoxin family. All plant-specific glutaredoxins can interact with TGA factors and 10 out of 17 tested glutaredoxins can suppress EIN3-induced *ORA59:LUC* activity in protoplasts (Chapter 5). However, their spatial and temporal expression pattern disqualifies several of these factors as being redundant, like for instance the flower-specific glutaredoxins ROXY1 and ROXY2. Therefore, we focussed on SA-inducible glutaredoxins ROXY18 (At1g03850), ROXY21 (At5g11930) and GRX480 for the generation of a triple knockout. In the *roxy18roxy21grx480* triple mutant, the *PDF1.2* was hyper-induced (Fig.S2) suggesting that some MYC2-mediated repression is relieved. However, SA-suppression of JA-induced *PDF1.2* expression was still detectable (Fig.S2). However, as SA might activate glutaredoxin activity by increasing the reducing power in the cytosol, constitutively expressed glutaredoxins might mediate the suppressive effect.

What is the target of glutaredoxins?

TGA factors as direct targets

It was postulated that ROXY1 redox-modifies the TGA factor PAN (PERIANTHIA) thereby suppressing its activity (Li et al., 2009). This hypothesis was based on the finding that a PAN mutant protein containing a Cys to Ser mutation in the putative activation domain cannot complement the *pan* phenotype. However, this result does not conclusively demonstrate that this critical cysteine is modified by ROXY1. For TGA1 it was shown that two cysteines are reduced after SA accumulation (Despres et

al., 2003). We have followed up the idea if TGA2 is directly redox-regulated by glutaredoxins by complementation of the *tga256* mutant with a TGA2 derivative containing a cysteine to serine exchange in the C-terminus of the protein. The two tested lines expressing the $TGA2_{C186S}$ derivative under the control of the *CaMV 35S* promoter complemented both the *tga256* phenotype with respect to the SA/JA cross-talk (Fig.S3). Therefore we rule out the possibility that glutaredoxins redox-modify TGA factors at the *ORA59* promoter and rather speculate that TGA factors function to recruit glutaredoxins to target promoters where they regulate other transcription factors or co-activators.

NPR1 and NPR1-like proteins

The crucial co-activator of many SA-driven processes, NPR1, constitutes a well-studied example for a redox-modulated protein in plants (Mou et al., 2003; Rochon et al., 2006; Tada et al., 2008). Upon reduction of critical cysteines by a thioredoxin, the multimeric NPR1 complex in the cytosol dissociates into NPR1 monomers which can subsequently enter the nucleus (Tada et al., 2008) to induce expression of target genes. However, NPR1 is not required for the suppression of the JA/ET pathway if increased ethylene levels are present (Leon-Reyes et al., 2009). In addition to NPR1, NPR1-like proteins NPR2, NPR3 and NPR4 can interact with TGA factors (Liu et al., 2005; Zhang et al., 2006). To elucidate their role in the SA-mediated cross-talk on the JA/ET pathway the *npr2npr3npr4* triple mutant was generated. However, no differences to wild-type plants were observed with respect to SA/ET cross-talk (Fig.S4).

EIN3 as a possible target

EIN3 function is crucial for ET and also for JA-activated *ORA59* transcription (Chapter 3,5). We have shown that GRX480 antagonizes EIN3-induced *ORA59* activity in protoplasts (Chapter 4) and that *35S:GRX480* mutants exhibit a similar induction pattern of JA/ET and JA marker genes compared to *ein3-1eil1-2* mutants. EIN3 is thus a candidate protein to be directly targeted by GRX480. Three scenarios how EIN3 activity can be affected by the catalytic activity of glutaredoxins can be envisioned. First; EIN3-stability is affected, second; the EAR-domain is activated or third; the activation potential of EIN3 might be abolished.

EIN3-activity is tightly regulated via its stability (Yoo et al., 2008). In order to test if GRX480 modulates the degradation of EIN3, we treated protoplasts transfected with EIN3, GRX480 and the *ORA59:LUC* reporter construct (Chapter 4) with the proteasome inhibitor MG132. If GRX480 would trigger the degradation of EIN3, we

would expect no suppressive effect in the presence of MG132. The EIN3-induced *ORA59* promoter activity was reduced by the MG132 treatment, probably due to stabilized JAZ repressors at the G-box. Sill a GRX480-mediated suppression was detectable (Fig.S5). Therefore, GRX480-mediated EIN3-degradation seems unlikely to represent a possible scenario for GRX480 action.

EIN3 also possesses a putative EAR (ERF-associated amphiphilic repression) motif at amino acid position 532 (Kagale et al., 2010) which is probably involved in transcriptional repression (Ohta et al., 2001). A repressive action of EIN3 was recently described to regulate the *SID2* (*SALICYLIC ACID INDUCTION DEFICIENT2*) promoter (Chen et al., 2009). We tested the hypothesis whether the interaction of GRX480 with EIN3 would make the EAR domain more accessible thereby facilitating the repression of *ORA59* activity. To pursue this idea, we mutated the EAR domain of EIN3 LNIPN to ANAAA and used this EIN3 variant to test its ability to trigger *ORA59* activity in protoplasts. We found that this mutated EIN3-derivative can activate *ORA59* activity like the wild-type EIN3. Still, *ORA59* activity was suppressed after additional transfection of the effector plasmid encoding *GRX480* (Fig.S6). We therefore conclude that the EAR motif of EIN3 is not involved in the regulation of the *ORA59* promoter.

The third scenario pursued the idea that reduction of a critical cysteine in EIN3 alters its activation potential. To test this hypothesis, we generated Cys to Ser mutations of each of the eleven cysteines in the EIN3 protein. Subsequently, the different derivatives were tested in transient assays for their *ORA59* activation potential. We obtained the EIN3 m(C402S) derivative which exhibits a reduced activation potential (Fig.S7). However, EIN3 m(C402S)-mediated activation was still antagonized by additional transfection of the effector plasmid encoding GRX480. It is not unlikely that the point mutation disturbs the protein integrity resulting in a lower activity. Other EIN3 inducible promoters like *ERF1* or *EBF2* should be tested to determine whether the cysteine has a general importance or whether it is it specific for the *ORA59* promoter. In yeast two hybrid assays, we were not able to demonstrate any interaction between EIN3 and GRX480 or TGA2 or any evidence for a trimeric complex between all three factors (data not shown).

R2R3-MYB transcription factors

Finally, the postulated MYB/JAZ complex at the G-box of the *ORA59* promoter could serve as a target for GRX-mediated redox modification. Especially MYB113 and MYB90, whose expression is ET and JA-inducible, are promising candidates as they contain nine conserved cysteines. Since their function at the *ORA59* promoter is

possible under conditions of elevated levels of ET and JA we could explain why the TGA2/GRX480 complex is operational under both inducing conditions. It should be tested in future transient assays if different R2R3-MYB factors can activate the *ORA59* promoter in protoplasts in a G-Box-dependent manner. In addition, a possible interaction between GRX480 and different MYBs and also JAZs should be addressed. Since our microarray analysis has revealed a cluster of ACC-induced genes that are activated by TGA factors without being repressed by SA, we can search for differences how these genes are regulated. If for instance induction of these genes would not depend on COI1, the JA regulatory cascade would be a good candidate to be regulated by glutaredoxins at the *ORA59* promoter.

In conclusion, this thesis has established a consistent working model which proposes the GRX/TGA interaction at the *ORA59* promoter as a central node for the modulation of the JA/ET defense pathway by SA and JA. Taking into account, that all land plant specific glutaredoxins interact with TGA factors and that the GRX/TGA interaction is crucial for proper anther and flower development, it seems likely that important redox processes are controlled by this interaction. Given the stringency of the negative effect of SA on the JA/ET pathway it seems likely that this regulation provided a strong selective advantage. The elucidation of glutaredoxin-mediated processes at the *ORA59* promoter might not only unravel important mechanisms within the innate immune system but it might also be important to understand developmental processes regulated by this complex.

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Supplementary material

Figure S1. The G-box is crucial for JA-induced *ORA59* promoter activity.

Figure S2. *PDF1.2* expression under SA/JA cross-talk conditions in the *roxy18 roxy21 grx480* triple mutant plants.

Figure S3. *PDF1.2* expression under SA/JA cross-talk conditions in *tga256 35S:TGA2$_{C186S}$* mutant plants.

Figure S4. *PDF1.2* expression under SA/ACC cross-talk conditions in *npr2npr3npr4* triple mutant plants.

Figure S5. GRX480 suppresses EIN3-activated *ORA59* promoter activity in transiently transformed mesophyll protoplasts in the presence of the proteasome inhibitor MG-132.

Figure S6. The putative EAR-domain of EIN3 plays no role in GRX480-mediated suppression of EIN3-induced *ORA59* promoter activity in transiently transformed mesophyll protoplasts.

Figure S7. Mutation of the cysteine at amino acid position 402 of EIN3 reduces the *ORA59* activation potential of EIN3 in transiently transformed mesophyll protoplasts, but is still subjected to GRX480-medaited suppression.

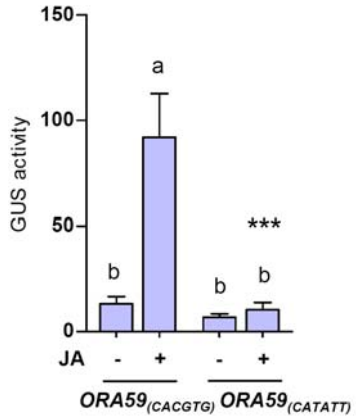

Figure S1. The G-box is crucial for JA-induced *ORA59* promoter activity.

Transgenic lines were generated where the G-box (CACGTG) of the *ORA59* promoter at bp position -333 to -328 was mutated to CATATT (Chapter 3, Fig.3d). Together with the wild-type constructs 15 independent *ORA59$_{(CATATT)}$:GUS* F2-lines were tested as a pool.. Each line induced with methyl jasmonate (MeJA) via the gaseous phase in glass containers treated with for 24 hours consists of 25 three-week old plants grown on soil in one pot. In each experiment the values of JA treated lines were set to 100%. The values (±SE) from one experiment are shown. Different letters indicate differences among treatments within a genotype (Student's *t*-test, $P < 0.05$). Asterisks represent significant differences between wild-type and *tga256* plants (two-way ANOVA, ***$P < 0.001$).

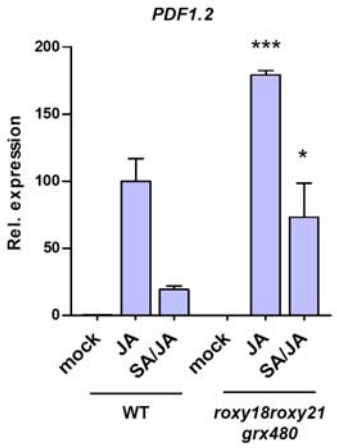

Figure S2. *PDF1.2* expression under SA/JA cross-talk conditions in the *roxy18 roxy21 grx480* triple mutant plants.

Four-week-old wild-type and *roxy18roxy21grx480* triple mutants plants grown on soil were treated for 24 h with MeJA in glass containers. For the SA/JA treatment plants were additionally sprayed with 1 mM SA. Control plants were sprayed with water in a glass container without MeJA. Relative *PDF1.2* transcript levels were determined by quantitative real-time RT-PCR analysis. The mean values (±SE) from four individual plants per genotype are shown. Asterisks represent significant differences between wild-type and the *roxy18roxy21grx480* triple mutant (two-way ANOVA, **P* < 0.05, ****P* < 0.001).

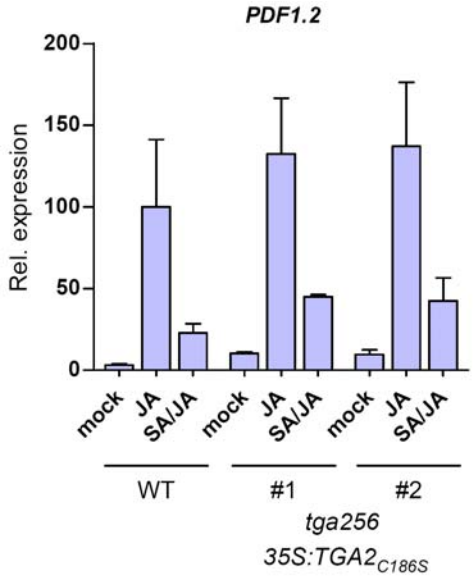

Figure S3. *PDF1.2* expression under SA/JA cross-talk conditions in *tga256 35S:TGA2*$_{C186S}$ mutant plants.

Four-week-old wild-type and two transgenic *tga256 35S:TGA2*$_{C186S}$ plants grown on soil were treated for 24 h with MeJA in glass containers. For the SA/JA treatment plants were additionally sprayed with 1 mM SA. Control plants were sprayed with water in a glass container without MeJA. Relative *PDF1.2* transcript levels were determined by quantitative real-time RT-PCR analysis. The mean values (±SE) from three individual plants per genotype are shown.

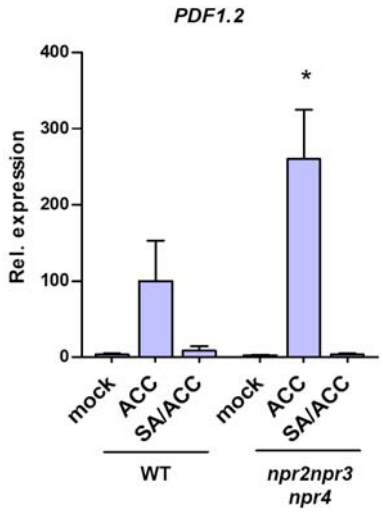

Figure S4. *PDF1.2* expression under SA/ACC cross-talk conditions in *npr2npr3npr4* triple mutant plants.

Four-week old soil-grown wild-type and *npr2npr3npr4* triple mutant plants were sprayed with 1 mM ACC or a combined treatment of 1 mM ACC and 1 mM SA for 24 hours. Water spraying serves as a control. The relative *PDF1.2* transcript levels were determined by quantitative real-time RT-PCR analysis. The relative expression values in ACC-treated wild-type plants were set to 100%. The mean values (±SE) from four individual plants per genotype are shown. Asterisks represent significant differences between wild-type and the *npr2npr3npr4* triple mutant (two-way ANOVA, **P* < 0.05).

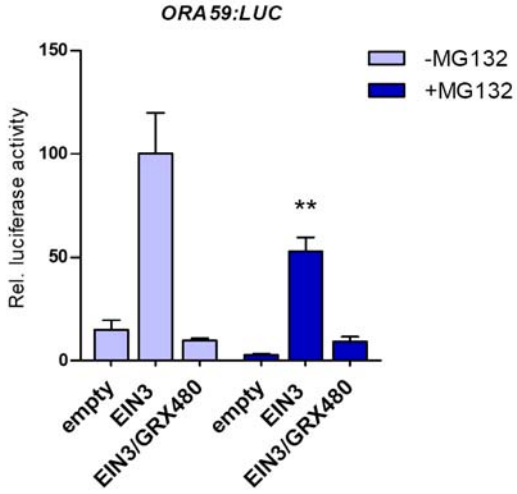

Figure S5. GRX480 suppresses EIN3-activated *ORA59* promoter activity in transiently transformed mesophyll protoplasts in the presence of the proteasome inhibitor MG-132.

ORA59 promoter sequences from base pair position -1 to -975 were fused to the firefly luciferase gene. Expression was analyzed in from wild-type mesophyll protoplasts using effector plasmids encoding EIN3 and GRX480 under the control of the *CaMV 35S* promoter in the presence or absence of 50µM MG-132. Relative LUC activities are expressed in arbitrary luminescence units, normalized to Renilla LUC activity. *ORA59* promoter activity in the presence of EIN3 and the absence GRX480 was set to 100%. Values are means of five replicates (±SE). Asterisks represent significant differences between the presence or absence of MG-132 (two-way ANOVA, ***P* < 0.01).

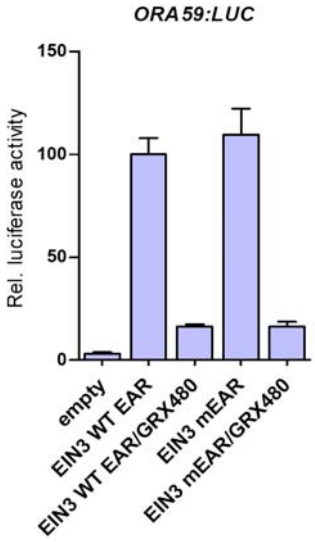

Figure S6. The putative EAR-domain of EIN3 plays no role in GRX480-mediated suppression of EIN3-induced *ORA59* promoter activity in transiently transformed mesophyll protoplasts.

ORA59 promoter sequences from base pair position -1 to -975 were fused to the firefly luciferase gene. Expression was analyzed in from wild-type mesophyll protoplasts using effector plasmids encoding EIN3 WT EAR, EIN3 mEAR and GRX480 under the control of the *CaMV 35S* promoter. Relative LUC activities are expressed in arbitrary luminescence units, normalized to Renilla LUC activity. *ORA59* promoter activity in the presence of EIN3 WT EAR and the absence GRX480 was set to 100%. Values are means of four replicates (±SE).

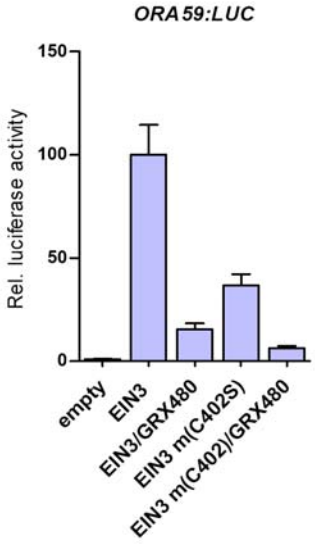

Figure S7. Mutation of the cysteine at amino acid position 402 of EIN3 reduces the *ORA59* activation potential of EIN3 in transiently transformed mesophyll protoplasts, but is still subjected to GRX480-medaited suppression.

ORA59 promoter sequences from base pair position -1 to -975 were fused to the firefly luciferase gene. Expression was analyzed in from wild-type mesophyll protoplasts using effector plasmids encoding EIN3, EIN3 m(C402S) and GRX480 under the control of the *CaMV 35S* promoter. Relative LUC activities are expressed in arbitrary luminescence units, normalized to Renilla LUC activity. *ORA59* promoter activity in the presence of EIN3 and the absence GRX480 was set to 100%. Values are means of four replicates (±SE).

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