



Development of a molecular reporter for cell-specific visualization of jasmonates

Dissertation

zur Erlangung des
Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der
Naturwissenschaftlichen Fakultät I – Biowissenschaften

der Martin-Luther-Universität
Halle-Wittenberg,

Vorgelegt von

Herrn Yulong Li

geb. am 09. Mai 1989 in Zoucheng, Shandong, China

Gutachter:

1. Prof. Dr. Bettina Hause
2. Prof. Dr. Ingo Heilmann
3. Prof. Dr. Jens Boch

Halle (Saale), 17. Januar 2018

Contents

1	Introduction.....	1
1.1	Biosynthesis of JA.....	2
1.2	Derivatives of JA.....	3
1.3	JA perceptions and signaling	5
1.3.1	SCF ^{COII} complex	6
1.3.2	Jasmonate ZIM-domain (JAZ) proteins	7
1.3.3	JAZ targeted transcription factors (TFs)	8
1.3.4	MYC2	9
1.3.5	G-box and JA-responsible cis-element (JARE).....	10
1.4	Cell or tissue specific detection of JA.....	11
1.5	Transcription activator-like effectors (TALE)	12
1.6	CSY4.....	13
1.7	Aim of the study.....	15
1.7.1	A system based on JAZ-mediated repression of MYC2	15
1.7.2	A system based on JAZ-CSY4 mediated controlling of the reporter gene expression	17
1.7.3	An approach based on a novel Jasmonate-Responsive Promoter elements (JARE).....	18
2	Material and methods.....	19
2.1	Material	19
2.1.1	Chemicals and supplies	19
2.1.2	Plants	19
2.1.3	Microorganisms	19
2.1.4	Primers and Plasmids.....	19
2.1.5	Fertilizer and media for cultivation of plants and microorganisms.....	21
2.1.5.1	Luria- Betani- Medium (LB)	22
2.1.5.2	LB Medium with agar	22
2.1.5.3	S.O.C. Medium (SOC).....	23
2.1.5.4	Murashige and Skoog (MS) culture medium	23
2.2	Microbiological Methods	23

2.2.1	Production of chemically competent <i>E. coli</i> cells	23
2.2.2	Transformation of <i>E. coli</i> cells	24
2.2.3	Production of electricity competent <i>A. tumefaciens</i> cells.....	24
2.2.4	Transformation of <i>A. tumefaciens</i> cells	25
2.3	Molecular biological methods	25
2.3.1	Isolation of genomic DNA	25
2.3.2	Isolation of Plasmid DNA	25
2.3.3	Isolation of RNA	25
2.3.4	DNase treatment to RNA.....	26
2.3.5	Synthesis of cDNA	26
2.3.6	Agarose Gel Electrophoresis	26
2.3.7	Polymerase Chain Reaction (PCR).....	27
2.3.8	Quantitative Real-Time PCR (qRT-PCR)	28
2.4	Cloning methods	30
2.4.1	Restriction of DNA.....	30
2.4.2	Gel extraction of DNA fragments	30
2.4.3	DNA-fragment purification	30
2.4.4	Sequencing.....	30
2.4.5	Gateway cloning	30
2.4.6	Vector construction with Golden Gate Cloning	31
2.5	Biological methods.....	32
2.5.1	Cultivation of plants	32
2.5.1.1	<i>Nicotiana benthamiana</i>	32
2.5.1.2	<i>A. thaliana</i> plants for stable transformation.....	33
2.5.1.3	Sterilization and germination of <i>A. thaliana</i> seeds	33
2.5.2	Transient transformation of <i>N. benthamiana</i>	33
2.5.3	Stable transformation of <i>A. thaliana</i>	33
2.5.4	Selection of transformants	34
2.5.5	Selection of single-insertion and homozygous transgenic plants	34
2.5.6	Gus activity staining	35
2.6	Photograph and microscopic works	36
2.6.1	Photograph with UV lamp	36
2.6.2	Photograph with Microscope.....	36

3	Results	37
3.1	A system based on JAZ-mediated repression of MYC2	37
3.1.1	Establishment of the synthetic promoter and TF constructs.....	37
3.1.1.1.	Synthetic promoter construct	37
3.1.1.2	Synthetic transcription factor construct	37
3.1.2	Analysis the GFP fluorescence of the <i>N.benthamiana</i> leaves after transient expression of transcription factor constructs	38
3.1.2.1	Infiltration experiments with the N-terminal fusions.....	38
3.1.2.2	Infiltration experiments with the C-terminal fusions.....	41
3.1.3	System built in stable transformed plants.....	42
3.1.3.1	Establishment of the constructs for stable transformation.....	42
3.1.3.2	Analysis of the GFP fluorescence in stable transformed <i>A.thaliana</i> lines	43
3.1.3.3	Analysis of GFP fluorescence in stable transformed <i>A.thaliana</i> lines upon JA treatment	46
3.1.4	Identification of the interaction between JAZ1 and TALE-MYC2 fusion variants.....	48
3.1.4.1	Interaction test using Yeast 2 Hybrid system	49
3.1.4.1.1	Establishment of the expression vectors	49
3.1.4.1.2	Interaction test in yeast	50
3.1.4.2	Interaction test using BiFC method	52
3.1.4.2.1	Establishment of the 2-in-1 expression vectors	53
3.1.4.2.2	Interaction test in mesophyll protoplasts of <i>A. thaliana</i>	54
3.1.4.2.3	Interaction test in mesophyll protoplasts of <i>N. benthamiana</i>	55
3.1.5	Analysis of the repression effect of JAZ1 on the transcriptional activity of the TALE-MYC2 fusions	56
3.1.5.1	Establishment of the constructs	57
3.1.5.2	Analysis of the transactivation assay	58
3.1.6	Overexpression of <i>JAZ1</i> in the high <i>GFP</i> expressing lines.....	59
3.1.6.1	Analysis the effects of overexpressed <i>JAZ1</i> on the <i>GFP</i> expression in <i>N.benthamiana</i> leaves	60
3.1.6.2	Analysis of the GFP fluorescence after overexpression of <i>JAZ1</i> in stable transformed <i>A.thaliana</i> lines.....	61

3.2	A system based on JAZ-CSY4 mediated controlling of the reporter gene expression.....	63
3.2.1	Establishment of the constructs for stable transformation.....	63
3.2.2	Screening of the transformants with expected GUS activity.....	64
3.3	A system based on a novel Jasmonate-Responsive Promoter Element (JARE).....	65
3.3.1	Establishment of the constructs containing JARE for infiltration.....	65
3.3.2	Infiltration experiments with the synthetic JARE promoter constructs ...	66
3.3.3	Establishment of the constructs containing the putative JARE for stable transformation.....	67
3.3.4	Analysis of the GFP fluorescence in stable transformed <i>A.thaliana</i> lines .	68
3.3.5	Root growth inhibition effect of JA on transgenic lines.....	69
4	Discussion.....	71
4.1	The system based on JAZ-mediated repression of TALE-MYC2 fusions.....	72
4.1.1	The artificial transcriptional network has high activity.....	72
4.1.2	MYC2-TALE fusions exhibited varying transcriptional activities	73
4.1.3	Analysis of GFP fluorescence upon JA treatment.....	74
4.1.3.1	Analysis of GFP fluorescence of <i>N.benthamiana</i> leaves transiently expressing transcription factor constructs upon JA treatment	74
4.1.3.2	Analysis of GFP fluorescence in stable transformed <i>A. thaliana</i> lines upon JA treatment.....	75
4.1.4	Interaction between JAZ1 and TALE-MYC2 fusions.....	76
4.1.4.1	Interaction tests reveal that MYC2-TALE fusions interact with JAZ1	76
4.1.4.2	Analysis of the repression effect of JAZ1 on the transcriptional activity of the TALE-MYC2 fusions using transactivation assay	79
4.1.5	Overexpression of JAZ1 as a repressor in stable transformed <i>A. thaliana</i>	81
4.2	A system based on JAZ-CSY4 mediated controlling of the <i>GUS</i> expression	81
4.3	A system based on Jasmonate-Responsive Promoter Element (JARE).....	83
4.3.1	Infiltration experiments with the synthetic JARE promoter constructs ...	83
4.3.2	Amplification of synthetic JARE activity using TALE.....	84

5	Summary.....	86
6	Appendix.....	105
7	Acknowledgments	115
8	Eidesstattliche Erklärung	116

List of Figures

Figure 1 Jasmonates response to developmental and environmental signals	1
Figure 2 Synthesis of jasmonic acid (JA) from α -linolenic acid generated from galactolipids.....	3
Figure 3 JA and its derivatives.....	4
Figure 4 A model of JA perception and signaling	6
Figure 5 Schematic representation of the JAZ1 protein and its conserved domains	7
Figure 6 Schematic representation of the MYC2 protein and its conserved domains.....	10
Figure 7 Schematic representation of the TALE protein and its conserved domains..	12
Figure 8 Schematic representation of a model for the detection of JA base on JAZ mediated repression of MYC2.....	16
Figure 9 Schematic representation of a model for the detection of JA base on JAZ-CSY4 mediated controlling of the reporter gene expression.....	17
Figure 10 Schematic representation of a model for the detection of JA base on JARE	18
Figure 11 Scheme of the synthetic promoter construct “ <i>promsyn::GFP</i> ”	37
Figure 12 Fragments of MYC2 and TALE used in synthetic transcription factor construct	38
Figure 13 Infiltrations experiments showed different level of GFP fluorescence in dependence on the different constructs	39
Figure 14 Schematic overview about the strength of <i>GFP</i> expression driven by the various constructs	40
Figure 15 Infiltrations experiments showed different level of GFP fluorescence of the positive control	41
Figure 16 Infiltrations experiments showed general low level of GFP fluorescence independence on the different constructs.	42
Figure 17 Scheme of the constructs for stable transformation	43
Figure 18 GFP fluorescence in the leaf of the single-insertion homozygous plants ...	44
Figure 19 Schematic overview about the levels of <i>GFP</i> expression detected from the single-insertion homozygous plants	45
Figure 20 GFP fluorescence after MeJA or COR treatment of single-insertion homozygous plants.	47

Figure 21 Schematic overview about the levels of GFP fluorescence detected from the single-insertion homozygous plants	48
Figure 22 Schematic representation of the prey and bait used in Y2H assay.....	50
Figure 23 Interaction test of the proteins of interest	52
Figure 24 Schematic of the constructs for 2-in-1 BiFC.....	53
Figure 25 Representation of mesophyll protoplasts from <i>A. thaliana</i> transformed with splitYFP expression vectors.	55
Figure 26 Representation of mesophyll protoplasts from <i>N. benthamiana</i> transformed with splitYFP expression vectors	56
Figure 27 Constructs used for the transactivation assay	58
Figure 28 Detection of <i>GUS</i> expression in transactivation assay.	59
Figure 29 Infiltrations experiments showed different level of GFP fluorescence in dependence on the different constructs	61
Figure 30 Scheme of the construct for overexpression of JAZ1.	62
Figure 31 RFP fluorescence in the seed coat of the positive transformats	62
Figure 32 GFP fluorescence in the JAZ1 overexpressed T1	63
Figure 33 Schematic of the constructs for stable transformation	64
Figure 34 GUS staining of the leaf tissues from T1 transformats	64
Figure 35 Schematic of the constructs for infiltration.	66
Figure 36 GFP fluorescence in <i>N. benthamiana</i> infiltrated leaves treated with phytohormones	67
Figure 37 Schematic of the constructs for stable transformation	68
Figure 38 Change of GFP fluorescence after MeJA or COR treatment in the single-insertion homozygous plants.	69
Figure 39 Root length of arabidopsis seedling in the presence of MeJA	70

List of Tables

Table 1. List of destination vectors and module donor vectors	20
Table 2. Concentration of antibiotics in bacterial selection medium.....	22
Table 3. Compositions of buffers for chemical competent cells production	24
Table 4. General scheme of PCR program	28
Table 5. General scheme for qRT-PCR program.....	29
Table 6. General scheme of restriction-ligation programs for Golden Gate cloning...32	
Table 7. List of level -1 vectors	105
Table 8. List of level 0 vectors.....	105
Table 9. List of level 1 vectors.....	106
Table 10. List of level 2 vectors.....	109
Table 11. Sequences of oligonucleotides for PCR.....	111

Abbreviations

12-OH-JA	12-hydroxy-JA
13-HPOT	(13-S)-hydroperoxy linolenic acid
13-LOX	13-lipoxygenase
<i>35S MP</i>	-46/+8 <i>CaMV</i> 35S-Minimal promotor
α -LeA	α -linolenic acid (18:3)
ABA	Abscisic acid
ACX	acyl-CoA oxidase
AOC	allene oxide cyclase
AOS	allene oxide synthase
Asn	Asparagine
Asp	Aspartic acid
<i>A. thaliana</i>	<i>Arabidopsis thaliana</i>
Bar	bialaphos resistance gene
BD	Binding domain
bHLH	basic-Helix-Loop-Helix
BiFC	Bimolecular fluorescence complementation
<i>CaMV</i>	<i>Cauliflower Mosaic Virus</i>
Cas	CRISPR-associated proteins
cDNA	complementary DNA
COI1	coronatine insensitive 1
COR	coronatine
CRISPR	Regularly Interspaced Short Palindromic Repeats
CSY4	Cas Subtype Ypeat protein 4

DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
<i>E. coli</i>	<i>Echerichia coli</i>
EDTA	Ethylenediaminetetraacetic acid
EGL3	Enhancer of Glabra 3
eV	Electron volt
forw	Forward
GA	Gibberellic acid
GBFs	G-box factors
GFP	Green fluorescent protein
GL3	Glabra 3
GUS	β -glucuronidase
HAD	Histone Deacetylase
IAA	Indole-3-aceticacid
JA	Jasmonic acid
JA-Ile	JA isoleucine
JAR1	Jasmonate Resistant 1
JARE	JA-responsible cis-element
Jas	Jasmonate associated domain
JAV	Jasmonate-associated VQ motif
JAZ	Jasmonate-ZIM-domain
JERE	Jasmonate-elicitor-responsive element
JID	JAZ interaction domain
JMT	Jasmonic acid carboxyl methyltransferase

KAT	3-ketoacyl-CoA thiolase
kDa	Kilodalton
LB	left border
LEU	Leucine
LUC	Luciferase
Mas	mannopine synthase <i>gene</i>
MeJA	Jasmonic acid methyl ester
MS	Murashige and Skoog culture medium
<i>N. benthamiana</i>	<i>Nicotiana benthamiana</i>
NC	Negative control
NINJA	Novel Interactor of JAZ
NLS	Nuclear Localization Sequences
N-ter	amino-terminal
OCS	octopine synthase gene
OD	Optical density
OE	Over expression
OPC-8	3-oxo-2 (2-pentenyl)-cyclopentane-1-octanoic acid
OPDA	12-oxophytodienoic acid
OPR3	OPDA reductase3
PC	Positive control
PCR	Polymerase chain reaction
PDF1.2	Plant Defensin 1.2
qRT-PCR	Quantitative Real-Time PCR
RB	Right border

rev	Reverse
RFP	Red fluorescent protein
RNA	Ribonucleic acid
rpm	Rounds per minute
RT	Room temperature
SA	Salicylic acid
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
SCF	SKIP–CULLIN–F-box complex
sgRNA	Single-guide RNA
T3SS	Type III secretion system
TAD	Transcriptional Activation Domain
TAE	Tris-Acetic acid -EDTA-buffer
TALE	Transcription Activator-like effector
TF	Transcription Factor
TPL	TOPLESS
TPRs	TPL-related proteins
TR	Transformation Rate
TT8	Transparent Testa 8
TTG1	Transparent Testa Glabra 1
UTR	untranslated region
VSP1	Vegetative storage protein 1
WT	Wild type
X-Gal	5-bromo-4-chloro-3-indolyl-beta-Dgalactopyanoside
X-gluc	5-bromo-4-chloro-3-indolyl glucuronide

Y2H	Yeast 2 Hybrid
YFP	Yellow Fluorescent Protein

1 Introduction

As sessile organisms, plants are constantly exposed to biotic and abiotic environmental factors. Plant development, although genetically determined, is adapted to specific local conditions and protective, as well as defense, reactions are initiated during stress situations (Schutzendubel and Polle, 2002). This adaptation in plants is controlled by phytohormones, which are low molecular weight signal molecules, synthesized in different tissues of the plant and acting in very low amounts. Phytohormones include, for example, auxin, abscisic acid, ethylene, gibberellic acid, salicylic acid and jasmonic acid (JA) (Schmelz *et al.*, 2003; Fahad *et al.*, 2015). In 1962, Jasmonic acid (JA) methyl ester was isolated from the essential oil of *Jasminum grandiflorum* (Demole, 1962), and JA synthesis and functions were investigated subsequently in decades later. Ubiquitous in the plant kingdom, JA and its derivatives, collectively known as jasmonates, belong to a family of lipid-derived signaling molecules that regulate many aspects of plant life, including defense against herbivores and pathogens, but also symbiotic interactions with mycorrhizal fungi by altering gene expression positively or negatively in a regulatory network in relation to other plant hormones (Ueda and Kato, 1980; Dathe *et al.*, 1981; Wasternack, 2007; Balbi and Devoto, 2008).

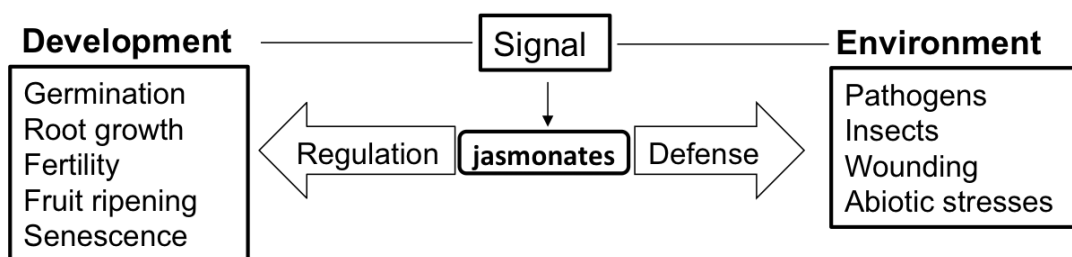


Figure 1 Jasmonates response to developmental and environmental signals. Jasmonates act as cellular regulators in diverse developmental processes, such as seed germination, root growth, fertility, fruit ripening, and senescence. In addition, jasmonates activate plant defense mechanisms in response to pathogens, insect, wounding, and environmental stresses such as drought, low temperature and salinity.

A role for JAs as signaling compounds regulating plant growth and development, and their involvement in numerous stress responses have been suggested mainly based on the accumulation of endogenous JAs as well as on the effects of application of exogenous JA, both of them correlating with JA-dependent processes. Levels of endogenous JAs are highest in young leaves, flowers, and fruit. Low levels of JA were found in roots and mature leaves (Creelman and Mullet, 1995) but increase after subjecting plants to wounding, UV light, water deficit, pathogens and ozone (Conconi *et al.*, 1996; McConn *et al.*, 1997; Rao *et al.*, 2000). The application of exogenous JAs induces the expression of a variety of genes that are also responsive to other stresses such as wounding and pathogen infection (Berger, 2002). The mode of action of jasmonates has been investigated by analysis of the effects of exogenous application of these compounds. This led to the identification of jasmonate responsive genes and determination of their expression and responsive promoter elements. In addition, jasmonate biosynthesis has been studied by identification of biosynthetic enzymes, use of inhibitors and determination of endogenous jasmonate levels. In addition to these traditional methods, insights into function of jasmonates were also provided by JA-insensitive or JA-deficient mutants, which allowed identification of tissues and processes where jasmonates are active (Berger, 2002; Memelink, 2005; Browse, 2009). Additionally, elevation of JA levels, expression of genes encoding enzymes of JA biosynthesis, and expression of JA-regulated genes were found to be positively correlated in a temporal manner and in dissected plant organs during distinct developmental processes or stress responses (Wasternack, 2007).

1.1 Biosynthesis of JA

In recent years, a great deal of information about the biosynthesis of JA was presented on reactions, genes, enzymes and finally regulation of JA biosynthesis (Wasternack, 2007 6; Acosta and Farmer, 2009; Browse, 2009; Schaller and Stintzi, 2009; Kombrink, 2012; Wasternack and Hause, 2013).

Jasmonates are synthesized through the octadecanoid pathway, where 12-oxophytodienoic acid (OPDA) is a central intermediate. The fatty acid substrate of JA biosynthesis is α -linolenic acid (18:3) (α -LeA) released from galactolipids of chloroplast membranes. Oxygenation of α -LeA is the initial step in JA biosynthesis. The initial reaction is the 13-lipoxygenase (13-LOX)-catalyzed insertion of molecular oxygen into position 13 of α -LeA most likely released from plastid envelope

membrane. Then (13-S)-hydroperoxy linolenic acid (13-HPOT) is converted by an allene oxide synthase (AOS) specific for 13-HPOT into an unstable allene oxide that is further processed by allene oxide cyclase (AOC). In the AOC-catalyzed reaction *cis*-(+)-OPDA is formed and is the final product of the plastid-located part of JA biosynthesis. OPDA is exported from the plastid and imported into the peroxisomes (Creelman and Mulpuri, 2002; Wasternack *et al.*, 2006; Wasternack, 2007) and is then reduced by a peroxisomal OPDA reductase (OPR3) into 3-oxo-2 (2-pentenyl)-cyclopentane-1-octanoic acid (OPC-8) (Strassner *et al.*, 2002). The final steps of JA synthesis are catalyzed by the three core enzymes of the β -oxidation cycle, namely acyl-CoA oxidase (ACX), the multifunctional protein (containing 2-*trans*-enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase activities), and 3-ketoacyl-CoA thiolase (KAT). Carboxyl side chains are shortened after three oxidation cycles leading to the formation of (+)-7-*iso*-JA (Fig.1) (Li *et al.*, 2005; Wasternack and Hause, 2013).

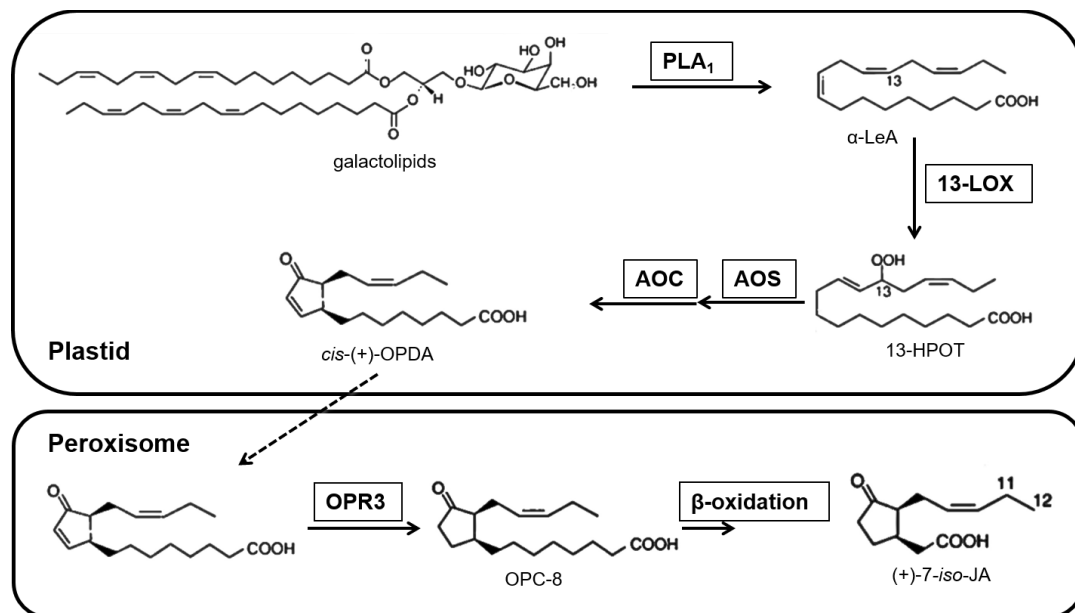


Figure 2 Synthesis of jasmonic acid (JA) from α -linolenic acid generated from galactolipids. PLA₁, phospholipase A1; α -LeA, α -linolenic acid; 13-LOX, 13-lipoxygenase; 13-HPOT, (13S)-hydroperoxyoctadecatrienoic acid; OPR3, OPDA reductase3; AOC, allene oxide cyclase; AOS, allene oxide synthase; OPC-8, 3-oxo-2-(2-pentenyl)-cyclopentane-1-octanoic acid; *cis*-(+)-OPDA; *cis*-(+)-12-oxophytodienoic acid

1.2 Derivatives of JA

JA can be enzymatically converted into numerous derivatives, some of which are not biologically active, such as 12-hydroxy-JA (12-OH-JA) and its sulfated or

glucosylated derivatives (Miersch *et al.*, 2008). However, others such as JA methyl ester (MeJA), and JA-amino acid conjugates (Pauwels *et al.*, 2009; Staswick, 2009) are well known to exhibit biological activity. Among the latter, JA isoleucine (JA-Ile) was recently shown to be the active form of jasmonates by mediating the binding of CORONATINE INSENSITIVE 1 (COI1) and JASMONATE ZIM DOMAIN (JAZ) proteins (see section 1.3) (Thines *et al.*, 2007).

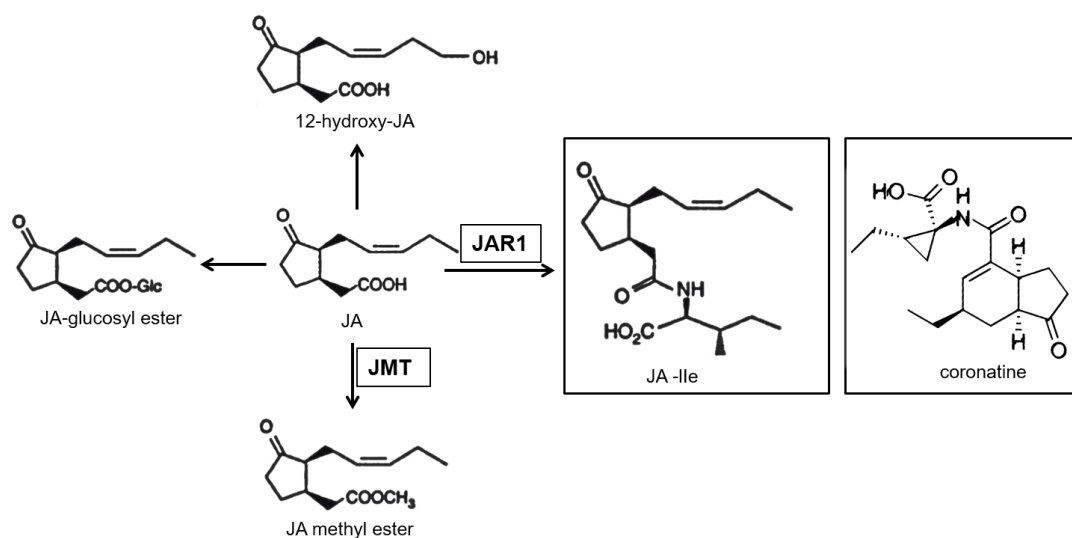


Figure 3 JA and its derivatives. JA can be transformed reversibly or irreversibly into a variety of derivatives, as 12-hydroxyjasmonic acid (12-OH-JA), JA–glucosyl ester, JA methyl ester, JA-isoleucine and other JA–amino acid conjugates. JAR1, Jasmonate Resistant1; JMT, Jasmonic acid carboxyl methyltransferase. In the boxes are bioactive compound JA-Ile and its analog Coronatine

MeJA is a naturally occurring compound, which is derived from jasmonic by the action of *S*-adenosyl-L-methionine: jasmonic acid carboxyl methyltransferase (JMT) (Seo *et al.*, 2001). It is a plant volatile that acts as an important cellular regulator mediating diverse developmental processes and defense responses. For example, it can induce the accumulation of defense chemicals such as phytoalexins or proteinase inhibitor proteins in plant (Farmer and Ryan, 1990). Plants produce MeJA in response to many biotic or abiotic stresses, in particular herbivory and wounding, as the signal of the original plant defense system. It can also spread by physical contact or through the air to produce a defensive reaction in unharmed plants. The unharmed plants absorb the airborne MeJA through either the stomata or diffusion through the leaf cell cytoplasm (Seo *et al.*, 2001). Therefore, MeJA is often used to study the biological function of jasmonates by spraying it to the plants.

MeJA has been considered as the bioactive signal in the JA-pathway for decades until the identification of JAR1, the jasmonoyl amino acid conjugate synthase and JAZ proteins (see section 1.3). The biological activity of MeJA was only apparent when converted to JA followed by its conjugation to JA-isoleucine (Tamogami *et al.*, 2008). JA and MeJA are just precursors of the bioactive molecule JA-Ile, which was shown to function directly in *COI1*-mediated signal transduction (see section 1.3) (Staswick and Tiriyaki, 2004; Stitz *et al.*, 2011). Identification of JAR1 was a breakthrough in looking for the real bioactive JA compound. *JASMONATE RESISTANT 1(JAR1)* locus is essential for pathogen defense. It encodes an enzyme that conjugates jasmonic acid to isoleucine (and other amino acids). The *jar1* mutant plant, which is deficient in JA-isoleucine (JA-Ile), shows insensitivity to JA or MeJA, but JA-Ile can complement this insensitivity (Staswick and Tiriyaki, 2004; Suza and Staswick, 2008). Furthermore, also coronatine (COR) is able to complement JA insensitivity in *jar1* mutant plant. COR is a phytotoxin produced by the bacterium *Pseudomonas syringae*, and is a structural mimic of JA-Ile (Krumm *et al.*, 1995). Due to its structure it has jasmonate-like properties. This was used to identify JA-insensitive mutants. The coronatine-resistant mutant *coil* is strongly insensitive to JA (Feys *et al.*, 1994; Xie *et al.*, 1998).

1.3 JA perceptions and signaling

In the last decades, significant progress has been made in identifying the key components of JA signaling (Xie *et al.*, 1998; Browse, 2009; Chung *et al.*, 2009; Koo and Howe, 2009; Memelink, 2009). In JA signal transduction pathway, a basic helix-loop-helix (bHLH)-type transcription factor MYC2 plays a central role and regulates diverse aspects of JA responses (Kazan and Manners, 2013). In normal conditions, the concentration of JA keeps at very low level; the transcriptional activity of MYC2 is repressed by JASMONATE ZIM DOMAIN (JAZ) proteins. A battery of stresses, including mechanical wounding, insect attack and pathogen infection, triggers a rapid increase of cellular JA levels. Synthesized JA is conjugated with isoleucine to form the active hormone JA-Ile, which is perceived by its receptor CORONATINE INSENSITIVE1 (COI1), an F-box protein that belongs to an E3 ubiquitin ligase. JA-Ile acts as a “molecular glue” to stimulate the interaction between COI1 and JAZ, which bring JAZs for degradation and therefore relieves their repression effect on MYC2 (Thines *et al.*, 2007).

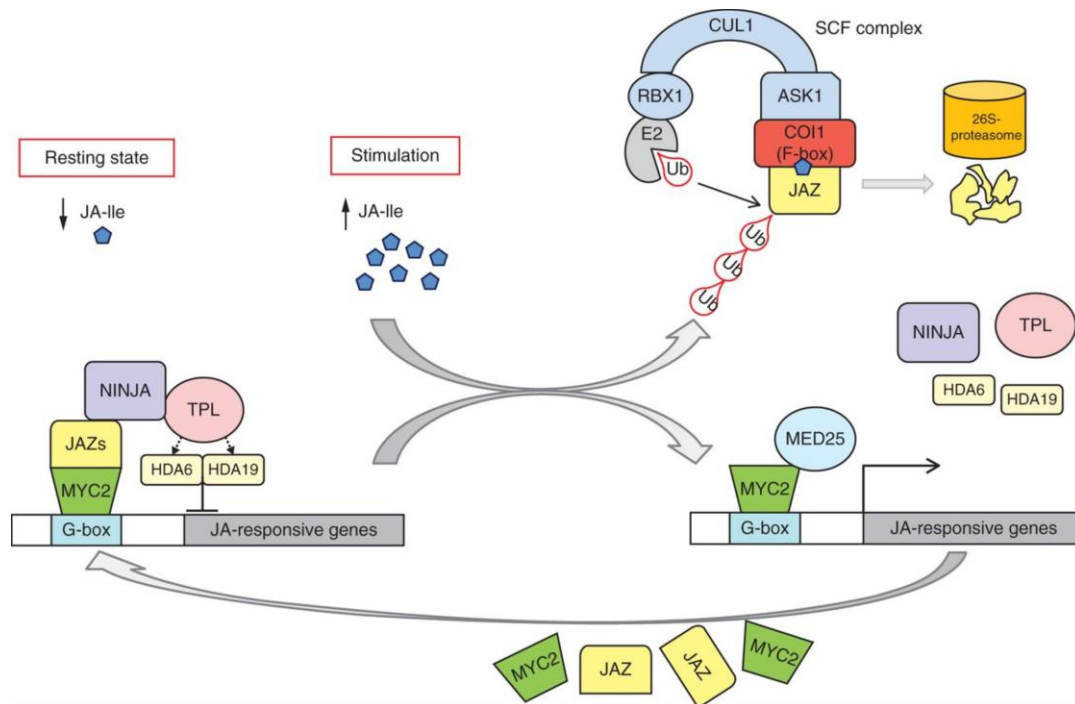


Figure 4 A model of JA perception and signaling. In the resting state (low JA-Ile level), JAZs bind MYC2 with co-repressors NINJA and TOPLESS repress transcription via HDA6 and HDA19. Upon stimulation (high JA-Ile level), JA-Ile stimulates the interaction between COI1 and JAZ, and then JAZs are subjected to ubiquitinylation and degradation by the 26S proteasome. Subsequently, the transcriptional activity of MYC2 is released to activate transcription of early JA-responsive mediated by MED25 (Wasternack and Hause, 2013).

1.3.1 SCF^{COI1} complex

The components involved in the JA perception were known after the identification of the *A. thaliana* mutant *coil*. This mutant shows insensitivity to the functional JA-Ile analog coronatine (see section 1.2.3) from the plant pathogen *Pseudomonas syringae*. Treatment with coronatine leads in the wild type *A. thaliana* to an inhibition of root growth, which does not occur in the *coil* mutant. *COI1*, mapped to a 90-kilobase genomic fragment is required for all JA-dependent responses tested in several plant species (Feys *et al.*, 1994; Xie *et al.*, 1998; Li *et al.*, 2004; Wang *et al.*, 2008). *COI1* is an F-box component of an SCF (SKIP–CULLIN–F-box) complex (Devoto *et al.*, 2002). *COI1* associates physically with AtCUL1, AtRbx1, and Arabidopsis Skp1-like proteins ASK1 or ASK2 to assemble ubiquitin-ligase complexes (Xu *et al.*, 2002). The complex is multiprotein E3 ubiquitin ligase catalyzing the poly-ubiquitination of target proteins, which are then subjected to proteasomal degradation. The F-box protein is the component conferring specificity for the substrate. In the case of *COI1*,

it recognizes the JAZ proteins, and targets them for 26S proteasome degradation in the presence of the jasmonates (Shan *et al.*, 2011; Fonseca *et al.*, 2014).

1.3.2 Jasmonate ZIM-domain (JAZ) proteins

Jasmonate ZIM-domain (JAZ) proteins have been identified as the targets of SCF^{COI1} (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2009). In *A. thaliana*, a gene family of 12 members encodes JAZ proteins. JAZ proteins consist of a TIFY and a Jas domain (Chini *et al.*, 2007; Thines *et al.*, 2007), which are necessary for dimerization of JAZ proteins and for binding to COI1, respectively (see Fig. 5) (Chini *et al.*, 2009; Chung and Howe, 2009). JAZ proteins act to repress transcription of jasmonate-responsive genes by recruiting the co-repressor TOPLESS (TPL) and TPL-related proteins (TPRs) through NOVEL INTERACTOR of JAZ (NINJA). Both NINJA and TPL proteins function as negative regulators of jasmonate responses (Szemenyei *et al.*, 2008; Pauwels *et al.*, 2010). TPL proteins act as general co-repressors via HISTONE DEACETYLASE HDA6 and HDA19 that affect multiple signalling pathways through the interaction with specific adaptor proteins (Kazan and Manners, 2008; Pauwels *et al.*, 2010; Wasternack and Hause, 2013).

Jasmonate treatment causes JAZ degradation and this degradation is dependent on activities of the SCF^{COI1} ubiquitin ligase and the 26S proteasome (Katsir *et al.*, 2008; Fonseca *et al.*, 2009). Furthermore, the (+)-7-*iso*-jasmonoyl-*l*-isoleucine (JA-Ile) conjugate, but not other jasmonate-derivatives such as JA, OPDA, or MeJA, promotes physical interaction between COI1 and JAZ1 proteins.

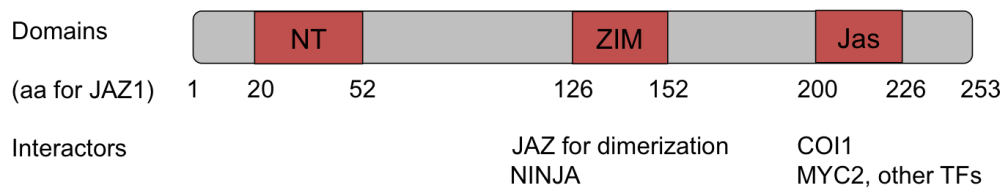


Figure 5 Schematic representation of the JAZ1 protein and its conserved domains. AtJAZ proteins have two highly conserved domains, ZIM and Jas domain. The ZIM domain (containing TIFY motif) is necessary for dimerization of JAZ proteins and interacts with NINJA. The C-terminal region containing the Jas domain is essential for interaction with COI1, MYC2 and other TFs (Pauwels and Goossens, 2011).

Similar to JAZs, JASMONATE-ASSOCIATED VQ motif (JAV) is also a negative regulator in JA signaling. JAV is degraded via the 26S proteasome in a COI1-dependent manner; however, JAV protein does not directly interact with COI1. JAV can interact with various regulators such as transcription factors WRKY28 and WRKY51. Degradation of JAV1 lead to activation of these downstream regulators, which subsequently regulate their respective downstream signal cascades (Hu *et al.*, 2013).

1.3.3 JAZ targeted transcription factors (TFs)

Transcription factors (TFs) bind specific elements of the promoters of JA-responsive genes to regulate the JA signaling. MYB21 and MYB24 are the R2R3-MYB transcription factors involved in the development of stamen (Mandaokar *et al.*, 2006). The over-expression of *MYB21* can partially rescue stamen filament length in JA biosynthetic and signaling mutants of *Arabidopsis* showing male sterility (Song *et al.*, 2011). Both of them can interact with JAZs indicating that JA regulates stamen development in *Arabidopsis* through JAZs mediated repression on MYB transcription factors. MYB transcription factors have two (R2 and R3) or three (R1, R2 and R3) DNA-binding motif repeats (Martin and Paz-Ares, 1997). Many MYBs interact with bHLH proteins and associate with a WD40 repeat protein to form a WD40-repeat/bHLH/MYB complex (Payne *et al.*, 2000; Zhang *et al.*, 2003). bHLH transcription factors have a basic helix-loop-helix domain that is responsible for sequence-specific DNA binding and homo- and heterodimer formation (Murre *et al.*, 1989; Murre *et al.*, 1994). WD repeat proteins have a 40 residues core region containing a glycine-histidine (GH) dipeptide and a tryptophan-aspartate (WD) dipeptide (Smith *et al.*, 1999). WD40-repeat/bHLH/MYB complex regulates many plant developmental processes, such as anthocyanin accumulation and trichome formation, which is a prominent JA/JA-Ile-dependent phenotype. For example, In *A. thaliana*, the WD40-repeat protein TRANSPARENT TESTA GLABRA1 (TTG1) recruits bHLH transcription factors, such as GLABRA3 (GL3), TRANSPARENT TESTA8 (TT8), or ENHANCER of GLABRA3 (EGL3) and R2R3 MYB transcriptional factors, such as MYB75, MYB90 to form the complex that mediates anthocyanin biosynthesis and trichome development. Most proteins mentioned above are identified as the targets of JAZs, indicating that JAZs can interact with the WD40-

Repeat/bHLH/MYB complexes to regulate jasmonate-mediated secondary metabolic production and other plant developmental processes (Qi *et al.*, 2011).

1.3.4 MYC2

133 bHLH transcription factors have been identified in *A.thaliana*. They are divided into 12 subfamilies, which have a range of different roles in plant development as well as metabolism (Heim *et al.*, 2003). Within each of these groups, conserved amino acid motifs are found outside the DNA binding domain. Members of same subgroups are likely to share similar functions, such as MYC3, MYC4 belong to the same subgroup IIIe as MYC2, acting additively with MYC2 to regulate specifically different subsets of the JA-dependent transcriptional response (Fernández-Calvo *et al.*, 2011; Song *et al.*, 2013). JASMONATE-ASSOCIATED MYC2-LIKE (JAM) is also included in the group III bHLH proteins, which are negative regulators of jasmonate responses (Sasaki-Sekimoto *et al.*, 2013).

MYC2, a nuclear-localized bHLH-type transcription factor, is to date the best-described TF inducing JA-mediated responses. MYC2 contains a bHLH domain in its carboxy-terminal domain conserved in plant bHLH proteins, which is required to form homo- or heterodimers with other TFs such as the MYC2-related bHLH TFs MYC3, and MYC4 (Fernández-Calvo *et al.*, 2011). Even though at least three NLS motifs reside outside the bHLH domain, the bHLH domain is still required for MYC2's nuclear localization (Lorenzo *et al.*, 2004; Kazan and Manners, 2013). A possible reason is that MYC2 requires dimerization before moving to the nucleus (Amoutzias *et al.*, 2008). It has a leucine zipper domain in the C-terminal also involved in the dimerization with specific TFs (Amoutzias *et al.*, 2008). In the amino-terminus, MYC2 contains a transcriptional activation domain (TAD), which can initiate the transcription by recruiting members of the Mediator complex such as MED25 (Fernández-Calvo *et al.*, 2011; Çevik *et al.*, 2012; Chen *et al.*, 2012). The JAZ Interaction Domain (JID) of MYC2 resides at the amino-terminus and is conserved among MYC proteins from several plant species (Fernández-Calvo *et al.*, 2011). In addition, MYC2 contains a phosphorylation site consisting of serine (S) residues (Kazan and Manners, 2013).

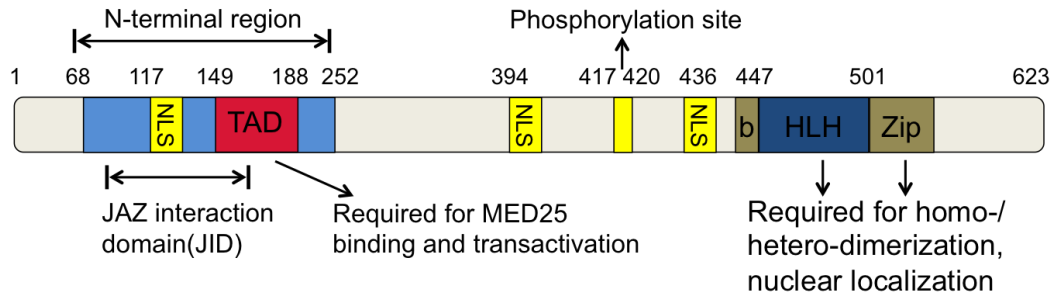


Figure 6 Schematic representation of the MYC2 protein and its conserved domains. MYC2 is composed of two highly conserved domains. In the N-terminal region, the JID is essential for interaction with the JAZs. The transcriptional activation domain (TAD) can initiate the transcription by recruiting the Mediator complex. In the C-terminal, the conserved bHLH domain is required for binding to the G-box sequence in target promoters and heterodimerization together with leucine zipper (Zip). Additionally, it has a phosphorylation site and several nuclear localization sequences (NLS) (Kazan and Manners, 2013).

Several biochemical experiments indicated that JAZs could directly interact with MYC2. The C-terminus containing the Jas domain was identified as the AtMYC2-interacting domain (Chini *et al.*, 2007). Yeast two-hybrid assays demonstrated a direct MYC2–JAZs interaction. Microarray analysis also supported that JAZ is a negative regulator of MYC2 function (Chini *et al.*, 2007). JA-Ile-triggered removal of JAZ proteins releases MYC2, enabling the transcriptional activator to regulate the expression of early jasmonate-responsive genes, including the genes encoding proteins of the JA biosynthesis, JAZ proteins and MYC2 itself (Lorenzo *et al.*, 2004; Chini *et al.*, 2007; Thines *et al.*, 2007; Pauwels *et al.*, 2008). The expression of *MYC2* is rapidly upregulated by JA, in a COI1 dependent manner. MYC2 differentially regulates the expression of two groups of JA-induced genes. The first group includes genes involved in defense responses against pathogens and is repressed by MYC2. The second group, integrated by genes involved in JA-mediated systemic responses to wounding, is activated by MYC2. MYC2 recognizes the G-box (5'-CACGTG-3') and G-box variants in the promoter of its target genes and regulates different branches of the JA pathway (Boter *et al.*, 2004; Lorenzo *et al.*, 2004; Chini *et al.*, 2007; Dombrecht *et al.*, 2007).

1.3.5 G-box and JA-responsible cis-element (JARE)

Plant *cis*-elements play important roles in global regulation of gene expression (Liu *et al.*, 2016). The G-box is a ubiquitous, *cis*-acting DNA regulatory element. It is a

hexameric motif CACGTG, found in many diverse plant genes and is an essential element for JA response (Kim *et al.*, 1992; Menkens *et al.*, 1995). Proteins known as G-box factors (GBFs) bind to G-box in a context-specific manner, mediating a wide variety of gene expression patterns. GBFs are usually bHLH or bZIP-type TFs, which MYC2 belongs to and MYC2 has strong affinity to G-box and G-box-related hexamers (Abe *et al.*, 1997; Boter *et al.*, 2004; Yadav *et al.*, 2005). Sequences flanking G-box are also important, since they determine whether TFs are capable of binding to the sequence, and they significantly influence the binding affinity (Ezer *et al.*, 2017). A genome-wide search revealed that 25% of early JA-responsive genes contain this *cis*-acting sequence, providing additional support for the potential importance of this sequence in MYC2-regulated expression of JA-responsive genes (Figueroa and Browse, 2012).

JARE is a novel identified jasmonate-responsive element in the *At JMT* promoter distinct from other JA-responsive elements previously reported, for example, G-box (see section 1.3.4) or other motifs (Dombrecht *et al.*, 2007; Memelink, 2009). It is also found in the promoter of other Arabidopsis JA-responsive genes, including *LOX2*, *COII*, *JAZs*, *WRKY70*, *PDF1.2*, *VSP1*, and *MYBs*. It regulates JA-responsive gene expression and contains a heptanucleotide sequence motif (G/C) TCCTGA. A multimerized JARE-containing construct could mediate JA-responsive induction of transcription showing that this element mediates response to JA (Seo *et al.*, 2013).

1.4 Cell or tissue specific detection of JA

To date a cell-specific detection of bioactive jasmonates is hardly possible, since the cell- and tissue-specific detection of jasmonates is hampered by their low concentration within plant cells or tissues and by the lack of suitable assay methods.

Mielke *et al.*, (2011) (Mielke *et al.*, 2011) generated and characterized jasmonate-specific antibodies and used them in combination with proper fixation and embedding methods to visualize jasmonates in leaf tissues. This antibody-based approach was developed to visualize JA directly in cross sections of plant material, in which JA was detected by immunolabeling within mechanically wounded leaves of tomato and Arabidopsis. But this available method to detect jasmonates on cell-specific level is invasive and does not allow studies on living plant tissues. Recently, a fluorescent biosensor termed Jas 9-VENUS was generated to follow JA perception *in vivo*

(Larrieu *et al.*, 2015). It is based on a functional Jas motif (see section 1.3.2) fused to the fast maturing VENUS variant of the yellow fluorescent protein and a N7 nuclear localization signal. This construct is expressed under the control of the constitutive CaMV 35S promoter leading to a basal fluorescence in all nuclei. Since the Jas9-VENUS degradation is dependent and specifically induced by bioactive JAs, decrease in fluorescence is indicative for JA responses and distribution *in planta* and can be monitored at the cellular scale. The disadvantage of this method lies in recording of a decreasing fluorescence to show the enhanced JA signal. Therefore, a new method to monitor the perception of biologically active jasmonates *in planta* should be developed, in which the fluorescent proteins rises following JA signaling. To construct these new sensors, transcription activator with DNA-binding specificities and RNA processing tool could be used.

1.5 Transcription activator-like effectors (TALE)

TALEs constitute a novel class of DNA-binding proteins with predictable specificity. They are employed by Gram-negative plant-pathogenic bacteria of the genus *Xanthomonas* that translocate different effector proteins via a type III secretion system (T3SS) into plant cells (Büttner and Bonas, 2010; Scholze and Boch, 2011). Inside the plant cell, TALEs localize to the nucleus, bind to target promoters, and alter the expression of plant genes, which possibly support the spread of the bacteria (Boch and Bonas, 2010; Büttner and Bonas, 2010).

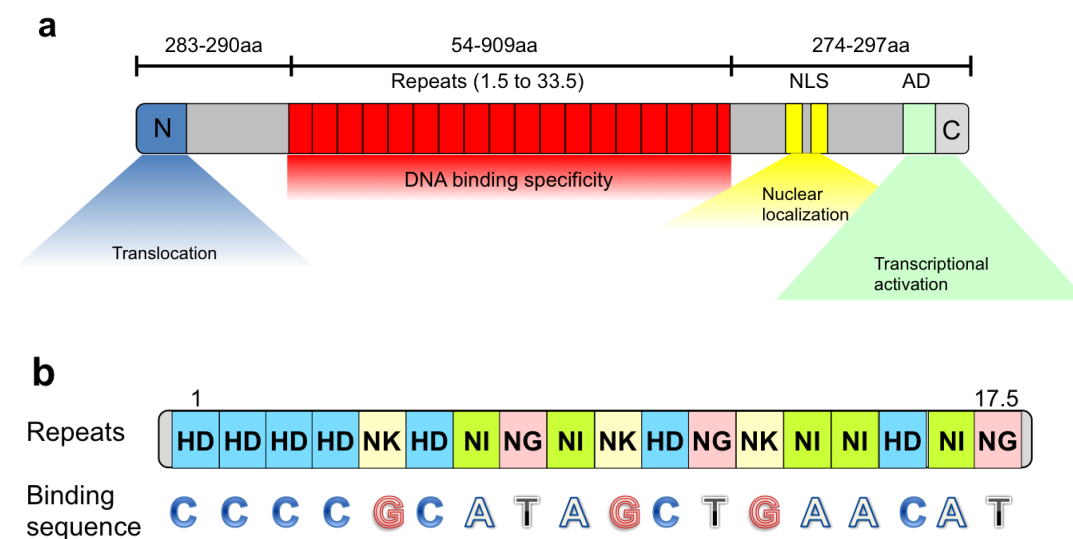


Figure 7 Schematic representation of the TALE protein and its conserved domains. (a) TALEs consist of three domains, the N-terminal region with translocation signal, a central domain for DNA binding

specificity and the C-terminal region consisting of two nuclear localization signals (NLS) and an acidic activation domain (AD). (b) Schematic view of an engineered repeats domain which can bind to the specific motif shown below. The specificity of each repeat is determined by a di-amino acid motif; e.g., NI repeats correspond to adenine, NG repeats to T, HD repeats to C and NK repeats to G. The N-terminally localized repeats correspond to the 5' end, whereas the C-terminal repeats correspond to the 3' end of the DNA box (Boch and Bonas, 2010).

TALEs are modular proteins that are composed of a N-terminal region containing the secretion and translocation signal for the T3SS, C-terminal region containing nuclear localization signals and a transcriptional activation domain, and a central DNA binding domain (Arnold *et al.*, 2009; Boch and Bonas, 2010; Bogdanove *et al.*, 2010). The central DNA binding domain consists of tandem repeats. Most TALEs have 17.5 repeats. However, the number of motif repeats can diverge between 1.5 to 33.5 times. Typically, each repeat has 34 amino acids (aa) and the last repeat is normally a half repeat (Büttner and Bonas, 2010). Each repeat confers recognition of one base pair (bp) in the DNA. Positions 12 and 13 in a typical 34-aa repeat are hypervariable indicating that the amino acids at position 12 and 13 probably interact directly with DNA bases (Boch *et al.*, 2009; Moscou and Bogdanove, 2009). Rearrangement of repeat modules allows the design of proteins with desired DNA-binding specificities. Engineered TALE with DNA-binding specificities is a useful tool in the study of the gene expression (Morbitzer *et al.*, 2010; Mahfouz *et al.*, 2011; Sanjana *et al.*, 2012).

1.6 CSY4

Bacteria have heavy adaption pressure exposed to bacteriophages. In order to escape from phage infection, bacteria established diverse defense mechanisms that allow the cell to recognize and distinguish foreign DNA from self- DNA and to survive exposure to invasive genetic elements. REGULARLY INTERSPACED SHORT PALINDROMIC REPEATS (CRISPR), in combination with CRISPR-associated proteins (Cas), forms the CRISPR/Cas systems. CRISPR provides acquired immunity against viruses and plasmids by targeting nucleic acid in a sequence specific manner (Horvath and Barrangou, 2010). CRISPR represents a family of DNA repeats occurring in the genome of various bacteria and archaea (Grissa *et al.*, 2007) and consists of an array of noncontiguous direct repeats separated by unique sequence elements (spacers). Spacer sequences are unique and in general derived from captured exogenous nucleic acid (Bolotin *et al.*, 2005; Mojica *et al.*, 2005; Pourcel *et al.*, 2005;

Barrangou *et al.*, 2007). The first stage of resistance is the acquisition of new spacers derived from the genetic material of infections (Barrangou *et al.*, 2007). The following stage is that the entire array is transcribed as a single pre-crRNA, which is bound to a single, multidomain protein Cas9 or to a multisubunit complex, forming the crRNA–effector complex and subsequently processed into small guide RNAs (crRNAs) by an endonuclease subunit of the multisubunit effector complex. The third stage is that crRNAs guide Cas proteins to recognize and silence potential genome invaders (Makarova *et al.*, 2006; Hale *et al.*, 2009; Wang and Quake, 2014). Therefore, spacers confer a sequence-specificity on the CRISPR resistance system. However, phages can overcome CRISPR defense by acquiring mutations in either the proto-spacer (the spacer target) or in the proto-spacer adjacent motif (PAM) (Barrangou *et al.*, 2007; Deveau *et al.*, 2010). PAMs are located next to the proto-spacer and are considered to function in spacer integration, targeting and to avoid self-targeting of CRISPR arrays (Mojica *et al.*, 2009; Marraffini and Sontheimer, 2010). For example, CRISPR/Cas9 system, belongs to Type II CRISPR, is the most famous and widely used in genome engineering (Hsu *et al.*, 2014). Cas9 has a HNH nuclease domain and a RuvC-like domain; each cleaves one strand of a double-stranded DNA. Cas9 can form a complex with a synthetic single-guide RNA (sgRNA), which can guide Cas9 to recognize and cleave target DNA. This system is used as an RNA-guided endonuclease to perform sequence-specific genome editing in bacteria, human cells and plants (Jinek *et al.*, 2012; Cong *et al.*, 2013; Shan, 2013).

The generation of crRNAs requires processing of the primary CRISPR RNA by endoribonuclease. Cas Subtype Y. pestis protein 4 (CSY4) was identified as an endoribonuclease of an *Yersinia pestis* subtype CRISPR/Cas system in the plant pathogen *Pectobacterium atrosepticum* (Przybilski *et al.*, 2011; Haurwitz *et al.*, 2012). Csy4 recognizes substrate RNA through a specific 28-nt hairpin sequence and cleaves it at the 3' end of the stem. Based on these specialties, CSY4 is used as a RNA processing tool for controlling mRNA stability and translation in recent research (Estarellas *et al.*, 2015; Qin *et al.*, 2015). The efficiency of Csy4-mediated processing on RNA depends on the position of the hairpin. CSY4 has high efficiency when the hairpin is located in the 5' UTR or immediately after the start codon but low efficiency when the hairpin is located in the 3' UTR prior to the poly (A) signal (Borchardt *et al.*, 2017).

1.7 Aim of the study

Among the jasmonates, JA-Ile is the only bioactive known to date. However, JA itself can be enzymatically converted into numerous derivatives, some of which are not biologically active, such as 12-hydroxy-JA (12-OH-JA) and its sulfated or glycosylated derivatives (Miersch *et al.*, 2008). However, others such as MeJA (Pauwels *et al.*, 2009), and JA-amino acid conjugates (Staswick, 2009) are well known to be biologically active. JA, JA-Ile and JAME cause physiological effects at very low concentrations (Creelman and Mulpuri, 2002) and act as systemic signaling molecules. Therefore, global hormone profiling seems not to be sufficient to conclude on specific functions of JA/JA-Ile. To broaden our knowledge on the mechanism of JA perception in response to biotic and abiotic stresses, the development but also the utilization of a detection system for JA at cell and tissue specific level will represent a major progress in jasmonate research.

Our study aims to develop and to use a new non-invasive detection system for active jasmonates. The envisaged system should be based on the rise of fluorescent proteins in living cells appearing just after specific induction by jasmonates. Such a monitoring of JA will allow getting insights with respect to the cell specific functions of JA in biotic interactions as well as in a number of developmental processes such as root, seedling, and flower and trichome development. Cell-specific patterns of gene expression as a read-out of cell-specific functions will lead to identification of JA-responsive genes, which could not be detected previously due to the dilution effect of extracting whole organs/tissues. With that, the identification of new putative components of JA signaling is expected.

To accomplish this goal, the following strategies were selected.

1.7.1 A system based on JAZ-mediated repression of MYC2

To build this system, the following strategy was selected: a synthetic promoter construct (see Fig.8a) should be developed that contains binding sites for a specific, synthetic TF and the encoding genes for reporter proteins, such as green fluorescent protein (GFP). Expression of GFP creates finally plants with fluorescence. This construct should be able to bind synthetic TFs (see Fig.8a), which will exhibit functional similarity to MYC2 and will be therefore repressed by JAZ proteins. As backbone for the synthetic TF, transcription activator-like effectors (TALEs) should

be used that originally have been identified as virulence factors in *Xanthomonas campestris* (see section 1.4) (Boch *et al.*, 2009).

Promoter and TF constructs should be expressed together in plants (see Fig.8b). It is expected that under normal conditions, the transcriptional activity of the designed transcription factor is repressed by JAZs, and an active JA signaling resulting in JAZ degradation will lead to liberation of the transcription factor and therefore to expression of the respective reporter gene *GFP*. *in planta* activity will allow the visualization of the spatial pattern of JA responses, thus being indicative for the occurrence of JA at cellular level.

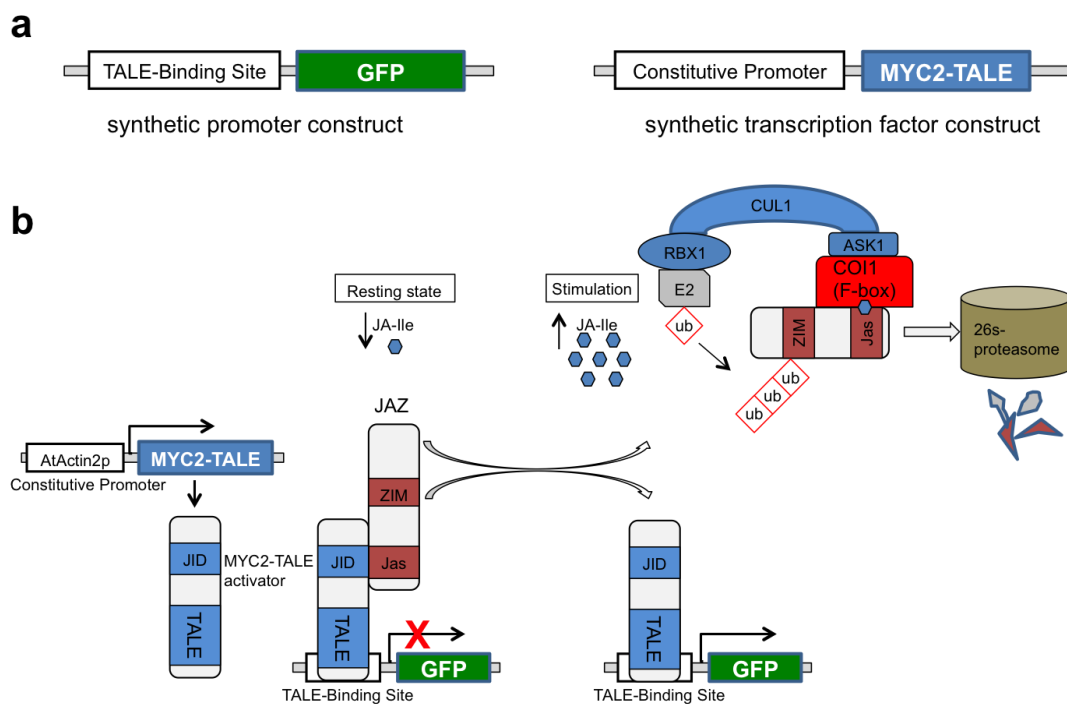


Figure 8 Schematic representation of a model for the detection of JA based on JAZ mediated repression of MYC2. (a) Synthetic promoter construct containing a TALE binding site in the promoter region and the GFP gene as reporter. Synthetic transcription factor construct containing MYC2 or derivatives of MYC2 fused with TALE under the control of a constitutive promoter. (b) In planta, the MYC2-TALE fusions are expressed constitutively under AtActin2 promoter, bind to the TALE binding site and are repressed by JAZ. The increase of the JA content will lead to the degradation of JAZ and the expression of GFP.

1.7.2 A system based on JAZ-CSY4 mediated controlling of the reporter gene expression

This system will be based on Csy4-mediated degradation of mRNA encoding a reporter protein and the JA-mediated degradation of JAZ1-Csy4 fusion protein. A reporter gene containing the hairpin sequence for Csy4 recognition should be expressed under a constitutive promoter. At the same time the JAZ1-Csy4 fusion protein will also be constitutively expressed and should target the hairpin sequence and cleave the transcription product leading to repression of reporter gene expression. Active JA signaling will lead the degradation of the JAZ1-Csy4 fusion protein and therefore eliminate the repression effect on mRNA of the respective reporter gene, resulting in the expression of reporter gene.

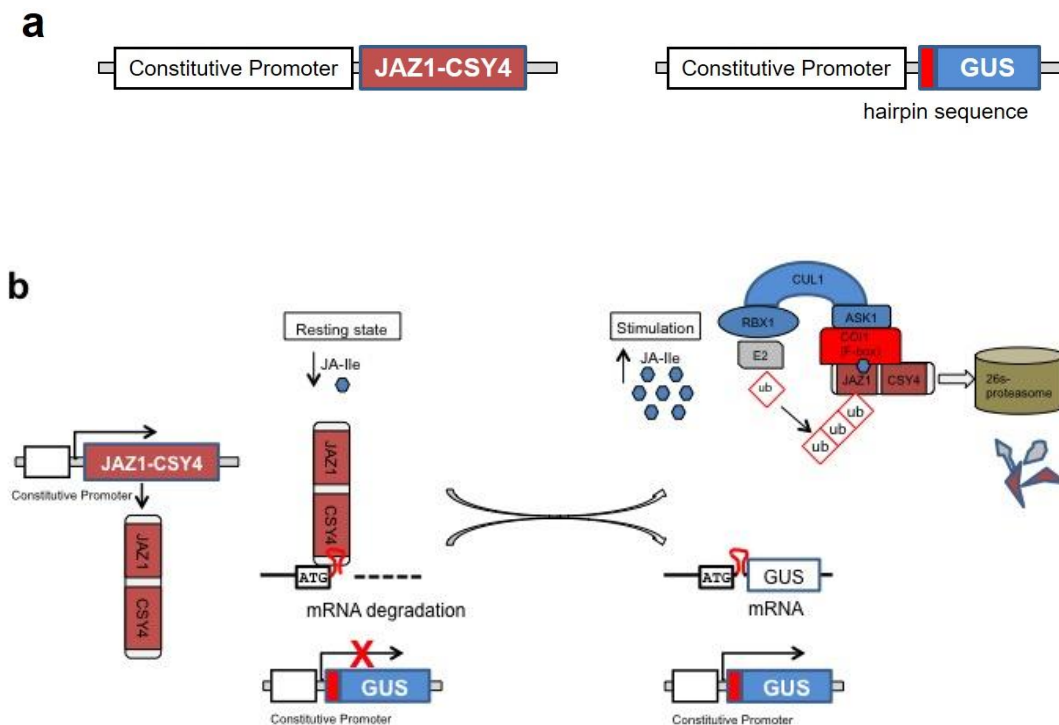


Figure 9 Schematic representation of a model for the detection of JA base on JAZ-CSY4 mediated controlling of the reporter gene expression. (a) JAZ1-CSY4 fusion and the reporter gene are both driven by a constitutive promoter. Inside the reporter gene coding sequence, there is a hairpin sequence, the binding site of CSY4. (b) In planta, the JAZ1-CSY4 fusions are expressed constitutively, bind to the hairpin binding site and cleave the mRNA of reporter gene. The increase of the JA content will lead to the degradation of JAZ and the expression of GUS.

1.7.3 An approach based on a novel Jasmonate-Responsive Promoter elements (JARE)

This system is based on a novel Jasmonate-responsive promoter elements (Seo *et al.*, 2013). A synthetic promoter construct (see Fig.10a) should be developed that contains multimerized JARE in promoter region and the encoding gene for GFP. Since this promoter is JA-responsive, the expression of the GFP will be indicative for the occurrence of JA at cellular level. In the case that the synthetic promoter is not strong enough to lead to sufficient expression of GFP, a second construct will be developed, which should result in amplification of the signal. For this, a promoter with the TALE binding site driving the encoding gene for GFP will be used in combination with the multimerized JARE in promoter region and encoding sequence for TALE. In this case, TALE as an amplifier will be produced JA dependently and will subsequently activate the expression of reporter gene.

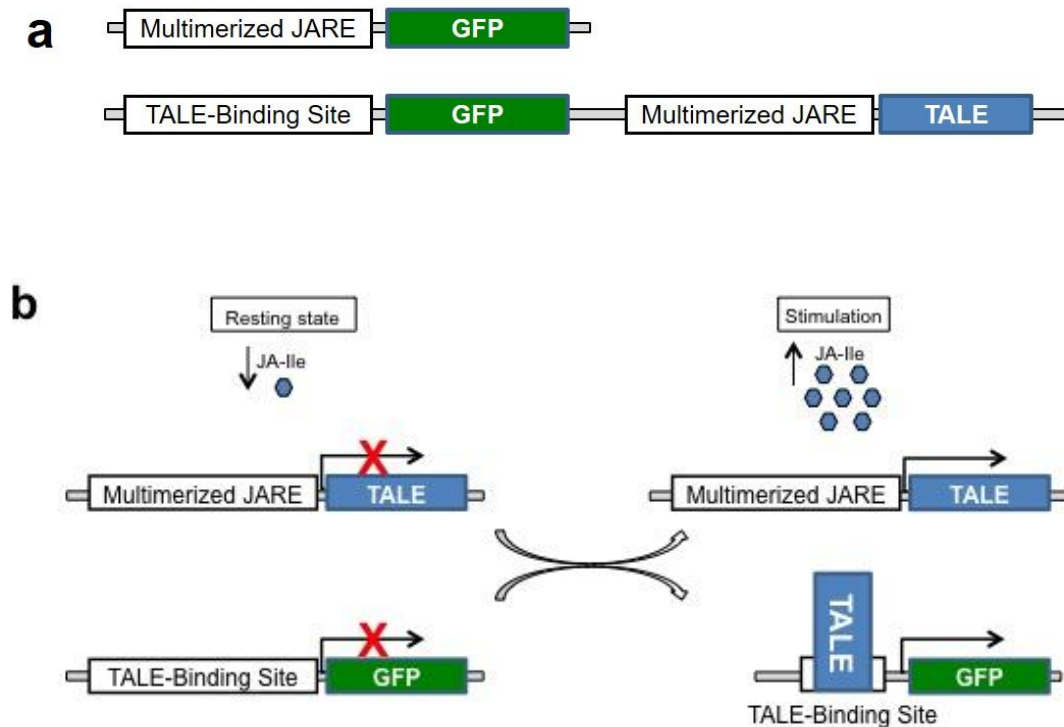


Figure 10 Schematic representation of a model for the detection of JA based on JARE. (a) The construct contains multimerized JARE in promoter region and the encoding gene for GFP. In case of that the synthetic promoter is too weak to lead to sufficient expression of GFP, a second construct containing a promoter with the TALE binding site driving the encoding gene for GFP will be used in combination with the multimerized JARE in promoter region and encoding sequence for TALE. (b) In planta, the expression of TALE driving by multimerized JARE can be induced by an active JA signaling and subsequently bind to the specific binding site, activate the expression of GFP.

2 Material and methods

2.1 Material

2.1.1 Chemicals and supplies

All chemicals, enzymes and supplies needed were obtained, if not mentioned separately, from the companies Life Technologies, Carl Roth, Sigma-Aldrich

2.1.2 Plants

Nicotiana benthamiana The *Nicotiana benthamiana* was used for transient expression assay.

Arabidopsis thaliana ecotype Columbia-0 was used as wild type plants and in stable transformation approaches.

Coi1-34 mutant lines were used for crossing with jasmonates inducible transgenic lines.

2.1.3 Microorganisms

Echerichia coli The *E. coli* strain DH10B was used for cloning. The Chromosomal Genotype of DH10B is F- mcrA Δ (mrr-hsdRMS-mcrBC) Φ 80dlacZ Δ M15 Δ lacX74 endA1 recA1 deoR Δ (ara,leu)7697 araD139 galU galK nupG rpsL λ - (Grant *et al.*, 1990). The DH10B strain was selected due to its high transformation efficiency for library construction and maintenance of large plasmids properties.

Agrobacterium tumefaciens The *Agr. Tumefaciens* strain GV3101 was used for the transformation of *Nicotiana benthamiana* leaves and stable transformation of *Arabidopsis thaliana*. The GV3101 strain is resistant to rifampicin and gentamicin. The GV3101 strain is selected because it was frequently used for many binary vectors for *Arabidopsis thaliana*.

2.1.4 Primers and Plasmids

All used primers were ordered from Eurofins MWG Operon. The software Geneious 6 was used for primer designing and the specific properties calculating. All primer

sequences are listed in the Appendix in Tab. 11. The destination vectors and important module donor vectors are listed in Tab. 1. Other plasmids are described in the corresponding chapters with an overview in the Appendix in Tab. 7; Tab. 8; Tab. 9 and Tab. 10.

Table 1. List of destination vectors and module donor vectors

Construct Name/ID	Description	Level	Resistance
pAGM759	N-terminus of AvrBs3 TALE without secretion peptide	-1	Kan
pICH70781	N-terminus of AvrBs3 TALE	-1	Carb
pICH75071	N-terminus of AvrBs3 TALE with NLS	-1	Carb
pAGM5421	TALE DNA binding part 1	-1	Carb
pAGM5432	TALE DNA binding part 2	-1	Carb
pAGM5443	TALE DNA binding part 3	-1	Carb
pICH73097	TALE EBE002 DNA binding part 1	-1	Carb
pICH73081	TALE EBE002 DNA binding part 2	-1	Carb
pICH73093	TALE EBE002 DNA binding part 3	-1	Carb
pICH72151	TALE C-terminal full length	-1	Carb
pICH72161	TALE C-terminal without activation domain	-1	Carb
pICH77444	TALE C-terminal without stop condon	-1	Carb
pICH80373	TALE truncated C-terminal without stop condon	-1	Carb
pICH73103	TALE with truncated N-ter	0	Spec
pICH41308	Level0 entry vector for CDS	0	Spec
pAGM1263	Level0 entry vector for 5'UTR	0	Spec
pAGM1276	Level0 entry vector for N-terminal fusion	0	Spec
pAGM1311	Level0 entry vector for fragment	0	Kan
pICH50581	Actin2 promoter	0	Spec
pICH41421	Nos terminator	0	Spec
pICH41432	Ocs terminator	0	Spec
pICH41531	GFP	0	Spec
pICH75111	GUS	0	Spec
pICH72400	G7 terminator	0	Spec
pICH87644	Actin2 promoter with TMV Ω +	0	Spec
pICH51277	Short 35s promoter with TMV Ω +	0	Spec
pAGT582-1	TALE EBE002 binding promoter	0	Spec
pICH77901	Mas terminator	0	Spec
pAGT3059	Arabidopsis actin2 promoter	0	Spec
pAGT861	6xHis tag	0	Spec
pICH46501	TMV(Tobacco mosaic virus) Ω +	0	Spec
pAGT3448	GUS containing Csy4 target site	0	Spec
pICH43201	Nosp-Bar expression cassette	1	Carb

pICH47742	Level1 position 2 vector	1	Carb
pICH47751	Level1 position 3 vector	1	Carb
pICH47781	Level 1 destination vector	1	Carb
pICH47852	Level 1 destination vector	1	Carb
pICH74043	Actin2 promoter-TALE	1	Carb
pICH41766	Linker	1	Spec
pICH67131	Kan expression cassette	1	Carb
pAGM25361	TALE binding promoter-GFP expression cassette	1	Carb
pAGM13163	Betacyanin synthesis associate enzyme 1	1	Carb
pAGM13175	Betacyanin synthesis associate enzyme 2	1	Carb
pAGM13187	Betacyanin synthesis associate enzyme 3	1	Carb
pICH41822	EL6 linker	1	Spec
pAGM23821	Nosp-Bar expression cassette	1	Carb
pICH54022	Dummy	1	Carb
pAGT3455	Actin2p-Csy4 expression cassette	1	Carb
pAGT3456	Actin2p-JAZ1: Csy4 expression cassette	1	Carb
pAGT4121	Actin2p-6xHis: GUS (containing Csy4 target site) expression cassette	1	Carb
pICH50892	L3E linker	1	Carb
pAGT4134	35sp-6xHis: GFP (containing Csy4 target site) expression cassette	1	Carb
pAGT4135	35sp-6xHis: GFP (containing Csy4 target site) expression cassette	1	Carb
pICH71081	TALE binding promoter-GFP expression cassette and BAR expression cassette	2	Carb
pAGM4673	Level 2 destination vector	2	Kan
pAGM4723	Level 2 destination vector	2	Kan
pAGM8031	Level 2 destination vector	2	Spec
pDG15	Donor vector with P1r P2 site	-	Kan
pDG26	Donor vector with P4-KpnI Xmal-P1r site	-	Amp
pDG27	Expression vector with R4 R2 site	-	Spec

2.1.5 Fertilizer and media for cultivation of plants and microorganisms

All media were sterilized by autoclaving for 20 min at 121 °C and 2 bar if not mentioned differently. Antibiotics were added after cooling the media to about 50 °C. Antibiotics were solved in H₂O_{dd}, if not mentioned differently and sterile-filtered (Rotilabo®- Spitzenfilter; 0, 22 µm).

Table 2. Concentration of antibiotics in bacterial selection medium

Antibiotic	Stock solution	Working concentration
Rifampicin*	50 mg/ml	50 µg/ml
Ampicillin	50 mg/ml	50 µg/ml
Carbenicillin	50 mg/ml	50 µg/ml
Kanamycin	50 mg/ml	50 µg/ml
Spectinomycin	100 mg/ml	100 µg/ml
X-Gal**	50 mg/ml	50 µg/ml
Glufosinate-ammonium***	-	6 mg/L

* Solved in methanol

** 5-bromo-4-chloro-3-indolyl-beta-Dgalactopyranoside. Sigma stock number: B4252

*** Main ingredients of herbicide Basta. Sigma stock number: 45520

2.1.5.1 Luria- Bertani- Medium (LB)

LB

1.0%(w/v) Tryptone

0.5%(w/v) Yeast extract

1.0%(w/v) Sodium Chloride

2.1.5.2 LB Medium with agar

LB with agar

1.0%(w/v) Tryptone

0.5%(w/v) Yeast extract

1.0%(w/v) Sodium Chloride

1.0%(w/v) Agar

pH=7.2

2.1.5.3 S.O.C. Medium (SOC)

SOC medium was used in the final step of bacterial cell transformation to obtain maximal transformation efficiency of *E. coli*.

SOC medium	for 500 ml
2.0%(w/v) Tryptone	10 g
0.5%(w/v) Yeast extract	2.5 g
10mM/L NaCl	0.292 g
2.5mM/L KCl	0.093 g
10mM MgCl ₂	0.476 g
10mM/L MgSO ₄	1.204 g
20mM/L Glucose	1.802 g

2.1.5.4 Murashige and Skoog (MS) culture medium

MS medium

MS basal mixture

1.0%(w/v) Sucrose

0.7%(w/v) Plant agar

Adjust pH to 5.6-5.7 with KOH

2.2 Microbiological Methods

2.2.1 Production of chemically competent *E. coli* cells

In this work *E. coli* strain DH10B was used. 4 ml LB-medium was inoculated with cells from a glycerol stock and were shaken at 220 rpm (Shaker, Fluke GmbH, Glottertal, Germany) over night at 37 °C. 4 ml of the overnight culture was transferred to 200 ml LB medium and shaken at 37 °C for around 3 h until an OD₆₀₀ of about 0.6 was reached. The culture was divided in 4 aliquots of 50 ml tubes and incubated for at least 10 min on ice. Samples were centrifuged (10 min, 3000 rpm, 4 °C) and the pellet was resuspended in 20 ml cold TFB1buffer on ice. Samples were

again centrifuged (10 min, 3000 rpm, 4 °C) and pellet was resuspended in 20 ml cold TFB2 buffer on ice. Then cells were aliquoted to 50 µl each in sterile tubes, shock frost in liquid nitrogen and stored at -80 °C.

Table 3. Compositions of buffers for chemical competent cells production

TFB1		TFB2	
Concentration	for 500 ml	Concentration	for 100 ml
30 mM CH ₃ COOK	1.475 g	100 mM MOPS (or PIPES)	2.095 g
10 mM CaCl ₂	0.735 g	75 mM CaCl ₂	1.1 g
100 mM RbCl	6.05 g	10 mM RbCl	0.12 g
15% Glycerol	75 ml	15% Glycerol	15 ml
Adjust pH to 5.8 (with 1 M CH ₃ COOH)		Adjust pH to 5.8 (with 1 M KOH)	

2.2.2 Transformation of *E. coli* cells

50 µl of cells were thawed on ice; 10 µl of the ligation reaction (see section 2.4.6) was added to the vial, mixed gently and incubated on ice for 30 min. The cells were incubated for exactly 90 sec at 42 °C and then placed on ice. 400 µl sterile SOC medium were added and shaken for 45 min at 450 rpm. 50-100 µl per transformation were plated on LB agar plates with the corresponding antibiotic (see Tab. M1) and X-gal for selection of positive transformants by blue-white screening of constructs that contain a LacZ selection marker. Plates were cultivated at 37 °C overnight. White colonies were analyzed by plasmid isolation and sequencing.

2.2.3 Production of electricity competent *A. tumefaciens* cells

A. tumefaciens cells were cultivated overnight in 4 ml LB medium containing rifampicin (28 °C, 220 rpm). 4 ml of the overnight culture was transferred to 400 ml fresh LB medium containing rifampicin and shaken at 28 °C for 16-18 h. The culture was divided in 8 aliquots of 50 ml tubes and centrifuged (3000 rpm, 4 °C, 10 min). The pellet was resuspended in 20 ml cold 10% glycerol on ice. The culture was centrifuged and resuspended in 20 ml cold 10% glycerol on ice again and mix cells to 2x 50 ml tubes. Cells were again centrifuged (3000 rpm, 4 °C, 10 min) and pellet was resuspended in cold 10% glycerol on ice (the volume was depend on the cell

numbers). Then cells were aliquoted to 25 μ l each in sterile tubes, shock frost in liquid nitrogen and stored at -80 °C.

2.2.4 Transformation of *A. tumefaciens* cells

The transformation of *A. tumefaciens* was performed by electroporation. Cells were thawed on ice. In parallel electroporation cuvettes (2 mm gap width, Eurogentec, Seraing, Belgium) were cooled on ice. 200-300 ng of plasmid DNA was added to the thawed cells. The cell/ plasmid suspension was pipetted into the cuvette, and inserted into the electroporator (BioRad Laboratories GmbH, Munich, Germany). Immediately after electroporation, 1 ml of LB-medium was added to the cuvette, to flush out the transformed cells and transferred to a 1.5 ml eppendorf tube. The transformed cells were incubated at 28 °C and 220 rpm for 3-4 h. 200 μ l of the transformed cells were plated on LB plates with selection antibiotics (see Tab. M1) and incubated at 28 °C for 2 days.

2.3 Molecular biological methods

2.3.1 Isolation of genomic DNA

100 mg frozen plant material was ground in a Retsch mill at 30 r/s for 2 min and kept frozen permanently with liquid nitrogen. The DNA isolation of plant material was done with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer instructions.

2.3.2 Isolation of Plasmid DNA

For miniprep of Plasmid DNA from a bacterial liquid culture the NucleoSpin® Plasmid Kit (MACHEREY-NAGEL GmbH & Co. KG, Neumann-Neander-Str. 6–8, 52355, Düren, Germany) was used according to the manufacturer instructions.

For midiprep of Plasmid DNA from a bacterial liquid culture the QIAGEN Plasmid Midi Kit (Qiagen, Hilden, Germany) was used according to the manufacturer instructions.

2.3.3 Isolation of RNA

For RNA isolation from plant material, the RNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany) was used according to the manufacturer instructions. The obtained RNA was quantified with a NanoDrop 1000 (peQLab Biotechnologie GmbH, Erlangen, Germany) and a quality control was done with a QIAxcel system from Qiagen.

2.3.4 DNase treatment to RNA

The RNA samples were diluted to the concentration under 200 ng/ μ l with RNase-free water first. 0.1 Volume 10x TURBO DNase Buffer and 1 μ L TURBO DNase were added into 34 μ L RNA and mixed gently. The reaction mixture were incubated at 37 °C for 20–30 min. DNase Inactivation Reagent (typically 0.1 volume, vortexed before using) were added and mixed well. The reaction mixture were incubated at room temperature for 5 min and mixed occasionally. The reaction mixture were then centrifuged at 10,000 x g for 1.5 min and RNA was transferred to fresh tube.

2.3.5 Synthesis of cDNA

For cDNA synthesis, 1 μ g of RNA in a volume of 10 μ l of H₂O was used. 1 μ l of a 100 mM Oligo (dT) primer was added, short mixed and incubated for 5 min at 70 °C and immediately incubated on ice for 5 min. 9 μ l mastermix including 1 μ l Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV), 4 μ l M-MLV RT 5x reaction puffer, 4 μ l dNTPs (2,5 mM), (Promega, Mannheim, Germany) were added to the RNA preparation which make volume up to 20 μ l and the following program was applied: 10 min at 40 °C, 50 min at 42 °C, 15 min at 70 °C. The obtained cDNA was diluted 10x to serve as template for PCR.

2.3.6 Agarose Gel Electrophoresis

The separation of PCR products, plasmid DNA or restricted DNA was performed in a 1% (w/v) agarose gel in 1x TAE buffer (40 mM Tris, 20 mM acetic acid (C₂H₄O₂: 100 %), 1 mM Na₂EDTA (pH 8.5)). 1 μ l/ 50 ml DNA stain G (SERVA GmbH, Heidelberg, Germany) was added to the heated medium for staining and visualization of the nucleic acid. Before loading the samples on the gel, 0.1 Volume 10 x loading buffer (10 x Fast Digest Green Buffer, Thermo Scientific) was added to the sample. The gel electrophoresis was conducted at 120 V in 1x TAE buffer. The negative load

of DNA fragments attract those to the anode and a mass dependent separation of the fragments follows. After electrophoresis the gels were analyzed with a gel documentation system.

Ruler, 1 kb plus, Thermo Scientific).

2.3.7 Polymerase Chain Reaction (PCR)

PCR is a method for targeted amplification of a DNA template strand with the help of two specific oligonucleotides that serve as primer and a heat resistant polymerase [Mullis *et al.*, 1986]. The procedure includes the three steps of DNA denaturation, annealing and elongation that are repeated in cycles (25-40x). The PCR reaction was performed with an Eppendorf MasterCycler® Gradient Thermocycler (Eppendorf AG, Hamburg, Germany). To check the primers were working or to find the most optimal melting temperature, the DreamTaq DNA polymerase (Thermo Fisher Scientific) was used. For further use in cloning applications, KOD Hot Start DNA polymerase (Sigma-Aldrich, 71086) was used, since it is a High fidelity DNA polymerase designed for accurate PCR amplification of long strand and GC- rich DNA templates for cloning and cDNA amplification applications.

DreamTaq PCR Reaction: Final volume		KOD PCR Reaction: Final volume	
	(10 µl)		(50 µl)
10x DreamTaq buffer	1 µl	10x buffer for KOD	5 µl
dNTP Mix (2 mM each)	0.5 µl	dNTP Mix (2 mM each)	5 µl
Forward Primer (10 µM)	0.2 µl	Forward Primer (10 µM)	1.5 µl
Reverse Primer (10 µM)	0.2 µl	Reverse Primer (10 µM)	1.5 µl
Template DNA	10-50 ng	Template DNA	10-50 ng
DreamTaq Polymerase	1 µl	MgSO ₄ (25 mM)	3 µl
H ₂ O _{dd}	add to 10 µl	KOD polymerase	1 µl
		H ₂ O _{dd}	add to 50 µl

Table 4. General scheme of PCR program: the annealing temperature (t_{opt}) was chosen primer-dependently with 3-6 °c below the specific melting temperature (t_m) of the primers.

Process	DreamTaq PCR Reaction	KOD PCR Reaction
Initial denaturation	3 min/95 °C	2 min/95 °C
35 cycles:		
Denaturation	30 sec/95 °C	20 sec/95 °C
Annealing	30 sec/ T_{opt}	10 sec/ T_{opt}
Extension	1 min per kb/ 72 °C	10 sec per kb/ 70 °C
Final elongation	5 min/ 72 °C	5 min/ 70 °C
End	∞ / 4 °C	∞ / 4 °C

2.3.8 Quantitative Real-Time PCR (qRT-PCR)

For quantification of mRNA in biological samples, Quantitative real-time polymerase chain reaction was used. Fluorescence signal was emitted by the report dye that should be proportional to the amplified nucleic acids. The quantification was assessed relative to the transcript amount of a non-regulated housekeeping gene. Crucial for quantification was the Ct-value that indicates the first PCR-cycle reaching a level of fluorescence (threshold) above the background (baseline). EvaGreen® Dye was used as fluorescence dye. The binding of EvaGreen® Dye to the double strand DNA induced an augmentation of fluorescence intensity, which correlates with the amount of template-DNA. The size of amplified fragments was 80-120 bp. qRT-PCR was performed with the CFX Connect™ Real-Time PCR System from BIO-RAD Laboratories, INC. (Munich, Germany). The data evaluation was done with the Bio-Rad CFX software.

qPCR Reaction: Final volume (10 μ l)

EvaGreen® Dye Master Mix	2 μ l
Forward Primer (2 μ M)	1 μ l
Reverse Primer (2 μ M)	1 μ l
Template cDNA	3 μ l
H ₂ O _{dd}	3 μ l

Table 5. General scheme for qRT-PCR program: the annealing temperature (T_{opt} , usually be 55 °c) was primer-dependent and was chosen 3-6 °c below the specific melting temperature(t_m) of the primers.

Process	Time	Temp (°C)
Initial denaturation	15 min	95
39 cycles:		
Denaturation	30 sec	95
Annealing	30 sec	T_{opt}
Plate read	-	-
Final elongation	10 sec	95°C
Melt Curve analysis	5 sec	65°C
	5 sec	95°C
Plate read	5 sec	-
End	∞	16 °C

Manager. The Ct-values of the target gene (TG) were normalized to the housekeeping genes (Reference gene: RG) with the following formula: $\Delta Ct = Ct_{TG} - Ct_{RG}$. The transcript accumulation of a gene from one sample was determined using the mean of 3 technical replicates. The negative potency of Ct resulted in the actual value of relative gene expression calculated with the following formula: Relative gene expression = $2^{-\Delta Ct}$

2.4 Cloning methods

2.4.1 Restriction of DNA

DNA restriction was performed with "fast digest" Enzymes from Thermo Fisher Scientific. 100-200ng DNA was used for a final reaction volume of 10 μ l according to the user's manual. To control the DNA restriction, an agarose gel electrophoresis was performed.

2.4.2 Gel extraction of DNA fragments

The DNA fragment of interest was separated with gel-electrophoresis and visualized on a UV-light table of the Geldocumentary system BioDoc Analyze (Whatmann Biometra, Göttingen, Germany) at an activation wavelength of 360nm. The fragment of interest was cut with a scalpel and the DNA was extracted from the gel with the MinElute[®] Gel Extraction Kit from Qiagen according to the user's manual.

2.4.3 DNA-fragment purification

To clean DNA-fragments gained from a PCR (see section 2.3.6) or restriction reaction (see section 2.4.1), SureClean Kit from Bioline GmbH (Luckenwalde, Germany) was used according to the user's manual.

2.4.4 Sequencing

All sequencing was done by the company MWG Biotech AG (Ebersberg, Germany). Evaluation of the quality of the sequencing file (nucleotide peaks) and the alignment with the original sequence was performed with the software Geneious 6.

2.4.5 Gateway cloning

The GATEWAY Cloning Technology is based on the site-specific recombination system. The gene of interest was amplified by specific primers resulted in flanking by attB site. The PCR product was purified (see section 2.4.3) and reacted with Donor Vector containing attP site by BP reaction to build Entry vector. Upon integration, the recombination between attB and attP sites generated attL and attR sites. Then attL recombination sites flanked the gene in Entry vector. The Entry Vector is transcriptionally silent. To produce the Expression Clone, the gene has to be

subcloned into a Destination Vector that contains all the sequence information necessary for expression and two attR recombination sites by LR reaction. For BP and LR reactions Invitrogen Gateway[®] kit was used according to the user's manual.

2.4.6 Vector construction with Golden Gate Cloning

The Golden Gate Cloning method was described by C. Engler (Engler *et al.*, 2008). Different from other cloning method, this method is not based on site-specific recombination but relies on the use of type IIs restriction enzymes. Type IIs restriction enzymes are able to cleave DNA outside of their recognition site, resulting in 5' or 3' DNA overhangs (depending on the enzyme) that can consist of any nucleotide. Therefore, 256 different overhangs can be created using a type IIs restriction endonuclease that produces a 4nt overhang. This property has been used to develop protocols for efficient assembly of multiple DNA fragments in a single ligation reaction and has multiple advantages: the enzyme recognition site is independent from the sequence of the gene of interest and would be eliminated after subcloning, the restriction sites could be designed to have different cleavage site sequences, allowing directional cloning and preventing religation of empty vector, no buffer incompatibility issues since only one restriction enzyme was used for all cleavage sites, close to 100% efficiency. Type IIs restriction enzymes BsaI (NEB R0535s) and BpiI (Thermo Fisher Scientific ER1012) were used.

Reaction mix

Destination vector	20 fmol *
Insert 1	20 fmol
Insert 2	20 fmol
.....	20 fmol
.....	20 fmol
Insert n	20 fmol
T4 ligase	1 μ l
Ligase buffer	1.5 μ l
BsaI or BpiI	0.5 μ l
H2O add to	15 μ l

*For 20 fmol: Vol (μ L) = 20 (fmol) x Size (bp) / conc (ng/ μ L) /1520

Table 6. General scheme of restriction-ligation programs for Golden Gate cloning
Programs for restriction-ligation

1 to 3 fragments	More than 3 fragments
2-4 h/37 °C	35 cycles: 2 min/37 °C
	5 min/16 °C
5 min/50 °C	5 min/50 °C
5 min/80 °C	5 min/80 °C

2.5 Biological methods

2.5.1 Cultivation of plants

2.5.1.1 *Nicotiana benthamiana*

Nicotiana benthamiana plants were grown in a greenhouse at 22-24 °C under long-day conditions (16 h light/8 h dark). 30 days old plants were used for infiltration.

2.5.1.2 *A. thaliana* plants for stable transformation

A. thaliana (ecotype Columbia-0) seeds were sprinkled onto wet soil in pots and were stratified at 4 °C for 2 d. Pots were transferred into the plant incubator at 22 °C under long-day conditions (16 h light/8 h dark), 70% Humidity for 10 d. Seedling were transferred into fresh wet soil in pots and cultivated in greenhouse for another 30 d.

2.5.1.3 Sterilization and germination of *A. thaliana* seeds

A. thaliana seeds were soaked in 100 µl disinfectant (64.7 µl H₂O_{dd}, 2 µl 1% Tween20, 33.3 µl NaClO) for 10 min in a 2 ml eppendorf tube. After that, add 100µl sterile H₂O_{dd}, centrifuge for several seconds and remove the supernatant liquid, then wash the seeds 3 times with sterile H₂O_{dd}.

Surface-sterilized seeds were sowed with sterile tip onto circular or square plates containing MS medium and 0.7% (w/v) agar, after 2 d of stratification grown with cycles of 16 h light and 8 h dark.

2.5.2 Transient transformation of *N. benthamiana*

For transient expression assays in *N. benthamiana*, leaves were infiltrated with *Agrobacterium tumefaciens* GV3101 harboring respective plasmids. Transformed *Agrobacterium tumefaciens* were plated on LB medium containing Rifampicin and antibiotics for 2 days at 28 °C. Positive transformants were cultivated in 4 ml LB with rifampicin and antibiotics correspondingly, overnight at 28 °C and 200 rpm. The OD₆₀₀ was measured and the needed amount of overnight liquid culture per infiltration was calculated. Cultures were adjusted to an OD₆₀₀ of 0.3 using dissolving buffer (10 mM MES (pH5.5), 10 mM MgSO₄). *N. benthamiana* leaves were pressure-infiltrated through the abaxial epidermis with a 1 ml syringe without needle. Successful infiltration was observed as a spreading “wetting” area. Infiltration areas were marked and leaves were analyzed 3-5 days after infiltration.

2.5.3 Stable transformation of *A. thaliana*

For *Agrobacterium*-mediated stable transformation of *A. thaliana*, the floral-dip technique was used (Clough and Bent, 1998). Transformed *Agrobacterium tumefaciens*, carrying the needed construct were plated on LB medium containing

Rifampicin and antibiotics for 2 days at 28 °C. Positive transformants were cultivated in 4ml LB with rifampicin and antibiotic, overnight at 28 °C and 200 rpm. The liquid cultures were transferred into 300 ml fresh LB with rifampicin and antibiotic, cultured overnight to the OD₆₀₀ around 1.2-1.6. The OD₆₀₀ was measured and the needed amount of overnight liquid culture was calculated as follows: $OD_{\text{target}} \times V_{\text{target}} = OD_{\text{actual}} \times V_{\text{culture}}$; with $OD_{\text{target}} = 0.8$ and $V_{\text{target}} = 500$ ml. The appropriate amount of overnight culture was centrifuged at 3000 rpm at room temperature for 30 min. The supernatant was removed and the pellet was resuspended in transformation buffer (5 % (w/v) sucrose, 0.05 % (v/v) Silwet-77). The plants (around 45 d old) with open flowers, many white buds ready to flower and few seeds capsules were choose and dipped into the cell suspension for 1min. The plants were lying down horizontally and covered with plastic bags to keep high humidity in dark for one day. Then the plants were transferred into greenhouse for getting seeds.

2.5.4 Selection of transformants

Seeds from primary transformants were sprinkled onto wet soil in pots and place in cold chamber for 2-5 days. Many hundreds of seeds were sowed as <2% would be transgenic. Numerous pots were prepared, labeled one as a "no spray" control (Ideally, it's a good idea to plant two additional control pots, one with wild-type seed and another with a known BASTA resistant line). Seedlings were sprayed with 200 mg/L basta solution (dilute commercial herbicide BASTA (200 mg/ml) 1:1,000 in water) at 8, 10, 13, 15days. Non-transformed plants would show yellowing of cotyledons from 13th day. 16-20 independent resistant plants were then transferred to new pots and covered inflorescences with plastic bags and stop watering at around 80th day. At 105th day, most seedpods would be brown; the plants were cut off and bags were stored for about 2 weeks to let seeds dry completely. At around 120th day, seedpods were broken on large white sheet of paper, blow off chaff and harvest seeds into microfuge tube. Be careful not cross contaminate the seeds.

2.5.5 Selection of single-insertion and homozygous transgenic plants

16 to 20 independent transgenic lines were identified in the T1 generation by Basta selection for each construct analyzed. To verify single-copy transgene insertion, about 100 seeds from each T2 transgenic line were sprinkled onto the MS plates containing

Glufosinate-ammonium. After 7 days incubation in plant incubator, the plates were analyzed for a 3:1 segregation ratio resistant to Basta. From the plants on these plates, 1/2 were heterozygous for the insert, 1/4 were homozygous for the insert, and 1/4 were WT which cannot survive on the selection plates. 12 resistant plants from each 3:1 segregation ratio plates were transferred into soil with forceps and grown in greenhouse up to seeds. The seeds (T3 generation) were harvested from each of the 12 plants and sprinkled onto the MS plates containing Glufosinate-ammonium. After 7 days incubation in plant incubator, the plates were analyzed and which with 100% resistant to Basta were the homozygous plants for the insert. GFP fluorescence was analyzed in homozygous T3 plants of two to four lines of each construct.

2.5.6 Gus activity staining

The GUS (β -glucuronidase) reporter system is a histochemical technique to analyze the activity of a promoter (in terms of expression of a gene under that promoter) either in a quantitative way or through visualization of its activity in different tissues (Jefferson *et al.*, 1987). The technique is based on β -glucuronidase an enzyme from the bacterium *Escherichia coli* which can transform the soluble colorless substrate 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) into an insoluble intensely blue final products.

The whole seedlings or leaf tissues were incubate in the GUS staining solution, vacuum infiltrated the solution into the objects, kept in darkness at 37 °C for several hours or until distinct blue staining appears (no longer than 24h). Photosynthetic tissues were cleared of chlorophyll by incubation in 95% ethanol.

GUS staining solution	Concentration
NaH ₂ PO ₄ (pH7.0)	100 mM
EDTA (pH7.0)	10 mM
K ₃ Fe(CN) ₆	0.5 mM
K ₄ Fe(CN) ₆	0.5 mM
X-Gluc*	1 mg/ml
Triton X-100	0.1%
H ₂ O _{dd}	-

*Predissolve the X-Gluc in a drop of *N, N*-dimethylformamide immediately prior to use. Staining solution can be stored at -20°C and reused several times.

2.6 Photograph and microscopic works

2.6.1 Photograph with UV lamp

To analyze transient expression assays in *N. benthamiana*, leaves at four to five days after infiltration were incubated in water, 50 µM Methyl Jasmonate solution or 1µM Coronatine solution for 24h. Leaves were photographed with a camera under UV light (wavelength: 365 nm) from the UV lamp (Blak-Ray[®] B-100AP High Intensity UV Lamp, Upland, CA, USA) in the dark room.

2.6.2 Photograph with Microscope

The homozygous T3 seeds were germinated on square MS plates and grown vertically in the plant incubator. The seedlings were sprayed with water, 50 µM Methyl Jasmonate solution or 1 µM Coronatine solution. After 24h, the GFP fluorescence was analyzed with Apotome microscope or confocal laser-scanning microscope LSM 700 (Zeiss, Jena, Germany) using 488 nm excitation and 495-557 nm as emission.

3 Results

3.1 A system based on JAZ-mediated repression of MYC2

This system to detect jasmonates based on JAZ-mediated repression of MYC2 needs two constructs: a synthetic promoter construct that contains binding sites for a specific, synthetic TF and the encoding gene for reporter protein GFP, and a synthetic TF which will exhibit functional similarity to MYC2 and will be therefore repressed by JAZ proteins. An active JA signaling resulting in JAZ degradation will lead to liberation of the TF and therefore to expression of the respective reporter gene GFP.

3.1.1 Establishment of the synthetic promoter and TF constructs

3.1.1.1 Synthetic promoter construct

The vector pICH71081 (see Table. M1) (supplied by S. Marillonnet) was used as synthetic promoter construct that contains the TALE binding site (EBE002) (see section 1.5) (5'- N (19) TCCCCGCATAGCTGAACATCTATATAAN (43) -3') for specific TALE part of synthetic TF and the encoding gene for reporter protein, GFP. It is expected that *GFP* expression would be switched on when TALE binds.



Figure 11 Scheme of the synthetic promoter construct “promsyn::GFP”. Synthetic promoter construct containing TALE binding site (EBE002) in the promoter region and the encoding sequence for GFP.

3.1.1.2 Synthetic transcription factor construct

The structure of MYC2 was analyzed to select appropriate domains that can be used for forming the synthetic TF (see section 1.3.4). Since JAZ interaction domain (JID) or the N-terminal region is responsible for the binding to JAZ, seven different MYC2 fragments containing JID domain or N-terminal region were cloned (see Fig.12a), and included JID domain only; N-terminus with JID domain; N-terminus with JID and TAD domain; JID and TAD domains; TAD domain; MYC2 without bHLH domain; and the full length MYC2.

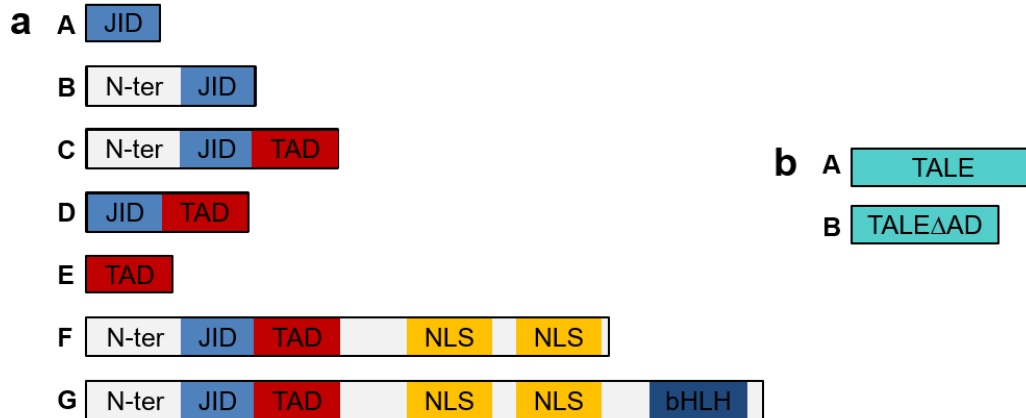


Figure 12 Fragments of MYC2 and TALE used in synthetic transcription factor construct. (a) Seven fragments of MYC2: JID domain, 93-160aa; N-terminus with JID domain, 1-160aa; N-terminus with JID and TAD domain, 1-188aa; JID and TAD domains, 93-188aa; TAD domain, 149-188aa; MYC2 without bHLH domain, 1-467aa; full length MYC2, 1-623aa. (b) Two variants of TALE: intact TALE with activation domain; TALE without activation domain.

Structure of TALE was also analyzed (see section 1.5). Repeat modules were engineered that confer binding to EBE002 specific sequence. Two variants of TALE were used (see Fig.12b), one is intact TALE with activation domain, and another one is the TALE without activation domain (47aa from the C-terminus were deleted).

The various fragments of MYC2 fused to N-terminus of TALE together with *actin2* promoter and *ocs* terminator were cloned to destination vector pICH47751 (see section 2.1.4). Combining all the variants, fourteen constructs were constructed from last cloning step. The fusions were transferred into *A.tumefaciens* (GV3101) (see section 2.1.3), which were used for transient expression assays in *N.benthamiana*.

3.1.2 Analysis the GFP fluorescence of the *N.benthamiana* leaves after transient expression of transcription factor constructs

3.1.2.1 Infiltration experiments with the N-terminal fusions

The constructs were co-infiltrated into *N. benthamiana* with the “*promsyn::GFP*” construct. The construct containing intact TALE with activation domain co-infiltrated with the “*promsyn::GFP*” construct was used as positive control (PC). The construct containing TALE without activation domain co-infiltrated with the “*promsyn::GFP*” construct was used as negative control 1 (NC1). Infiltration only with the “*promsyn::GFP*” construct was used as negative control 2 (NC2).

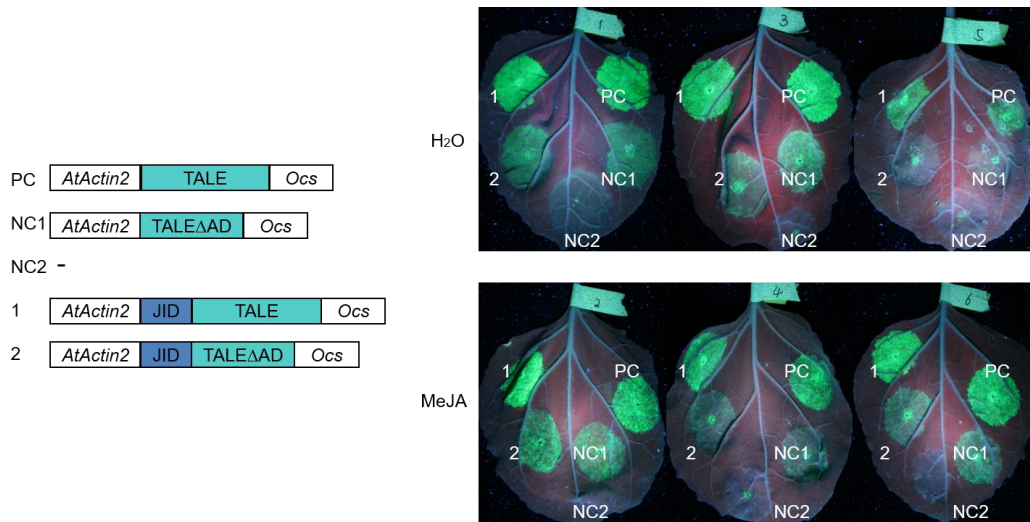


Figure 13 Infiltrations experiments showed different level of GFP fluorescence in dependence on the different constructs (for construct numbers see legend to Fig. 14). The experiment was performed in independent triplicates with similar results; one representative example is shown.

All infiltrations were done in triplicates using leaves from different plants. The photos of the leaves were taken using an UV lamp five days after infiltration (Fig. 13). NC2 convincingly showed that the expression of *GFP* was absent if TALE is not expressed. This means the expression of *GFP* cannot be activated without TALE. In contrast, NC1 led to expression of *GFP* that was very low but was still visible. This phenomenon led to the conclusion that TALE without activation domain also can activate the expression of *GFP* slightly. In contrast, PC showed strong fluorescence indicative for high expression.

In order to summarize the results, a schematic overview was created and the plus sign was used to represent the strength of *GFP* expression (Fig. 14). It was visible that the construct containing MYC2 domains fused to TALE without activation domain led to a *GFP* expression which was as weak as in the negative control 1 and the construct containing MYC2 domains fused to TALE with activation domain led to a *GFP* expression which was as strong as in the positive control except the construct containing full length MYC2 fused to the N-terminus of intact TALE with activation domain. This led to the conclusion that after addition of full length MYC2 to the N-terminus of TALE with activation domain, its activity can be repressed most probably by the activity of JAZ proteins.

	Construct Schematic	GFP fluorescence	
		H ₂ O	MeJA
PC	AtActin2 TALE Ocs	+++++	+++++
NC1	AtActin2 TALE Δ AD Ocs	++	++
NC2	-	-	-
1	AtActin2 JID TALE Ocs	+++++	+++++
2	AtActin2 JID TALE Δ AD Ocs	++	++
3	AtActin2 N-ter JID TALE Ocs	++++	++++
4	AtActin2 N-ter JID TALE Δ AD Ocs	+	+
5	AtActin2 N-ter JID TAD TALE Ocs	++	++
6	AtActin2 N-ter JID TAD TALE Δ AD Ocs	+	+
7	AtActin2 JID TAD TALE Ocs	++++	++++
8	AtActin2 JID TAD TALE Δ AD Ocs	+	+
9	AtActin2 TAD TALE Ocs	++++	++++
10	AtActin2 TAD TALE Δ AD Ocs	+	+
11	AtActin2 N-ter JID TAD NLS NLS TALE Ocs	++++	++++
12	AtActin2 N-ter JID TAD NLS NLS TALE Δ AD Ocs	++	++
13	AtActin2 N-ter JID TAD NLS NLS bHLH TALE Ocs	+	+
14	AtActin2 N-ter JID TAD NLS NLS bHLH TALE Δ AD Ocs	-	-

Figure 14 Schematic overview about the strength of GFP expression driven by the various constructs (“-“ means there are almost no GFP fluorescence). PC: positive control, intact TALE with activation domain; NC1: negative control 1, TALE without activation domain; NC2: negative control 2; 1: JID domain of MYC2 fused to the N-terminus of intact TALE with activation domain; 2: JID domain of MYC2 fused to the N-terminus of TALE without activation domain; 3: MYC2-N-terminus + JID domain fused to the N-terminus of intact TALE with activation domain; 4: MYC2-N-terminus + JID domain fused to the N-terminus of TALE without activation domain; 5: MYC2-N-terminus + JID + TAD domains fused to the N-terminus of intact TALE with activation domain; 6: MYC2-N-terminus + JID + TAD domains fused to the N-terminus of TALE without activation domain; 7: JID + TAD domains of MYC2 fused to the N-terminus of intact TALE with activation domain; 8: JID + TAD domains of MYC2 fused to the N-terminus of TALE without activation domain; 9: TAD domain of MYC2 fused to the N-terminus of intact TALE with activation domain; 10: TAD domain of MYC2 fused to the N-terminus of TALE without activation domain; 11: MYC2 without bHLH domain fused to N-terminus of intact TALE with activation domain; 12: MYC2 without bHLH domain fused to N-terminus of TALE without activation domain; 13: Intact MYC2 fused to N-terminus of intact TALE with activation domain; 14: Intact MYC2 fused to N-terminus of TALE without activation domain. All the constructs above were co-infiltrate with the synthetic promoter construct PICH71081. The experiment was performed in independent triplicates with similar results.

But due to the high variations in the positive controls that did not show always high *GFP* expression, it is highly uncertain to draw final conclusions (Fig. 15). To trigger the *GFP* expression, the leaves were floated on 50 μ M MeJA for 0.5 h, a treatment that leads to degradation of JAZ proteins followed by expression of JA-induced

genes. Two days after MeJA treatment, there was, however, not more GFP fluorescence visible in the leaf area transformed with the constructs.

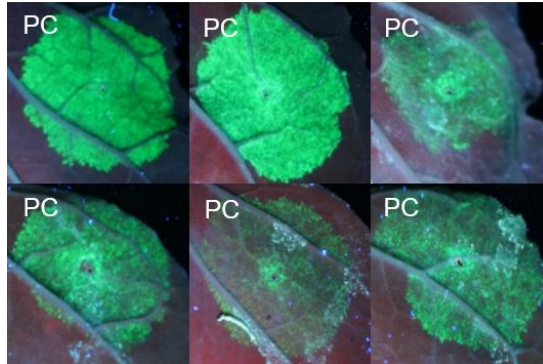


Figure 15 Infiltrations experiments showed different level of GFP fluorescence of the positive control. PC: positive control. Infiltrations were done in sextuplicates using leaves from different plants. PC showed strong but high variational fluorescence.

3.1.2.2 Infiltration experiments with the C-terminal fusions

Based on the results of infiltrations experiments with the N-terminal fusions, none of these fusions were defined as the applicable synthetic transcription factor in this system. Therefore, the C-terminal fusions were created. The same parts of MYC2 as described above were used (see section 3.1.1.2). Again, two variants of TALE (with or without activation domain) were combined at their C-terminus with MYC2-parts using the Golden Gate cloning system. After transfer into *A. tumefaciens*, these fusions were also tested by infiltration into *N. benthamiana* leaves. The results showed that NC1 and NC2 cannot activate the expression of *GFP* as expected, but even the positive control led to a very low *GFP* expression. Other C-terminal fusions constructs co-infiltrated with “*prom_{syn}::GFP*” did not lead to a *GFP* expression, neither before nor after MeJA treatment (only two example constructs shown in Fig. 16).

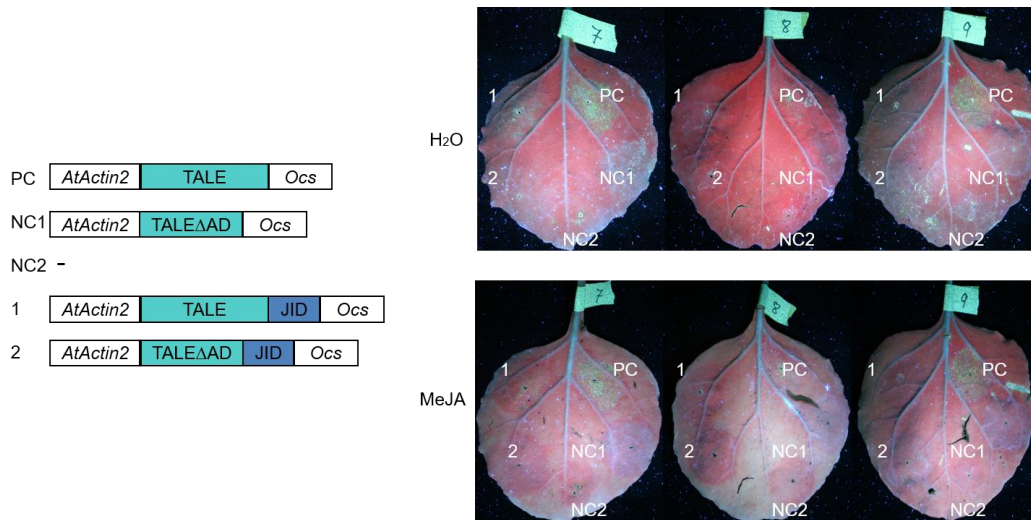


Figure 16 Infiltrations experiments showed general low level of GFP fluorescence independence on the different constructs. PC: positive control, intact TALE with activation domain; NC1: negative control 1, TALE without activation domain; NC2: negative control 2; 1: JID domain of MYC2 fused to the C-terminus of intact TALE with activation domain; 2: JID domain of MYC2 fused to the C-terminus of TALE without activation domain. The experiment was performed in independent triplicates with similar results.

3.1.3 System built in stable transformed plants

Since the reproducibility of the *N. benthamiana* infiltration experiments was not good, stable transformation was the second way to test this system in the plant. The constructs were made as described below for stable transformation with *A.thaliana*.

3.1.3.1 Establishment of the constructs for stable transformation

The constructs have to contain three different and independent cloning cassettes on the same vector backbone pAGM4673 (see section 2.1.4). The first one is the *Nos* promoter that drives the *Bar* gene leading to plant's resistance against the herbicide BASTA as a selectable marker. The second one contains the *GFP* encoding sequence as well as the promoter to which the TALE can bind (EBE002). The third one consists of the N-terminal or C-terminal fusions constructed before (see section 3.1.1.2 and 3.1.2.2) driven by the *Actin2* promoter. The construct containing the first and second cassettes serves as negative control. For positive control, the third cassette is the *Actin2*-promoter driven intact TALE containing the activation domain.

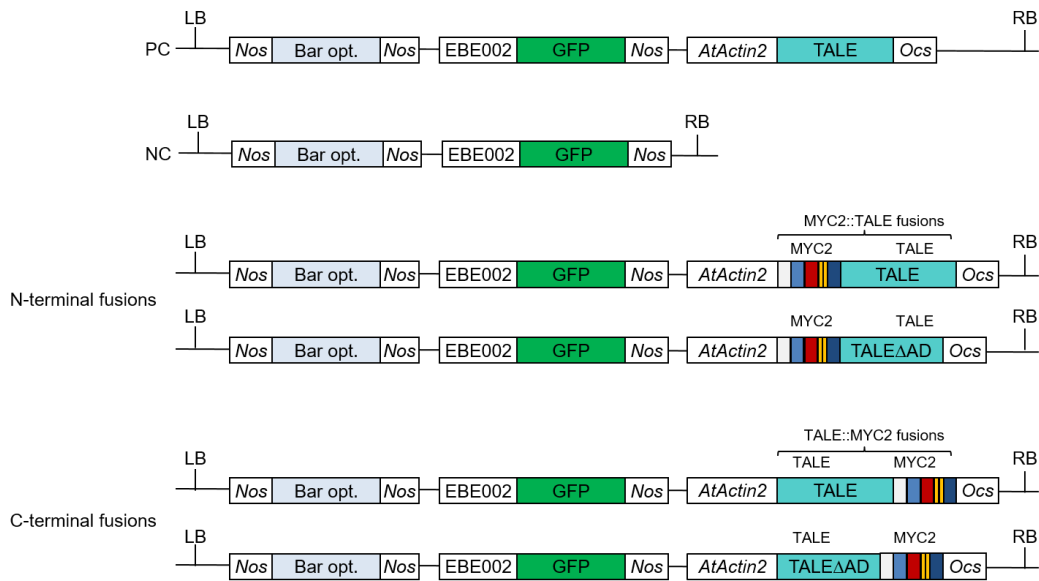


Figure 17 Scheme of the constructs for stable transformation. PC: positive control; NC: negative control; N-terminal fusions: two variants of TALE (with or without activation domain) combine at their N-terminal with MYC2-parts; C-terminal fusions: two variants of TALE (with or without activation domain) combine at their C-terminus with MYC2 parts. Bar: bialaphos resistance (bar) gene.

3.1.3.2 Analysis of the GFP fluorescence in stable transformed *A.thaliana* lines

All constructs were transferred into *A. tumefaciens* (GV3101), which then were used for floral dipping. Resulting transgenic lines (T1) were selected by spraying with BASTA. Lines with a single insertion that were homozygous were selected from T1 and T2 seeds by germination on BASTA and calculation of the ratio of BASTA resistant and sensitive plants.

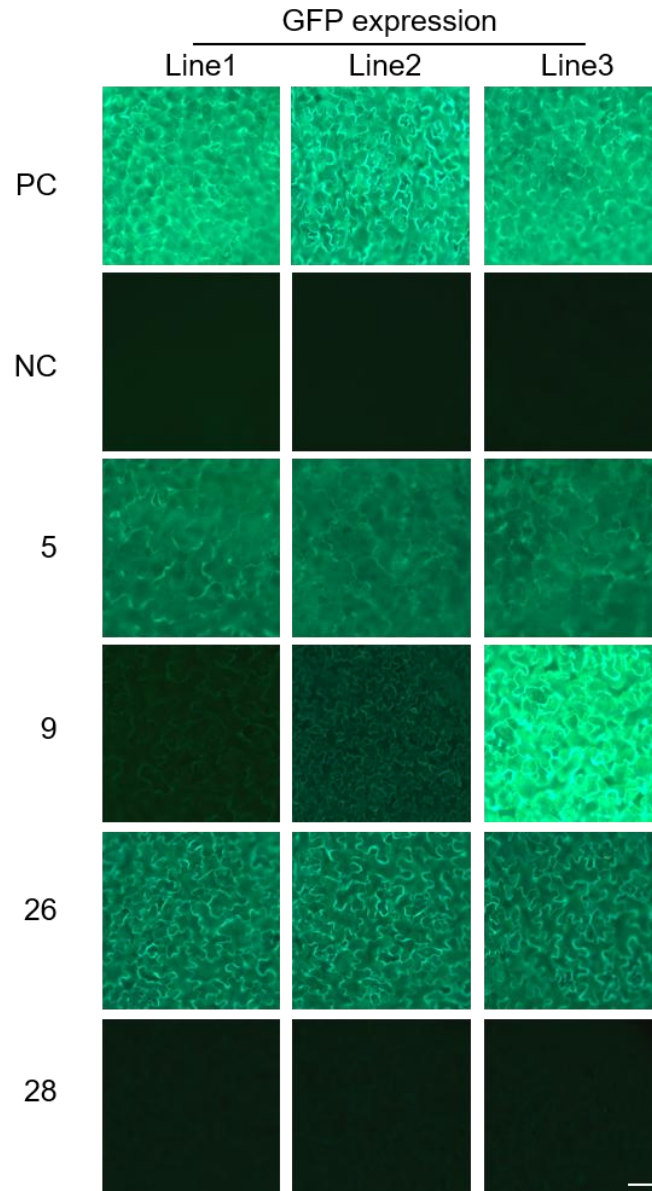


Figure 18 GFP fluorescence in the leaf of the single-insertion homozygous plants. PC: positive control; NC: negative control; for construct numbers see legend to Fig. 19; Line1, 2, 3 means the sublines expressing the same construct. The microscopic analysis was carried out using 488 nm excitation and 495-557 nm as emission. The scale bar is 100 μm for all micrographs. For each line, at least three seedlings were analyzed. One representative micrograph is shown here.

The GFP fluorescence of the single-insertion homozygous plants was checked with 7-days old seedlings using an epifluorescence microscope. The results showed that positive control led to *GFP* expression resulting in green fluorescence in all cells. In contrast, transformants expressing the negative control exhibited no GFP fluorescence. T3 lines expressing the same construct showed the same level of GFP fluorescence such as construct 5, 26, 28 (for construct numbers see legend to Fig. 19).

However, some T3 lines expressing other constructs showed different levels of GFP fluorescence, such as construct 9 (Fig. 18).

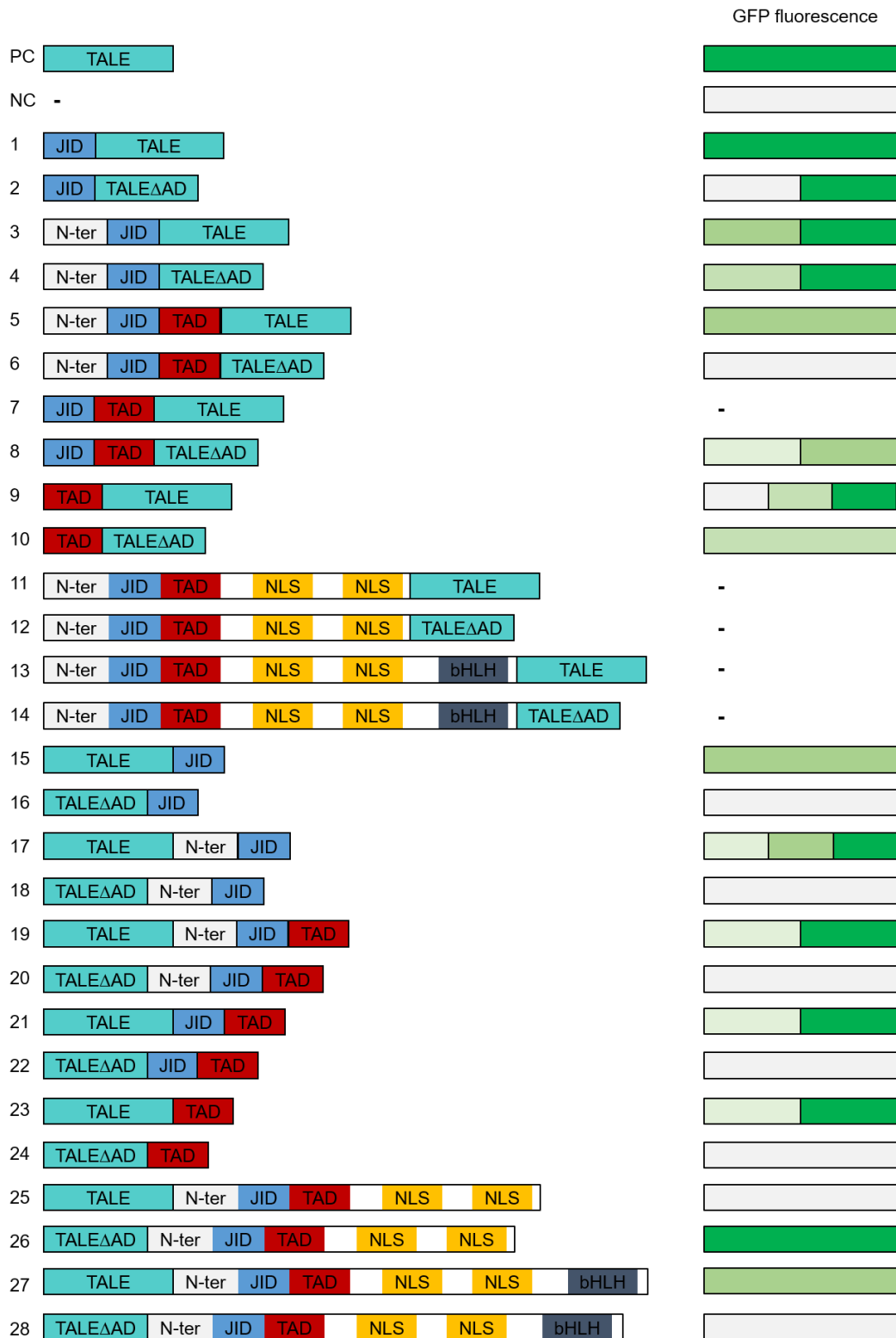


Figure 19 Schematic overview about the levels of GFP expression detected from the single-insertion homozygous plants. White means there are almost no GFP fluorescence; light green means the middle

level of GFP fluorescence; green means the highest level of GFP fluorescence; “-“means no single-insertion homozygous lines were screened with corresponding construct. For each construct, at least three lines were checked.

In order to summarize the results, a schematic overview was created and a color code was used to represent the levels of GFP fluorescence (Fig. 19). In general, the lines expressing different constructs lead to various levels of GFP fluorescence. It was visible that all the T3 lines expressing construct 1 or 26 lead to *GFP* expression as high as positive control. Some of the T3 lines expressing construct 2, 3, 4, 9, 17, 19, 21 or 23 showed high GFP fluorescence as positive control, however, other lines expressing these constructs showed low or no GFP fluorescence. All the T3 lines expressing construct 6, 16, 18, 20, 22, 24, 25 or 28 exhibited no *GFP* expression like the negative control. The lines expressing construct 5, 8, 15 or 27 lead to middle strength *GFP* expression.

3.1.3.3 Analysis of GFP fluorescence in stable transformed *A.thaliana* lines upon JA treatment

The lines, which showed lower levels of GFP fluorescence, were in keeping with the assumption that JAZ proteins might bind to the TALE-MYC2 fusion, towards repressing the *GFP* expression. To trigger the *GFP* expression, 50 μ M MeJA or 1 μ M coronatine solution was sprayed to the seedlings, which would lead to degradation of JAZ proteins. 24h after treatment, GFP fluorescence was checked and compared with non-treated seedlings. Several examples were shown in Fig. 20.

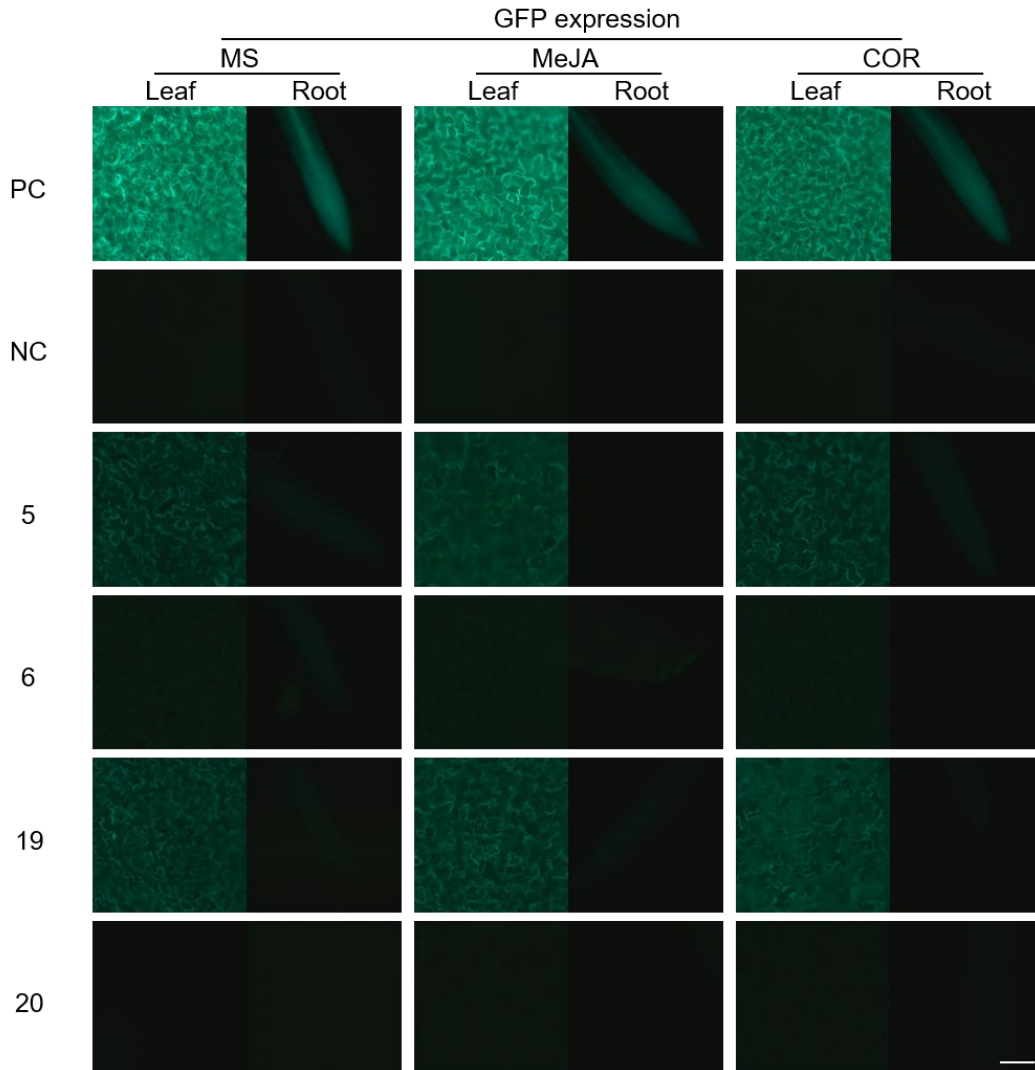


Figure 20 GFP fluorescence after MeJA or COR treatment of single-insertion homozygous plants. For construct numbers see legend to Fig. 19. The microscopic analysis was carried out with 488 nm as excitation and 495-557 nm as emission. The scale bar is 100 μm for all micrographs. For each line, at least three seedlings were checked. One representative of them is shown here.

The GFP fluorescence of both the leaf and root tip was checked from at least three different seedlings with an epifluorescence microscope. The positive control led to high strength *GFP* expression and negative control did not show GFP fluorescence neither before nor after MeJA or COR treatment. In comparison to the control, there was, no more GFP fluorescence visible in the leaf area or root tip of the seedlings expressing other constructs.

In order to summarize the results, a schematic overview was created and a color code was used to represent the levels of GFP fluorescence (Fig. 21).

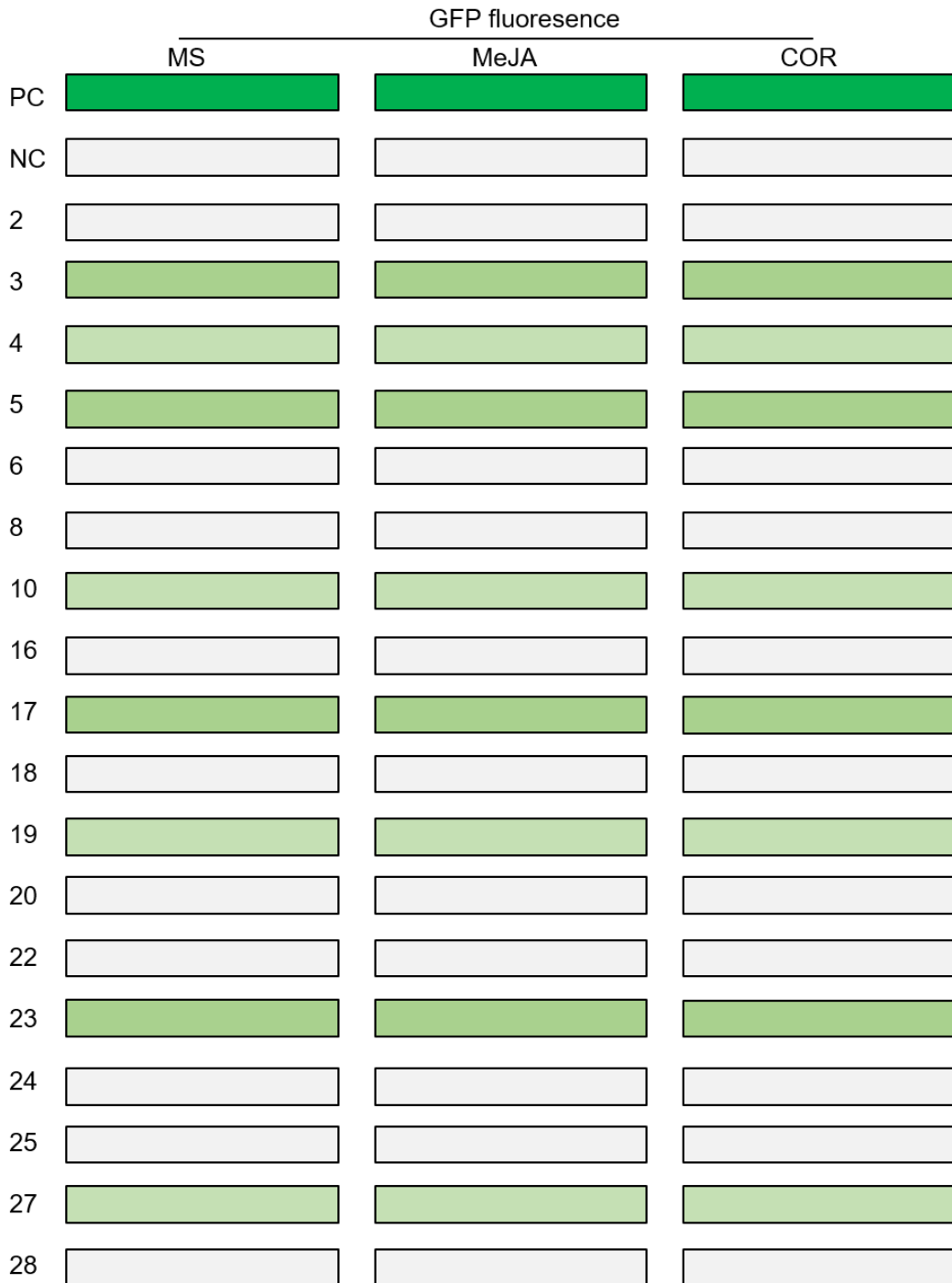


Figure 21 Schematic overview about the levels of GFP fluorescence detected from the single-insertion homozygous plants. White means there are almost no GFP fluorescence; light green means the middle level of GFP fluorescence; green means the highest level of GFP fluorescence. For construct numbers see legend to Fig. 19. For each line, at least three seedlings were checked.

3.1.4 Identification of the interaction between JAZ1 and TALE-MYC2 fusion variants.

3.1.4.1 Interaction test using Yeast 2 Hybrid system

Yeast 2 Hybrid (Y2H) is a method for the detection of protein-protein interaction. This system is based on the use of the transcription factor Gal4 which contains a binding domain (BD_{Gal4}) for binding to the promoter of corresponding target genes and a domain for the activation of transcription (AD_{Gal4}). Two proteins of interest are fused to BD_{Gal4} and AD_{Gal4} , called bait and prey, respectively, such that interaction between these two proteins reconstitutes a functional Gal4-TF that then drive reporter gene expression. The experiments were performed with the help of Carolin Alfs in her master thesis.

3.1.4.1.1 Establishment of the expression vectors

The various TALE-MYC2 fusions were cloned into the expression vector pAGT1939 as prey. In this vector TALE: MYC2 fusions were fused to the C-terminus of AD_{Gal4} driven by a constitutive *ADH1* promoter as well as the *TRP1* gene for the synthesis of tryptophan (TRP1). Full length MYC2 fused to the C-terminus of AD_{Gal4} was used as positive control due to the well-known interaction between JAZ1 and MYC2 (Fernández-Calvo *et al.*, 2011). TALE fused to the C-terminus of AD_{Gal4} was used as negative control. JAZ1 was cloned into the expression vector pAGT1940 as bait. In this vector JAZ1 was fused to the C-terminus of BD_{Gal4} driven by *ADH1* promoter as well as the *LEU2* gene for the synthesis of leucine (LEU2). As an additional control, the empty vector $BD_{Gal4}eV$ was used in combination with the corresponding prey plasmid (Fig. 22).

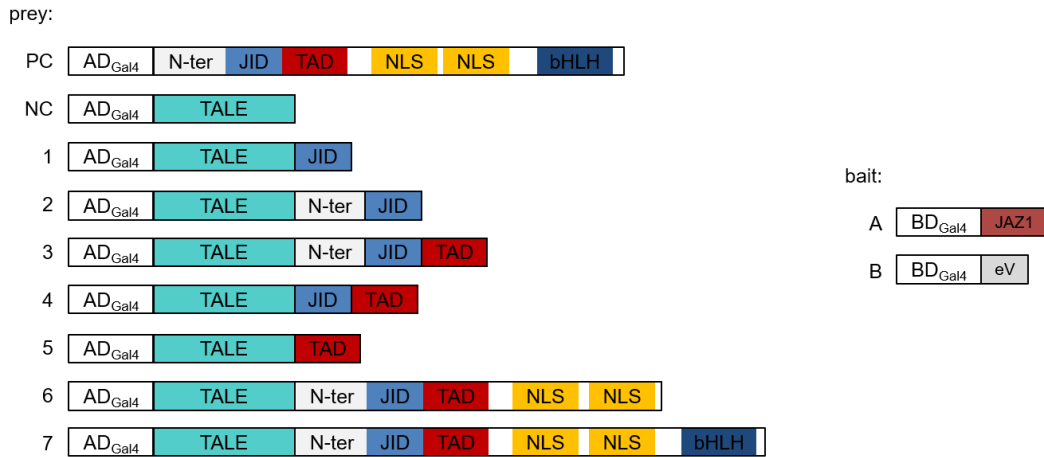


Figure 22 Schematic representation of the prey and bait used in Y2H assay. TALE-MYC2 fusions were fused to the C-terminus of ADGal4 as prey. JAZ1 was fused to the C-terminus of BDGal4 as bait. PC: positive control; NC: negative control; eV: empty vector. The domains of MYC2 were introduced in section 3.1.1.2.

3.1.4.1.2 Interaction test in yeast

Expression vectors containing bait and prey were co-transformed into a *S. cerevisiae* strain pJ69, which has no functional Gal4 gene and is an auxotrophic strain of adenine and histidine. For each test three independent transformations were performed. ADE2 and HIS3 for the synthesis of the base adenine and histidine were used as reporter genes. Interaction of prey and bait protein leads to the functional reconstitution of Gal4-TF. This results in the expression of the reporter genes, thus enabling the growth of yeast on the selection medium.

Various dilution steps of a liquid culture of the yeast strains were dropped onto transformation selective plates (SD-TL, without leucine and tryptophan). The transformation was checked by growth on selective plates. The positive transformants were subsequently grown in SD-TL liquid medium and were dropped onto selective plates without leucine and tryptophan (SD-TL), as well as without adenine (SD-TLA) or histidine (SD-TLH) after various dilution stages. As expected, the plaque on selective plates showed that all of the prey plasmids were successfully co-transformed with the bait plasmids. The positive control could grow on both selective mediums SD-TLA and SD-TLH showing that MYC2 can interact with JAZ1. The Negative control could not grow on selective media SD-TLA or SD-TLH showed that TALE cannot interact with JAZ1. For the clones co-transformed with prey 1, 2, 3, 4, 5 and

bait A, no growth was visible on the both selective media SD-TLA and SD-TLH suggesting that no interaction takes place between these prey and bait proteins. For the clones co-transformed with prey 6 and bait A, and prey 7 and bait A, similar intensity of growth could be observed on the selective medium SD-TLH. Growth was also detectable for both clones on SD-TLA selective medium, but the intensity of the growth is weaker than that on the transformation control (SD-TL) and on SD-TLH selective medium. Clones co-transformed with bait B instead of bait A did not show any growth on both selection media as expected because no protein was fused to BD_{Gal4} (Fig. 23). Together with all these results, only TALE fused with full length MYC2 and TALE fused with MYC without bHLH domain showed an interaction with JAZ1.

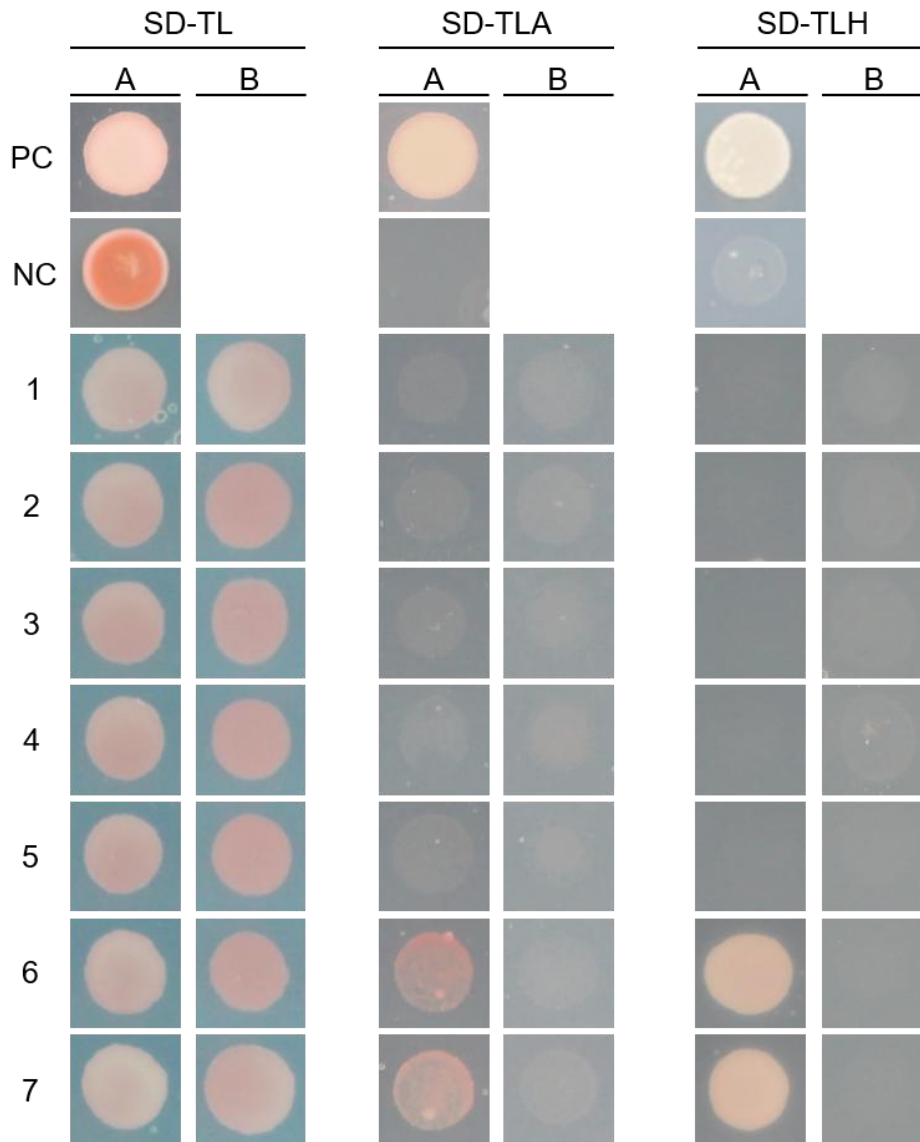


Figure 23 Interaction test of the proteins of interest. PC: ADGal4: MYC2 (prey) and BDGal4: JAZ1 (bait); NC: ADGal4: TALE (prey) and BDGal4: JAZ1 (bait); SD-TL: Synthetic Defined Medium without leucine and tryptophan; SD-TLA: Synthetic Defined Medium without leucine, tryptophan and adenine; SD-TLH: Synthetic Defined Medium without leucine, tryptophan and histidine. For each test three independent transformations were performed and showed the same results. (Adopted from Alfs, 2017)

3.1.4.2 Interaction test using BiFC method

Another method for the investigation of protein-protein interaction is the bimolecular fluorescence complementation (BiFC). The potential interaction partners are fused with the N-terminus or C-terminus half of the yellow fluorescent protein (YFP) (nYFP / cYFP, splitYFP). These partial fragments cannot fluoresce alone. In the case of interaction between the fusion proteins, the N and C terminus fragments of the

fluorescence protein are brought into proximity, which leads to reconstitution to a functional fluorophore (Kudla and Bock, 2016).

3.1.4.2.1 Establishment of the 2-in-1 expression vectors

The 2-in-1 cloning according to (Grefen and Blatt, 2012) was used for the establishment of vectors for the BiFC. For the generation of 2-in-1 vectors, the coding genes for the fusion proteins to be investigated were cloned into Gateway donor vectors, which lead to these fusions flanked by specific attL recombination sites. The recombination took place between the donor vectors and expression vector pBiFC2-in-1-NN, which insert the TALE: MYC2 fusions and JAZ1 into specific site. In the expression vector, nYFP and cYFP halves were fused to the N-terminus of TALE: MYC2 fusions and JAZ1, respectively, driven by 35S promoter. At the same time, an expression cassette coding for the red fluorescent protein (RFP) driven by 35S promoter serves as a transformation control (Fig. 24).

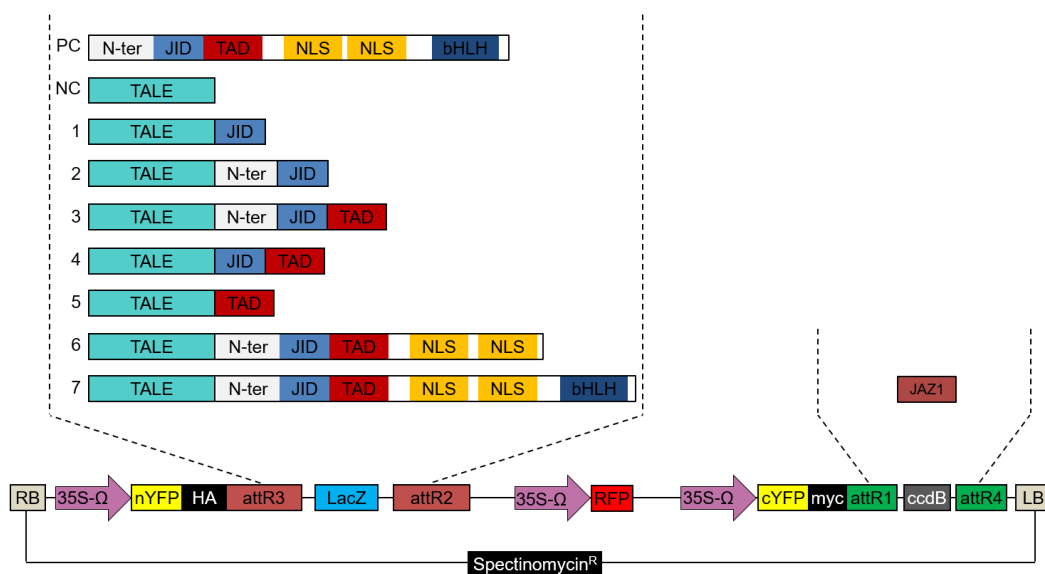


Figure 24 Schematic of the constructs for 2-in-1 BiFC. The coding genes of the proteins to be examined were cloned into the expression vector pBiFC-2-in-1-NN. In this expression vector, nYFP and cYFP halves were fused to the N-terminus of TALE: MYC2 fusions and JAZ1 respectively. Positive control: full length MYC2; Negative control: intact TALE. The domains of MYC2 were introduced in section 3.1.1.2.

3.1.4.2.2 Interaction test in mesophyll protoplasts of *A. thaliana*

The generated vectors were subsequently transformed into mesophyll protoplasts from *A. thaliana*. The evaluation was carried out with the confocal laser-scanning microscope LSM780. Three independent transformations were performed. An RFP fluorescence signal could be observed in the mesophyll protoplasts for all investigated constructs indicating a successful transformation. The transformation rate, however, was very low with about 6-15% for the potential interaction partners (1 to 7) compared to the positive control. For the positive control, a very strong but irregular nuclear-localized YFP fluorescence signal could be detected. For the negative control, no YFP fluorescence signal could be detected in the nucleus as expected. A very weak nuclear-localized YFP fluorescence signal compared to the positive control was detectable in mesophyll protoplasts transformed with expression vectors 6 and 7. This suggested a weak interaction between these two TALE-MYC2 fusions and JAZ1. No YFP fluorescence was detected in mesophyll protoplasts transformed with expression vector 1, 2, 3, 4 or 5 (Fig. 25).

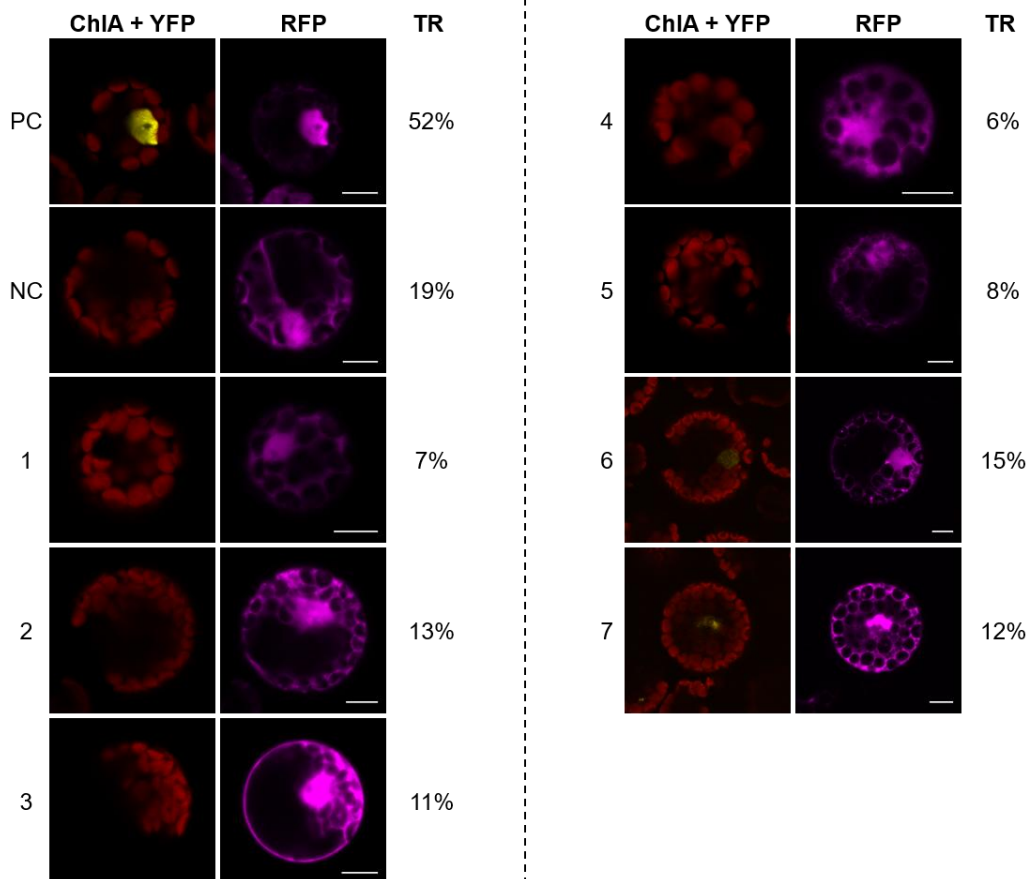


Figure 25 Representation of mesophyll protoplasts from *A. thaliana* transformed with splitYFP expression vectors. The images of the YFP fluorescence are superimposed with the chlorophyll A (ChlA) fluorescence (red) as well as the RFP fluorescence (magenta). TR indicates the transformation rate. The microscopic analysis of the mesophyll protoplasts was performed at an excitation wavelength of 514 nm (YFP) and 594 nm (ChlA / mRFP) as well as a detection range of 518 to 564 nm (YFP), 570 to 625 nm (mRFP) and 655-722 nm (ChlA), The scale bars are 10 μ m. For each test three independent transformations were performed and showed the same results. One representative protoplast is shown for each combination. (Figure from Alfs, 2017)

3.1.4.2.3 Interaction test in mesophyll protoplasts of *N. benthamiana*

Due to the low transformation rate in mesophyll protoplasts from *A. thaliana*, the BiFC experiments were repeated in mesophyll protoplasts of *N. benthamiana*. As can be seen from the RFP fluorescence signal, all analyzed expression vectors could be successfully transformed. Compared with the rate of transformation of the mesophyll protoplasts from *A. thaliana* that of *N. benthamiana* was significantly higher showing 57-82%. A uniformly strong YFP fluorescence signal could be observed in the nucleus for positive control. Similar to the results determined with *A. thaliana*

mesophyll protoplasts, a nuclear-localized YFP fluorescence signal could be detected in mesophyll protoplasts transformed with expression vectors 6 and 7 only (Fig. 26).

Together with the results of the BiFC in mesophyll protoplasts from *A. thaliana* and *N. benthamiana*, the fusions TALE with full length MYC2 and with MYC without bHLH domain can be considered as interaction partners of JAZ1.

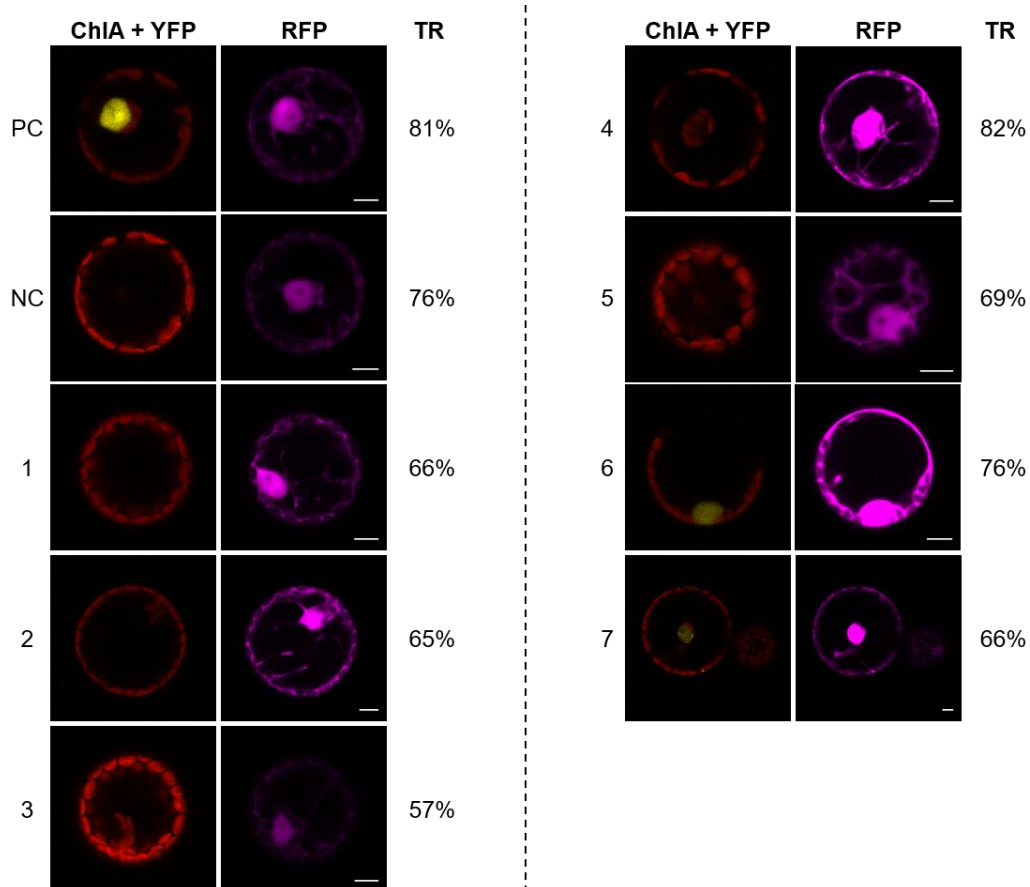


Figure 26 Representation of mesophyll protoplasts from *N. benthamiana* transformed with splitYFP expression vectors. The images of the YFP fluorescence are superimposed with the chlorophyll A (ChlA) fluorescence (red) as well as the RFP fluorescence (magenta). TR indicates the transformation rate. The microscopic analysis of the mesophyll protoplasts was carried out at an excitation wavelength of 514 nm (YFP) and 594 nm (ChlA / mRFP) as well as a detection range of 518 to 564 nm (YFP), 570 to 625 nm (mRFP) and 655722 nm (ChlA). The scale bars are 10 μ m. For each test three independent transformations were performed and shown the same results. One representative protoplast is shown for each combination. (Figure from Alfs, 2017)

3.1.5 Analysis of the repression effect of JAZ1 on the transcriptional activity of the TALE-MYC2 fusions

The results of the Y2H and BiFC showed that the fusions of TALE fused with full length MYC2 and with MYC without bHLH domain can interact with JAZ1. The following step was to test whether JAZ1 can repress *in vivo* the transcriptional activity of these TALE-MYC2 fusion transcription factors.

3.1.5.1 Establishment of the constructs

In the transactivation assay, GUS was used as a reporter under the control of the synthetic promoter (*EBE002*). By means of transient co-transformation of reporter and the respective activators in mesophyll protoplasts from *A. thaliana*, it was first to test whether the fusion proteins containing TALE bind to the synthetic promoter and activate *GUS* expression leading to a measurable enzyme activity. The measured *GUS* expression reflects the activity of the TALE-MYC2 fusion transcription factors. In addition, it should be checked whether the additional co-transformation with the repressor leads to a reduction of *GUS* expression. The repressor was JAZ1, which is constitutively expressed under the control of the *35S* promoter. The normalization of the *GUS* activity occurred through the use and co-transformation of luciferase (LUC) under the control of the *35S* promoter (transformation control). For the activator constructs, full length TALE driven by *AtActin2* promoter was used as positive control; co-transformation without the activator served as negative control; the fusions consisting TALE or TALE without activation domain (TALE Δ AD) fused with MYC2 or MYC2 without bHLH domain driven by *AtActin2* promoter were used as various activators (Fig. 27).

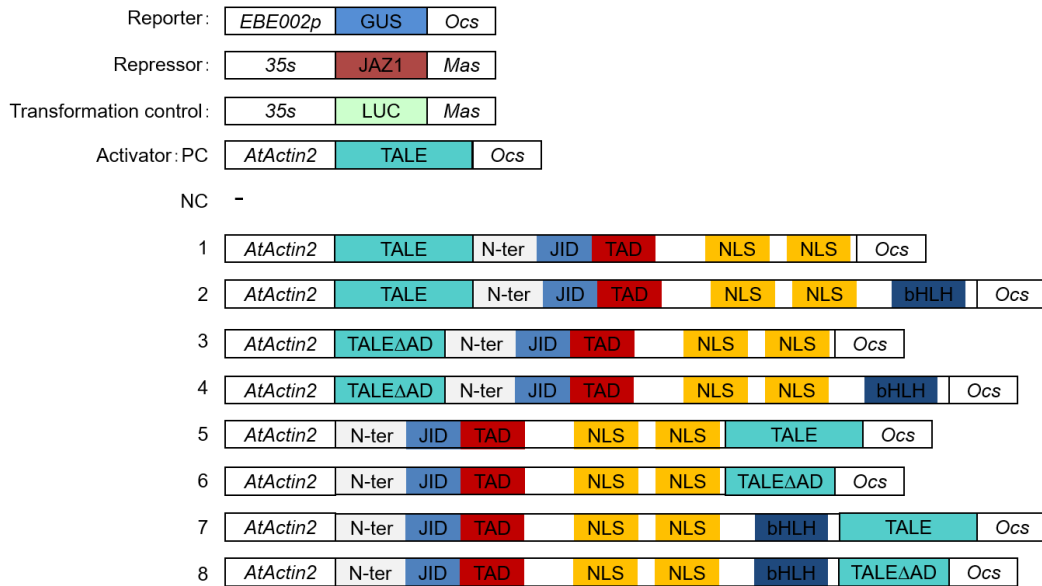


Figure 27 Constructs used for the transactivation assay. As a reporter, GUS was under the control of a synthetic promoter (EBE002). The activators were TALE-MYC2 fusion variants, with TALE being fused N-terminally (1 to 4) or C-terminally with MYC2 or MYC2 without bHLH domain (5 to 8). For the normalization of the measured GUS activity, LUC under the control of the 35S promoter was co-transformed as the transformation control.

3.1.5.2 Analysis of the transactivation assay

The mesophyll protoplasts were transformed with the corresponding constructs and the GUS activity was measured using a fluorometer. The relative GUS activity tested with and without repressor is shown in Fig. 28. As expected, after the transformation of the reporter construct alone, no GUS activity could be detected. If this was co-transformed with the positive control activator, the GUS activity was measurable. It can be assumed that TALE can bind to the synthetic promoter and lead to expression of *GUS*. The expression of *GUS* was not influenced after the mesophyll protoplasts additionally co-transformed with the repressor construct because JAZ1 cannot interact with TALE and thus does not lead to any repression.

Except of activator 3 and 4, *GUS* expression could be detected for all activators (without repressor). For the activator 1, 2, and 8, the relative *GUS* expression is less than that of the positive control. However, an increased GUS activity could be detected for activator 5, 6 and 7. The co-transformation with the repressor construct resulted in a towards to reduced 26.8%, 11.7%, 21.3%, 7.4% relative GUS activity for activator 2, 5, 6, 8, respectively and no change for activator 1 (Fig. 28). This

suggested that JAZ1 could repress the transcriptional activity of these TALE-MYC2 fusion transcription factors at least partially. Among these activators, activator 2 is the most promising construct which containing intact TALE fused C-terminally with full length MYC2.

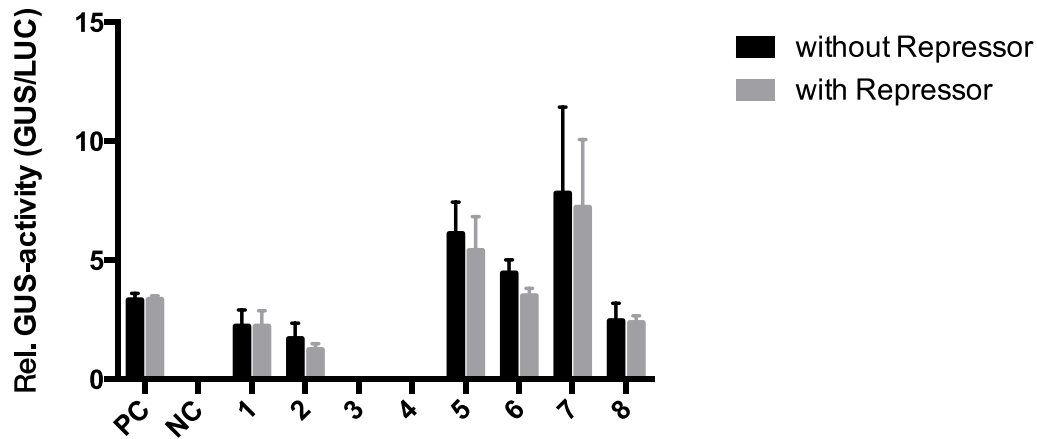


Figure 28 Detection of GUS expression in transactivation assay. The relative (rel.) GUS activity from mesophyll protoplasts is transiently transformed with the reporter construct and the associated potential activators (Fig. 27). The transformation control was carried out to normalize the GUS activity. The black columns represent the GUS activity detected from mesophyll protoplasts co-transformed with Transformation control, Reporter, Activator construct. The gray columns represent the GUS expression activity after co-transformation with Repressor construct. The experiment was performed in independent triplicates. Shown are the mean values of all three measurements. Error bars resemble the standard deviation. For statistical analysis, a two-tailed t-test was performed between values for expression without and with repressors. Significance levels are $P < 0.05$. The result showed that there was no significant repression.

3.1.6 Overexpression of *JAZ1* in the high *GFP* expressing lines

In some stable transformed lines such as lines expressing the construct 26, 27 (for construct numbers see legend to Fig. 19), the level of the GFP fluorescence was very high even as in positive control lines. This might mean that JAZ proteins did not repress the transcriptional activity of the TALE-MYC2 fusion transcription factors effectively. In consideration of the results obtained from the transactivation assay, one possible reason is that there is not enough JAZ protein binding to these TALE-MYC2 fusions and therefore they cannot repress *GFP* expression. The following step was to test whether overexpressed *JAZ1* can repress the transcriptional activity of these TALE-MYC2 fusion transcription factors. First is to check whether overexpressed *JAZ1* will affect the expression of *GFP* in transient assay.

3.1.6.1 Analysis the effects of overexpressed *JAZ1* on the *GFP* expression in *N.benthamiana* leaves

The constructs were co-infiltrated into *N. benthamiana* with the synthetic promoter “*promsyn::GFP*” construct that contains TALE binding site (*EBE002*). The construct containing intact TALE driven by *AtActin2* promoter co-infiltrated with the construct pICH71081 was used as positive control (PC). Infiltration with the construct containing *JAZ1* driven by *35S* promoter only was used as negative control 1 (NC1). Infiltration with the “*promsyn::GFP*” construct only was used as negative control 2 (NC2). All infiltrations were done in triplicates using leaves from different plants. The photos of the leaves were taken using an UV lamp five days after infiltration (Fig. 29). NC1 did not lead to expression of *GFP* since the construct used did not containing the coding sequence of *GFP*. NC2 convincingly showed that the expression of *GFP* was absent if TALE is not expressed. This means the expression of *GFP* cannot be activated without TALE. In contrast, PC showed strong fluorescence indicative for high expression meaning that TALE can bind to the synthetic promoter and lead to expression of *GFP* (also visible in Fig. 13). The expression of *GFP* was not influenced after the additionally co-infiltration of the construct containing *JAZ1* driven by *35S* promoter or the synthetic promoter, to which TALE can bind.

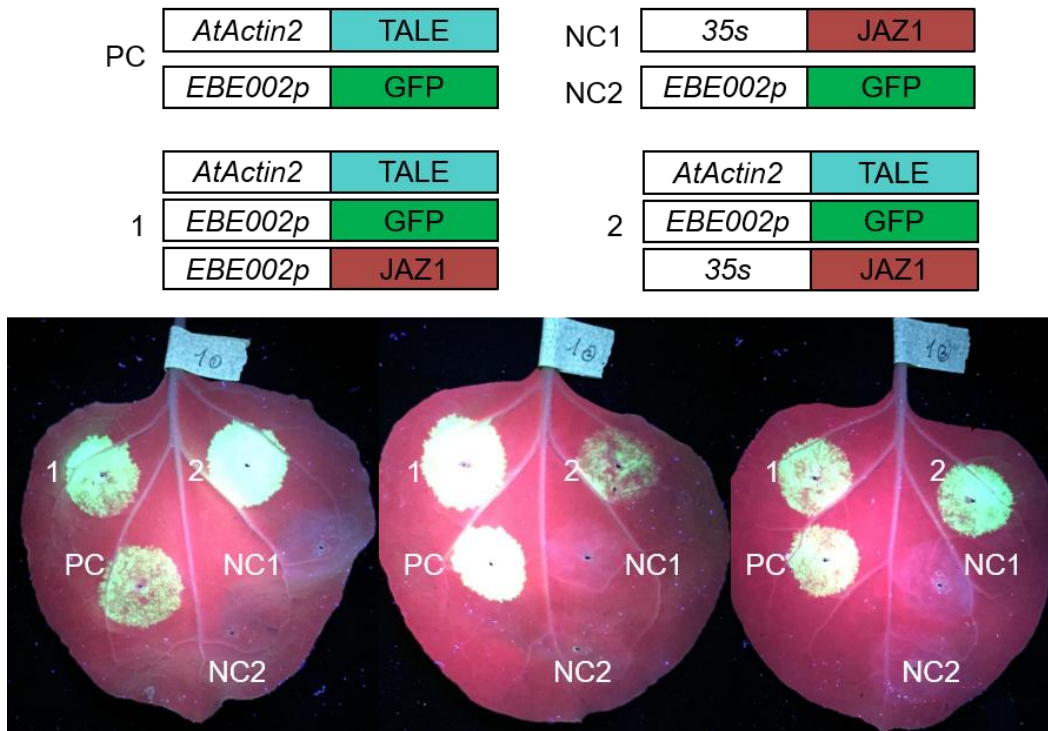


Figure 29 Infiltrations experiments showed different level of GFP fluorescence in dependence on the different constructs. PC: the construct containing intact TALE driven by *AtActin2* promoter co-infiltrated with the construct pICH71081; NC1: infiltration with the construct containing *JAZ1* driven by 35S promoter; NC2: infiltration with the “promsyn::GFP” construct; 1: the construct containing intact TALE driven by *AtActin2* promoter co-infiltrated with the “promsyn::GFP” construct as well as the construct containing *JAZ1* driven by the synthetic promoter to which TALE can bind; 2: the construct containing intact TALE driven by *AtActin2* promoter co-infiltrated with the “promsyn::GFP” construct as well as the construct containing *JAZ1* driven by 35S promoter. The experiment was performed in independent triplicates with similar results.

3.1.6.2 Analysis of the GFP fluorescence after overexpression of *JAZ1* in stable transformed *A.thaliana* lines

The construct used contains two independent cloning cassettes on the same vector backbone. The first one is the 35S promoter that drives *JAZ1* coding sequence. The second one contains the coding gene for the red fluorescent protein (RFP) driven by the seed coat-specific promoter *OLE1* as a selectable marker (Fig. 30).

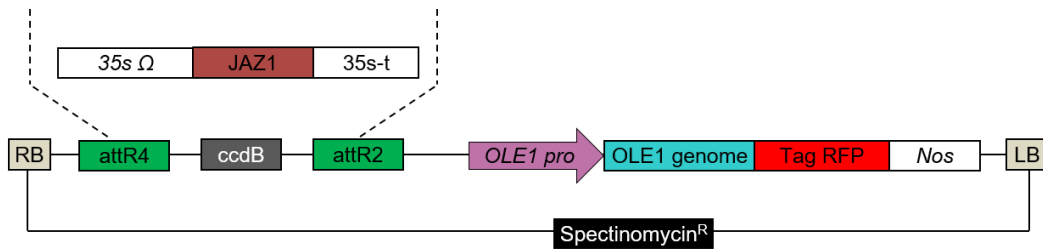


Figure 30 Scheme of the construct for overexpression of JAZ1. The coding gene of JAZ1 driven by 35S promoter was cloned into the specific site by Gateway cloning.

The construct was transferred into *A. tumefaciens* (GV3101), which then were used for floral dipping with the stable transformed lines expressing construct 26 or 27 (for construct numbers see legend to Fig. 19). Resulting transgenic lines (T1) were selected with the RFP fluorescence using a fluorescence microscope (Fig. 31).

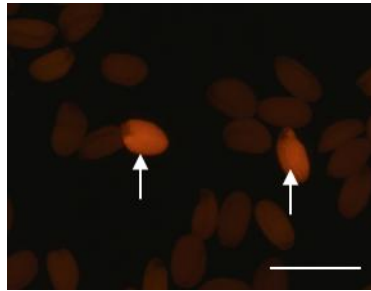


Figure 31 RFP fluorescence in the seed coat of the positive transformants (arrows). The scale bar is 1 mm.

The GFP fluorescence of the T1 plants was checked on 7-days old seedlings using an epifluorescence microscope. The results showed that *JAZ1* overexpressed in positive control lines did not affect *GFP* expression both in the leaf area and root tip. This led to the same conclusion as the transient expression analysis (section 3.1.6.1) showing that over expression of *JAZ1* does not influence the *GFP* expression. *JAZ1* overexpressed in negative control lines led to no or very low *GFP* expression both in the leaf and root tip. Comparing the lines only expressing construct 26 or 27, over expression of *JAZ1* in these lines did not change the GFP fluorescence level neither before nor after MeJA or COR treatment (Fig. 19 and Fig. 32). These results led to the conclusion that the high *GFP* expression in the lines expressing construct 26 or 27 was not due to the lack of *JAZ1*.

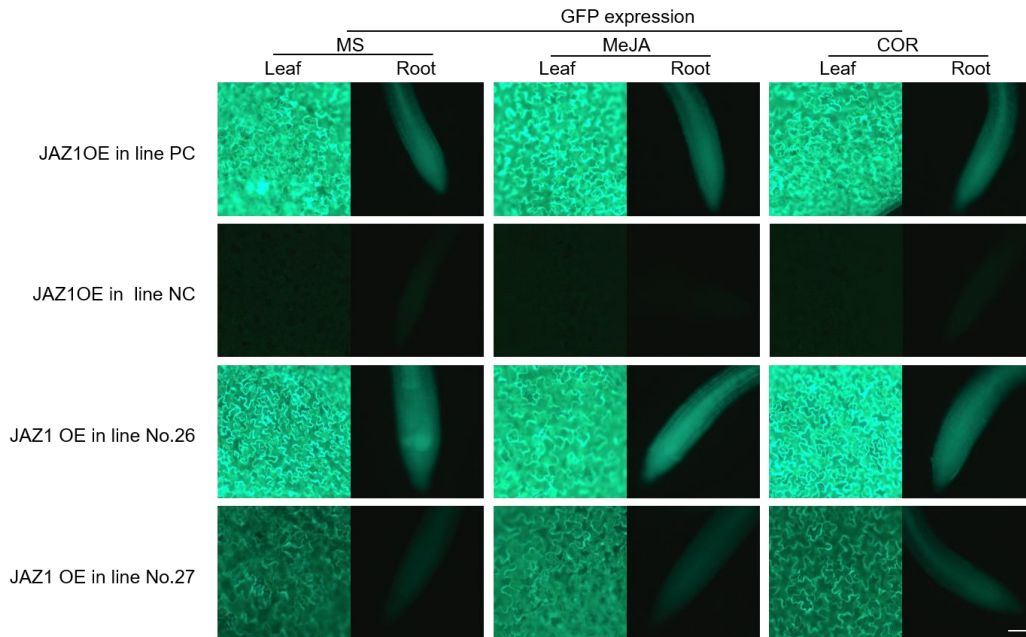


Figure 32 GFP fluorescence in the JAZ1 overexpressed T1. OE: overexpression. For construct numbers see legend to Fig. 19. The microscopic analysis was carried out using 488 nm as excitation and 495-557 nm as emission. The scale bar is 100 μ m. For each independent line, at least three seedlings were checked, one of them is shown as representative example.

3.2 A system based on JAZ-CSY4 mediated controlling of the reporter gene expression

3.2.1 Establishment of the constructs for stable transformation

The constructs used in this system have to contain different and independent cloning cassettes on the same vector backbone pAGM8031 (see section 2.1.4). The first one is the *Nos* promoter that drives the *Bar* gene leading to plant's resistance against the herbicide BASTA as a selectable marker. The second one contains the GUS encoding sequence as well as the hairpin sequence (5'-AGTTCAGTCCGTATAGGCAGCTAAGAAAA-3') for CSY4 recognition driven by *AtActin2* promoter. The construct containing only these first and second cassettes serves as positive control. For the negative control, the third cassette consists of the *AtActin2* promoter driving the *CSY4* gene. For the test construct, the third cassette consists of the *AtActin2* promoter driving the *JAZ1* fused C-terminally with *CSY4* gene sequence (cloning modules for the second and third cassettes were provided by Tom Schreiber).

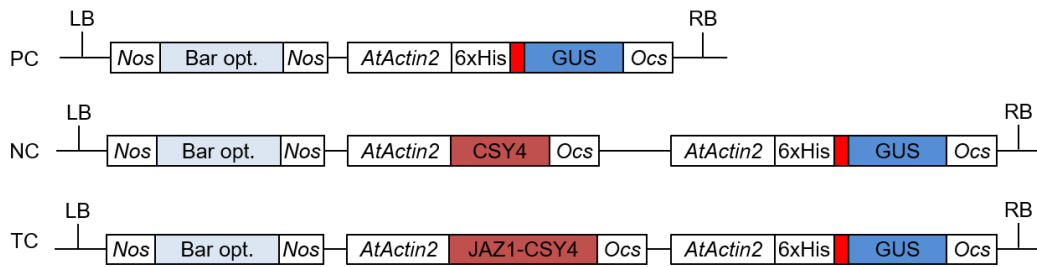


Figure 33 Schematic of the constructs for stable transformation. PC: positive control; NC: negative control; TC: test construct; Bar: bialaphos resistance (bar) gene; 6xHis: a polyhistidine-tag; GUS: β -glucuronidase

3.2.2 Screening of the transformants with expected GUS activity

All constructs were transferred into *A. tumefaciens* (GV3101), which then were used for floral dipping. Resulting transgenic lines (T1) were selected by spraying with BASTA. Leaves from the T1 transformants were either directly incubated in the GUS staining solution or wounded followed by floating on water for 24h. Leaves were stained in darkness at 37 °C for 4h. Chlorophyll was removed by incubation in 95% ethanol. At least three leaves from different transformants were checked.

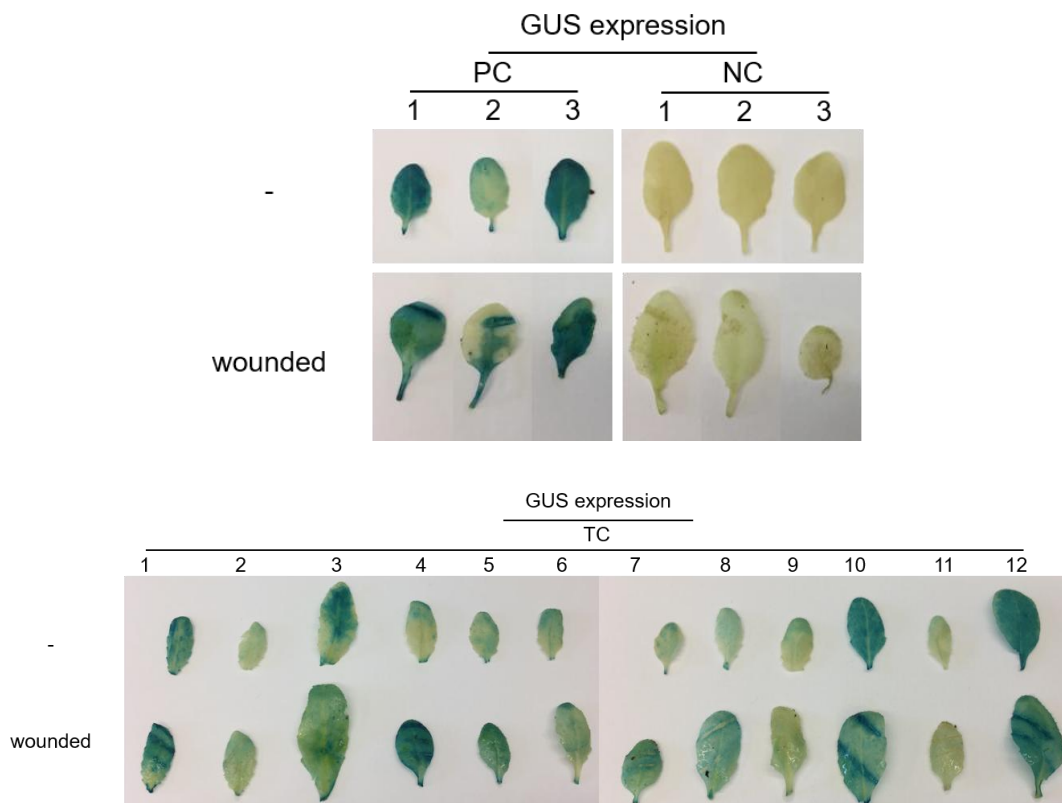


Figure 34 GUS staining of the leaf tissues from T1 transformants. Wounding was carried out with forceps. The numbers depict different transformants.

The analysis showed that the positive control lead to a high *GUS* expression driven by the constitutive promoter *AtActin2*. The negative control did not show *GUS* expression, indicating that *GUS* expression was repressed by constitutively expressed *CSY4* through binding to the mRNA of *GUS*. The leaves expressing test construct showed various *GUS* expression levels. Some of them showed high *GUS* expression as positive control, others showed low *GUS* expression even as negative control. To trigger the *GUS* expression, the leaves were wounded, a treatment that should lead to degradation of JAZ1-CSY4 fusion proteins followed by release of the repression on *GUS* expression. The results showed that wounding does not change the *GUS* expression in positive control or negative control. Among the twelve T1 transformants, the wounded leaves from transformants 1, 4, 5, 7 and 8 showed higher *GUS* expression than the nonwounded leaves indicating that wounding results in degradation of JAZ1-CSY4 and triggers the *GUS* expression. These five transformants were considered as candidate plants and the single insertion homozygous lines will be selected in future works.

3.3 A system based on a novel Jasmonate-Responsive Promoter Element (JARE)

This approach to detect jasmonates is based on the precisely described Jasmonate-Responsive Element in the *AtJMT* (jasmonic acid carboxyl methyltransferases) promoter (Sasaki-Sekimoto *et al.*, 2013). The JARE is distinct from other JA-responsive elements previously reported and a multimerized JARE-containing construct responds to MeJA. On this basis, constructs containing multimers of the JARE linked to a minimal promoter were created and tested to see whether they are able to mediate JA-responsive transcription.

3.3.1 Establishment of the constructs containing JARE for infiltration

A region containing the putative JARE was duplicated 4 or 8 times and fused with the TATA-box sequence of the *CaMV 35S* promoter and a *HTA6-GFP* coding sequence that can localize the fluorescent protein into the nucleus. Two different terminators *ocs* and *mas* were used. These fusions were also first tested by infiltration into *N. benthamiana* leaves.

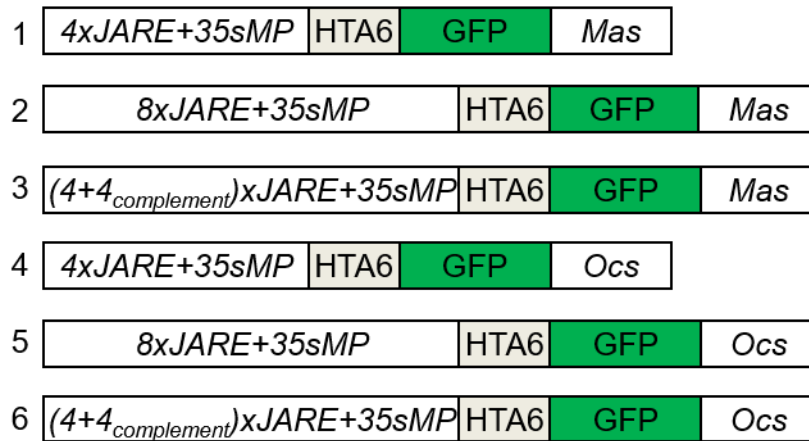


Figure 35 Schematic of the constructs for infiltration. 4xJARE: 4 repeats of JARE-containing promoter region; 8xJARE: 8 repeats of JARE-containing promoter region; (4+4complement) xJARE: 4 repeats of JARE-containing promoter region and 4 repeats of the complement sequences; 35sMP: 35S minimal promoter; HTA6: histone H2A 6.

3.3.2 Infiltration experiments with the synthetic JARE promoter constructs

The photos of the leaves were taken using fluorescence microscope five days after infiltration. In order to summarize the results, a schematic overview was created and the color code was used to represent the levels of GFP fluorescence (Fig. 36). From the third day after infiltration, the leaves were incubated with MeJA and several other hormones or only water. The construct of 35S-minimal promoter recombined to the *HAT6-GFP* was used as negative control. The tetramer of JERE (Jasmonate-elicitor-responsive element) (Menke *et al.*, 1999) recombined to the *HAT6-GFP* was used as positive control. As expected, the negative control showed that the expression of *GFP* was absent and showed no response to any exogenous hormone or wounding. The positive control showed strong basal fluorescence when incubated on water, which was slightly reduced after incubation with different hormones. Except for construct 1, GFP fluorescence was visible for all constructs incubated on water and was less than that of the positive control. For the construct 2, 4, and 5, the *GFP* expression was reduced or no change after incubation with different hormones. For the construct 1, 3, and 6, the *GFP* expression was slightly induced after incubation with IAA or wounding compare to which incubate on water. Additionally, for construct 3 and 6, the *GFP* expression was also slightly induced after incubation with SA compare to

which incubate on water. This suggested that the synthetic JARE promoter constructs, especially the construct 1, 3, and 6, are responsive to the hormones.

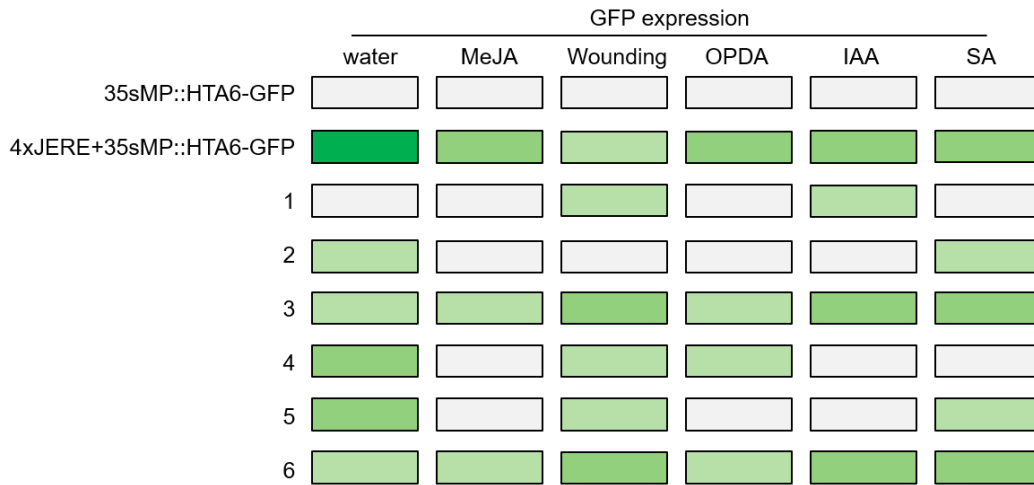


Figure 36 GFP fluorescence in *N. benthamiana* infiltrated leaves treated with phytohormones. Negative control (35s-MP-HAT6-GFP): the construct contains 35S-minimal promoter recombined to the HAT6-GFP; Positive control (4xJERE-HAT6-GFP): the construct contains tetramer of JERE recombined to the HAT6-GFP; OPDA: 12-oxophytodienoic acid; IAA: Indole-3-acetic acid; SA: Salicylic acid; for construct numbers see legend to Fig. 35. The experiment was performed in three independent replicates with similar results.

3.3.3 Establishment of the constructs containing the putative JARE for stable transformation

The results of the infiltration experiments led to a conclusion that the multimers of the JARE-containing promoter is a phytohormone-responsive but very weak promoter. The expression of the reporter gene can be induced slightly driven directly by the JARE-containing promoter (according to Kati Mielke, 2014). To enhance the induction, TALE was cloned under the control of the JARE-containing promoter as well as the reporter *GFP* gene driven by the synthetic promoter, which TALE can bind (EBE002). Since the high transcriptional activity of TALE, the induction can be enhanced.

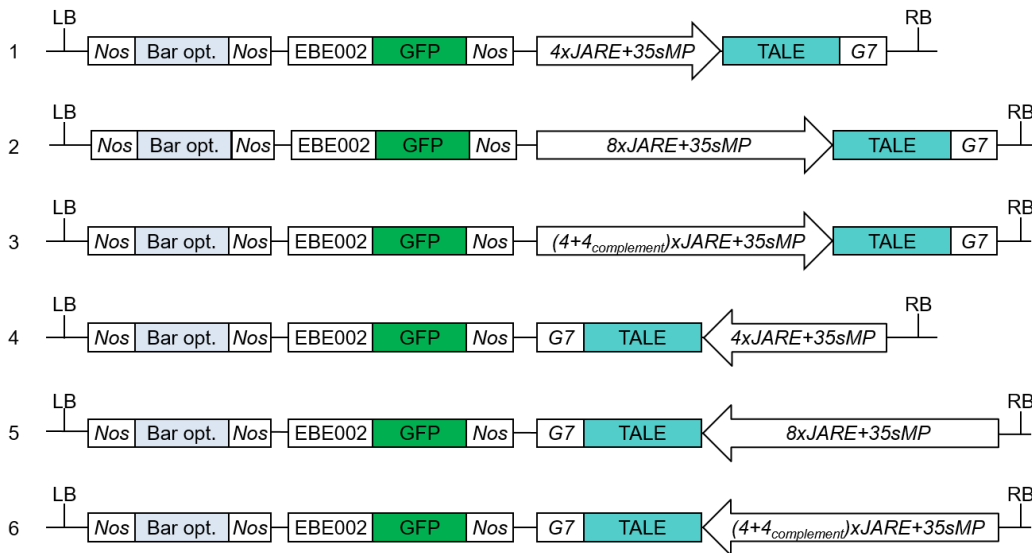


Figure 37 Schematic of the constructs for stable transformation. Bar: bialaphos resistance (bar) gene; EBE002: binding site for TALE; 4xJARE: 4 repeats of JARE-containing promoter region; 8xJARE: 8 repeats of JARE-containing promoter region; (4+4complement) xJARE: 4 repeats of JARE-containing promoter region and 4 repeats of the complement sequences; 35sMP: 35S minimal promoter

The constructs have three different and independent cloning cassettes on the same vector backbone pAGM4723 (see section 2.1.4). The first one is the *Nos* promoter that drives the *Bar* gene leading to plant's resistance against the herbicide BASTA as a selectable marker. The second one contains the *GFP* encoding sequence as well as the promoter to which the TALE can bind (EBE002). The third one consists of a region containing the putative JARE was duplicated 4 or 8 times and fused with the TATA-box sequence of the *CaMV* 35S promoter and the TALE coding sequence. Two different orientations of the third cloning cassette were used.

3.3.4 Analysis of the GFP fluorescence in stable transformed *A.thaliana* lines

All constructs were transferred into *A. tumefaciens* (GV3101), which then were used for floral dipping. Resulting transgenic lines (T1) were selected by spraying with BASTA. Lines with a single insertion that were homozygous were selected from T1 and T2 seeds by germination on BASTA and calculation of the ratio of BASTA resistant and sensitive plants.

The GFP fluorescence of the single-insertion homozygous plants was checked with 7-days old seedlings using a confocal laser-scanning microscope. The results showed that the GFP fluorescence of seedlings from some lines selected could be induced by

MeJA or coronatine treatment (Fig. 38). The T3 lines expressing construct 2 showed low *GFP* expression both in leaf and root tip. The *GFP* fluorescence in the root tip was induced after MeJA and coronatine treatment and the *GFP* fluorescence in the leaf was induced only after coronatine treatment. The T3 lines expressing construct 3 showed low *GFP* expression in leaf and high *GFP* expression in root tip. After MeJA and coronatine treatment, the *GFP* fluorescence in the leaf was induced, at the same time, the zone showing green fluorescence was getting larger in the root tip. The T3 lines expressing construct 6 showed low *GFP* expression both in leaf and root tip. After MeJA and coronatine treatment, the *GFP* fluorescence both in the leaf and root tip was strongly induced.

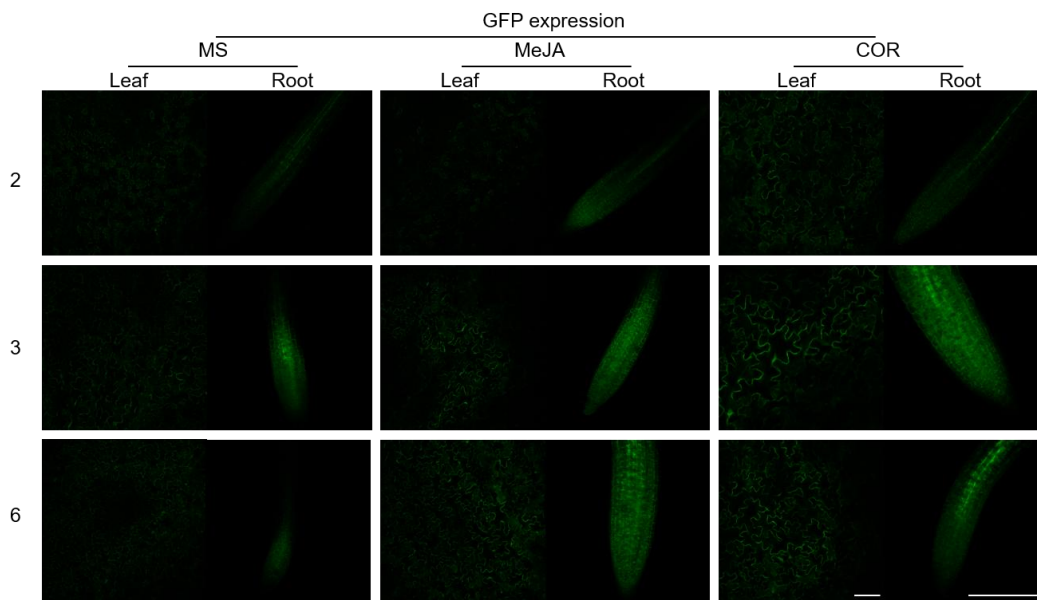


Figure 38 Change of *GFP* fluorescence after MeJA or COR treatment in the single-insertion homozygous plants. For construct numbers see legend to Fig. 37. The microscopic analysis was carried out with 488 nm as excitation and 495-557 nm as emission. The scale bars are 100 μ m. For each construct, at least two lines were checked. For each line, at least three seedlings were checked. One representative example is shown here.

3.3.5 Root growth inhibition effect of JA on transgenic lines

Inhibition of root growth is a prominent JA dependent phenotype (Staswick *et al.*, 1992). To investigate if the root growth of the responsive transgenic lines could be inhibited by JA, seeds from T3 lines were germinated on the MS plate containing 0, 1, 10, 50 μ M MeJA respectively. At least ten plants of each line were checked 7 days after germination. Root length was measured using scale and Image J software.

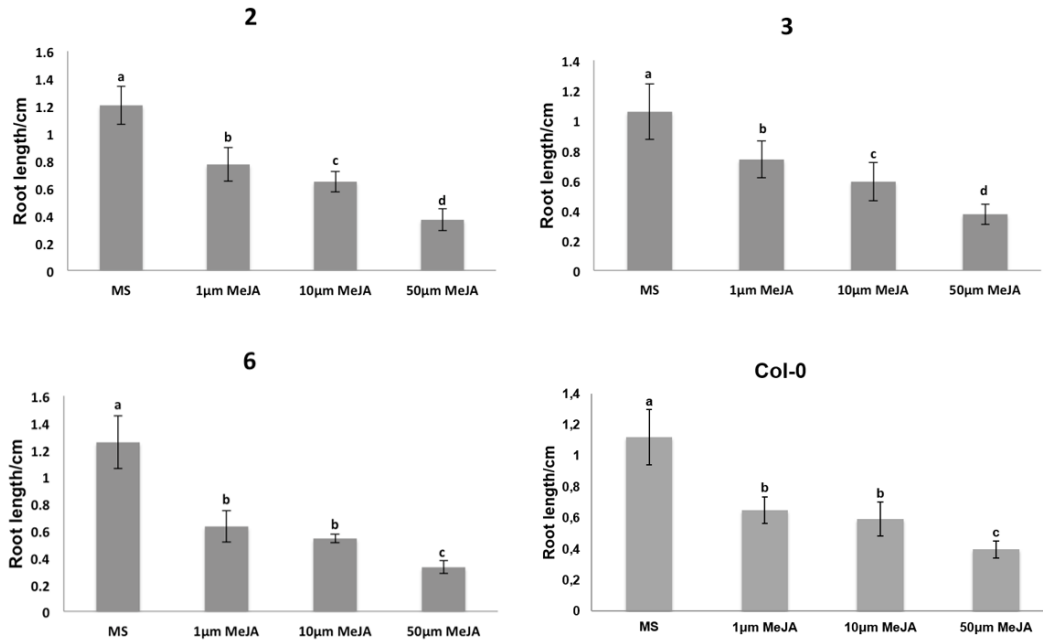


Figure 39 Root length of Arabidopsis seedling in the presence of MeJA. 15 to 20 seeds of each line were distributed on the MS Petri dish containing the indicated concentration of MeJA. Root length was measured 7 days after incubation at 22°C. Values are given as the mean \pm SD (n=10). Different letters above columns indicate significant differences among values (two-tailed t-test, $P < 0.05$).

The results showed that the root growth on MeJA-containing plates is inhibited significantly compared to absence of MeJA. On the 1 μ M MeJA- containing plates, the root length is 55-64% of that on the MS plates. On the 50 μ M MeJA- containing plates, the root length is only 25-30% of that on the MS plates. The results indicated that the root growth of these transgenic lines could be inhibited by JA supporting the fact that JA signalling is not altered by expression of the respective constructs.

4 Discussion

Jasmonic acid (JA) and its derivatives, collectively known as jasmonates, belong to a family of lipid-derived signaling molecules that regulate many aspects of plant life, including defense against herbivores and pathogens, but also symbiotic interactions with mycorrhizal fungi (Jung *et al.*, 2012; Yan and Xie, 2015). Jasmonates alter gene expression positively or negatively in a regulatory network (Wasternack and Hause, 2013). Most functions of jasmonates have been identified through analysis of mutants that are JA-insensitive or affected in the biosynthesis of JA. To broaden our knowledge on the mechanism of JA perception in response to biotic and abiotic stresses, the development but also the utilization of a detection system for JA at cell and tissue specific level represents a major challenge in jasmonates research.

Until now, there are only two techniques available to detect jasmonates tissue- and cell-specifically, 1): To visualize JA directly in cross sections of plant material, an antibody-based approach was developed, in which JA was detected by immunolabeling within mechanically wounded leaves of tomato and Arabidopsis (Mielke *et al.*, 2011). This available method to detect jasmonates on cell-specific level is invasive and does not allow studies on living plant tissues. 2): a fluorescent biosensor was generated to follow JA perception *in vivo* (Larrieu *et al.*, 2015). It is based on a functional Jas motif fused to the fast maturing VENUS variant of the yellow fluorescent protein and a N7 nuclear localization signal expressed under the control of the constitutive CaMV 35S promoter, leading to a basal fluorescence in all nuclei. Since the Jas9-VENUS degradation is dependent and specifically induced by bioactive JAs, decrease in fluorescence is indicative for JA responses.

Different from previously developed detection methods, this study aimed to develop and to use a new non-invasive detection system for active jasmonates. The envisaged system should be based on the rise of fluorescent proteins in living cells appearing just after specific induction by jasmonates. To accomplish this goal, three strategies were followed in this study. The first one is the system based on JAZ-mediated repression of MYC2, the second one is the system based on JAZ-CSY4 mediated controlling of the reporter gene expression, and the third one is an approach based on Jasmonate-Responsive Promoter element (JARE).

4.1 The system based on JAZ-mediated repression of TALE-MYC2 fusions

In this system, synthetic promoter and TF constructs were created and expressed together in plants to establish an artificial transcriptional network for the expression of the specific reporter gene *GFP*.

4.1.1 The artificial transcriptional network has high activity

Engineered TALE with DNA-binding specificities was used as the backbone for the synthetic TF since it is a useful tool in the study of the gene expression and transcription factors (Morbitzer *et al.*, 2010; Mahfouz *et al.*, 2011; Sanjana *et al.*, 2012). The synthetic promoter contains an 18-base-long DNA-binding domain, named EBE002, flanked by a 19-base-long degenerate sequence immediately upstream, and a consensus TATA box downstream. The TATA box is followed by a 43-base-long degenerate sequence and the ATG start codon (see section 3.1.1.1) (Kay *et al.*, 2007; Römer *et al.*, 2007; Kay *et al.*, 2009; Brückner *et al.*, 2015). In according to the study showing that this artificial transcriptional factor could lead to reporter gene expression as strong as the viral *35S* promoter (Brückner *et al.*, 2015), the engineered TALE, which binds to the constant EBE002 sequence, was used as the positive control transcription factor. The results from transient expression assay in *N.benthamiana* leaves (see section 3.1.2.1) and stable transformed *A.thaliana* (see section 3.1.3.2) showed very high GFP fluorescence indicating the high activity of this synthetic transcription system. However, in infiltration experiments, positive control showed variations that did not show always high *GFP* expression. One possible reason is that in transient expression assay, the copy numbers of T-DNA expressed into the genome of *N.benthamiana* is difficult to control. Different copy numbers led to different *GFP* expression levels. Even if the positive control showed high variations, it still showed strongest GFP fluorescence compared to infiltrations with other constructs (Fig. 15).

In infiltration experiments, the construct containing TALE without activation domain co-infiltrated with the “*promsyn::GFP*” construct was used as negative control 1 (NC1). Infiltration only with the “*promsyn::GFP*” construct was used as negative control 2 (NC2) (see section 3.1.2.1). The results showed that NC1 lead to a visible expression of *GFP* indicating that TALE without activation domain still has

transcriptional activity which can activate the expression of *GFP* slightly. However, it is also possible that, due to the high load of T-DNAs that are transferred, transient assays in *N. benthamiana* leaves lead to background transcriptional activity. So far, hardly anything is known about whether TALE without activation domain has transcriptional activity or not. At least, studies showed that the C-terminus of TALE is important for TALE binding activity, and ~68 amino acids of the C terminus should be preserved to keep the highest level of TALE activity (Szurek *et al.*, 2001; Zhang *et al.*, 2011). No GFP fluorescence could be seen in NC2 illustrate that the synthetic promoter does not have any activity in the absence of the TALE, confirming the high specificity of the synthetic promoters.

4.1.2 MYC2-TALE fusions exhibited varying transcriptional activities

To establish the synthetic transcription factor, several fragments or full length MYC2 were fused N-terminally or C-terminally to intact TALE and TALE without activation domain. The results from infiltration experiments in *N. benthamiana* leaves with N-terminal fusions showed that the construct containing MYC2 domains fused to TALE without activation domain led to a *GFP* expression which was as weak as in the NC1. The construct containing MYC2 domains fused to TALE with activation domain led to a *GFP* expression, which was as strong as in the positive control (Fig. 14). This revealed that MYC2-TALE fusions have diverse transcriptional activities ranging from 0 to almost 100% of the TALE activity. Although MYC2 contains a transcriptional activation domain in its N-terminus, the fusions of MYC2 to TALE without activation domain did not show transcriptional activities in the range of fusions of MYC2 to intact TALE. This led to the conclusion that the activation domain of MYC2 cannot fully cover the deficiency of the original activation domain of TALE. Possible reason might be that different activation domains stimulate initiation and elongation at different times and via different residues with the paired binding domain (Stringer *et al.*, 1990; Brown *et al.*, 1998).

Same with the results from infiltration experiments in *N. benthamiana* leaves, large variations in expression levels of *GFP* in stable transformed *A.thaliana* lines expressing different constructs were also observed. Surprisingly, different T3 lines expressing the same construct exhibited various levels of GFP fluorescence, although

they are all single-insertion homozygous lines. This might be caused by different insertion positions in the *A.thaliana* genome. Possible reason is that even though they have the same insertion numbers, the insertion position might be different. The position in the *A.thaliana* genome where T-DNA is inserted could be close to an enhancer or insulator sequence, which can lead to undesirable consequences such as gene silencing or overexpression (Peremarti *et al.*, 2010).

4.1.3 Analysis of GFP fluorescence upon JA treatment

In this artificial transcriptional system, MYC2-TALE fusion transcription factors should not only be able to bind synthetic promoter, but should also exhibit functional similarity to MYC2. Therefore, their activity should be repressed by JAZ proteins. JAZ proteins are the targets of SCF^{COI1} and act to repress transcription of jasmonate-responsive genes by recruiting the co-repressor TPL and TPRs through NINJA (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007). Jasmonate treatment causes JAZ degradation and this degradation is dependent on activities of the SCF^{COI1} ubiquitin ligase and the 26S proteasome (Katsir *et al.*, 2008; Fonseca *et al.*, 2009). To trigger the release of MYC2-TALE fusions, the *N. benthamiana* leaves or *A.thaliana* seedlings were treated with MeJA or coronatine leading to degradation of JAZ proteins that then result in the expression of *GFP*.

4.1.3.1 Analysis of GFP fluorescence of *N.benthamiana* leaves transiently expressing transcription factor constructs upon JA treatment

To trigger the *GFP* expression, the infiltrated leaves were floated on 50 μ M MeJA for 0.5 h. Two days after treatment, however, no increase in GFP fluorescence was visible in the leaf area transformed with the constructs. Except for full length MYC2 fused to N-terminus of intact TALE, for all constructs containing MYC2 fused to intact TALE, a possible induction was not visible since they showed high *GFP* expression as much as positive control even before JA treatment. For the constructs that contained MYC2 fused to TALE without activation domain, an induced expression of *GFP* was also not detected. This might due to the deletion of activation domain from TALE leading to low transcriptional activity of the MYC2-TALE transcription factors. For the construct containing full length MYC2 fused to N-

terminus of intact TALE, the *GFP* expression seems to be repressed by the activity of JAZ proteins before JA treatment, but due to the low reproducibility of the infiltration assay, it is uncertain to draw final conclusion.

The phenomenon in infiltration experiments with the C-terminal fusions showed this low reproducibility of the infiltration assay again. The results showed that even the positive control led to a very low *GFP* expression, which is a little bit higher than NC1 and NC2, however, the positive control and the negative controls used are the same with the infiltration experiments with the N-terminal fusions. Other C-terminal fusions constructs co-infiltrated with “*prom_{syn}::GFP*” did not lead to a *GFP* expression as negative controls, neither before nor after MeJA treatment (see section 3.1.2.2). The use of different *A. tumefaciens* strains, the OD of the infiltration solution, age and the water content of the leaves or temperature have an impact on *A. tumefaciens* mediated transient expression assay in *N. benthamiana*, which might explain the low reproducibility of the infiltration assay (Dillen *et al.*, 1997; Wroblewski *et al.*, 2005).

4.1.3.2 Analysis of GFP fluorescence in stable transformed *A. thaliana* lines upon JA treatment

Since the reproducibility of the *N. benthamiana* infiltration experiments was not good, stable transformation was carried out to build this artificial transcriptional system in *A.thaliana*. The constructs contained three independent cloning cassettes on one vector backbone including a Bar gene as a selectable marker, the *GFP* encoding sequence and the promoter to which the TALE can bind (EBE002) as well as the N-terminal or C-terminal MYC2-TALE fusions constructed (see sections 3.1.1.2, 3.1.2.2 and 3.1.3.1).

The GFP fluorescence of the single-insertion homozygous plants was checked with 7-days old seedlings using an epifluorescence microscope. The lines, which showed lower levels of GFP fluorescence, were used due to the assumption that JAZ proteins might bind to the TALE-MYC2 fusion, towards repressing the *GFP* expression. Seedlings from these lines were sprayed with 50 μ M MeJA or 1 μ M coronatine solution to trigger JAZ degradation. Unfortunately, the results showed that no increase in GFP fluorescence was visible in the leaf or root tip of seedlings (Fig. 20 and 21). This led to the conclusion that low levels of GFP fluorescence were possibly

not due to JAZ proteins binding to the TALE-MYC2 fusions, but MYC2 fragments fused to TALE exhibited reduced transcriptional activity. Additionally, binding affinity of fusion proteins to the synthetic promoter might be reduced since the property or function of the fusion protein might be changed (Chen *et al.*, 2013).

4.1.4 Interaction between JAZ1 and TALE-MYC2 fusions

In the artificial transcriptional system, MYC2-TALE fusion transcription factors should exhibit functional similarity to MYC2 and therefore be repressed by JAZ proteins. Several biochemical experiments indicated that JAZs directly interact with MYC2. The C-terminus containing the Jas domain was identified as the AtMYC2-interacting domain. Yeast-two-hybrid assays demonstrated a direct MYC2–JAZs interaction (Fernández-Calvo *et al.*, 2011; Pauwels and Goossens, 2011). Microarray analysis also supported that JAZ is a negative regulator of MYC2 function (Chini *et al.*, 2007). JAZ Interaction Domain (JID) of MYC2 (93-160aa) resides at amino-terminus and is conserved among MYC proteins from several plant species and is sufficient for the interaction with most JAZ proteins including JAZ1 (Fernández-Calvo *et al.*, 2011; Kazan and Manners, 2013).

4.1.4.1 Interaction tests reveal that MYC2-TALE fusions interact with JAZ1

The interaction between the protein JAZ1 and TALE-MYC2 fusions was first analyzed by means of a yeast-2-hybrid system, which should give an impression of whether the fusion of TALE to MYC2 has an influence on this interaction, and which MYC2 domains (see 1.3.4 and Fig. 12a) are essential for the interaction with JAZ1 in this context. Six fragments of MYC2 and the full length MYC2 that should interact with JAZ1 were fused to the C-terminus of TALE to consist C-terminal MYC2-TALE fusions. For the analysis, JAZ1 was fused to the BDGal4 as a bait plasmid; C-terminal MYC2-TALE fusions were fused to ADGal4 as the prey plasmids.

The results showed that MYC2 interacts with JAZ1 confirming the previous studies. In contrast to the observations with the derivatives of MYC2, except fusions TALE:MYC2 and TALE:MYC2 Δ bHLH, no interaction with JAZ1 could be detected for any other C-terminal MYC2-TALE fusions. In previous studies using yeast-2-hybrid assays, it was shown that derivatives of MYC2 without C-terminus or bHLH domain

could interact with JAZ1. The deletion of the N-terminus, however, led to a loss of the interaction (Fernández-Calvo *et al.*, 2011). Although six fragments of MYC2 contain the crucial domain for interaction with JAZ1, the protein interaction property of MYC2 was probably changed after fusion with TALE.

In order to evaluate the interaction of the TALE-MYC2 fusions with JAZ1 within plant cells, BiFC was additionally applied using splitYFP-2in1 constructs for the detection of protein-protein interaction *in planta* (Grefen and Blatt, 2012). For this purpose, C-terminal MYC2-TALE fusions analogously to the yeast-2-hybrid experiments were N-terminally fused with nYFP and JAZ1 was N-terminally fused with cYFP on the backbone of 2in1 vector (Fig. 24). As a transformation and expression control, the splitYFP-2in1 vector additionally contains an expression cassette with RFP under the control of the 35S promoter.

RFP fluorescence was detected for all investigated potential interaction partners, as well as the accompanying controls. Thus, a successful transformation of the plasmids and expression of the fusion proteins can be assumed (Fig. 25). Analogous to the results from the yeast-2-hybrid experiments, the fusion TALE: MYC2 and TALE: MYC2 Δ bHLH could also be identified as interaction partners of JAZ1. However, the intensity of the YFP fluorescence signal was much weaker compared to the positive control (interaction between MYC2 and JAZ1, Fig. 25). This suggests the same conclusion as drawn from the yeast-2-hybrid experiments. The interaction between JAZ1 and MYC2 might be disturbed by the fusion of MYC2 with TALE. In addition, mesophyll protoplasts transformed with TALE-MYC2 fusion constructs showed a conspicuously low transfection rate with 6-13%. It is known that the transfection rate of mesophyll protoplasts from *A. thaliana* can be up to 90% (Sheen, 2001). The low transfection rate may be due to the size of the splitYFP-2in1 vectors, which are approx. 15-17 kb. The use of smaller vectors with approximately 7 to 11 kb experimentally showed much higher transfection rates of *A. thaliana* mesophyll protoplasts. Due to the low transfection rate in *A. thaliana* mesophyll protoplasts, transfection of mesophyll protoplasts from *N. benthamiana* was carried out. Here, a higher transfection rate with splitYFP-2-in-1 vectors was obtained in pre-experiments. Moreover, mesophyll protoplasts from *N. benthamiana* appeared to be more robust. First, for all the analyzed potential interaction partners and the accompanying controls, RFP fluorescence could be detected correspondingly; a successful

transformation of the plasmids and expression of the fusion proteins could be assumed (Fig. 26). Compared to *A. thaliana*, a markedly higher transfection rate was observed for mesophyll protoplasts from *N. benthamiana*, which was between 57 and 82%. Also, using mesophyll protoplasts from *N. benthamiana*, the fusion TALE:MYC2 and TALE:MYC2 Δ bHLH could be identified as interacting partners of JAZ1 (Fig. 26). As already described for the experiments in mesophyll protoplasts from *A. thaliana*, it was also observed in this case that the YFP fluorescence signal of the interaction partners was weaker than that of the positive control. Thus, the result determined by transient transfection of mesophyll protoplasts from *A. thaliana* was confirmed.

With regard to the results obtained from yeast-2-hybrid and BiFC, the question arises whether the deletion of the two NLS in C-terminus of MYC2 has an influence on the interaction with JAZ1. JAZ1 and MYC2 are nuclear localized proteins indicating that the interaction should take place in the cell nucleus (Chini *et al.*, 2007; Thines *et al.*, 2007). Since the MYC2 has an additional NLS at N-terminus and TALE has two functional NLS in the C-terminus, which can mediate transport into the nucleus (Yang and Gabriel, 1995), it can be assumed that the deletion of the NLS from MYC2 has no influence on the transport of the fusion protein from the cytoplasm into the nucleus (Çevik *et al.*, 2012; Chen *et al.*, 2012). Thus, all of the TALE-MYC2 fusions should be able to be transported into the nucleus and an interaction with JAZ1 should be possible.

As mentioned above, the YFP fluorescence signal of the interaction partners was much weaker than that of the positive control. It can be assumed that the fusion with TALE has a negative influence on the binding between JAZ1 and MYC2. Possibly, the fusion of TALE to the N-terminus of MYC2 leads to a disturbance of the tertiary structure in the N-terminal region of MYC2 by interactions between charged amino acid. This can hinder the correct folding to a functional protein. In previous studies it has been shown that fusion proteins can often not be expressed stably and that the biological activity can be impaired (Chen *et al.*, 2013). Reason for this may be structural disturbances between proteins that may cause fusion proteins to be folded incorrectly (Bai *et al.*, 1996). Although the expression in some cases can be improved by changing the orientation of components of the fusion proteins (Zhao *et al.*, 2007), the disability is often not effectively reduced due to the short distance between the

domains. By using so-called linkers, a larger distance between the individual proteins can be generated, which allows independent folding, improves the stability of the fusion proteins, and can reduce adverse interactions (Arai *et al.*, 2001; Lu and Feng, 2008). The use of a suitable linker between TALE and MYC2 might improve the interaction between JAZ1 and the TALE-MYC2 fusions. Since the orientation of a fusion can have an influence on the folding and activity of a protein complex (Hong *et al.*, 2006; Zhao *et al.*, 2007), the investigations of N-terminal MYC2-TALE fusions should also be considered.

4.1.4.2 Analysis of the repression effect of JAZ1 on the transcriptional activity of the TALE-MYC2 fusions using transactivation assay

Since the fusions TALE: MYC2 and TALE: MYC2 Δ bHLH were positively tested for the interaction with JAZ1 with the aid of yeast-2-hybrid and BiFC, a transactivation assay was carried out. It should be checked whether the TALE-MYC2 fusions (activator) can activate the activity of the synthetic promoter (EBE002) (reporter) and whether the JAZ1 (repressor) can repress the transcriptional activity of the TALE-MYC2 fusions *in vivo*.

The TALE-MYC2 fusion constructs were transiently transformed into mesophyll protoplasts from *A. thaliana*. It was shown that, with the exception of the two constructs *AtActin2*:: TALE Δ AD: MYC2 Δ bHLH and *AtActin2*:: TALE Δ AD: MYC2, all fusions could activate *GUS* expression. If no activity can be detected, it is an indication that the catalytic domains might be misfolded or important parts necessary for their activity have been deleted. As mentioned above, the orientation of the fusion (TALE fused N-terminally or C-terminally to MYC2) can have an influence on the folding and activation of the protein complex (Hong *et al.*, 2006). This was clearly indicated by the results obtained, since the TALE: MYC2 fusions have less activity than MYC2: TALE fusions. Thus, if MYC2 is fused to N-terminus of TALE, the folding of the protein may be more advantageous for the activity of the protein complex. The function of activation domains, however, could be masked when MYC2 is fused to C-terminus of TALE since the activation domain of MYC2 is localized in the N-terminus and the activation domain of TALE is localized in the C-terminus. Moreover, TALE is a very large protein (molecular weight of 82 kDa)

(Stella *et al.*, 2013). If TALE is fused to the N-terminus of MYC2 or MYC2 Δ bHLH, the tertiary structure in the N-terminal region of MYC2 may possibly be disturbed by interactions between charged amino acid, which can consequently interfere with the correct folding to a functional protein.

The deletion of the activation domain (AD) of TALE reduced the activity of the fusions. However, when MYC2 was fused to N-terminus of TALE without activation domain, GUS activity could be observed. When MYC2 was fused to C-terminus of TALE without activation domain, no GUS activity could be measured. The results revealed again that MYC2 fused to N-terminus of TALE was more advantageous than MYC2 fused to C-terminus of TALE for the activity of the protein complex. When full length MYC2 or MYC2 without bHLH was fused to N-terminus of intact TALE, much higher GUS activity was measured than positive control. It is possible that MYC2 has additional positive effect on activity of TALE, two activation domains are presumably not sterically hindered, the mediator complex can be recruited and the transcription takes place.

These two TALE-MYC2 fusions previously tested by means of yeast-2-hybrid and BiFC are positive for an interaction with JAZ1. Thus, it is expected that the activity of the activator MYC2-TALE fusions are repressed by JAZ1 leading to a reduction in the relative GUS activity. The results showed that co-transformation with repressor construct resulted in tendency to reduced relative GUS activity, but this was not significant. It is well known that mechanical injury of *A. thaliana* leaves leads to a rapid increase of JA level (Reymond *et al.*, 2000; Glauser *et al.*, 2008). Since the preparation of protoplasts is based on the mechanical and enzymatic disintegration of *A. thaliana* leaves (Yoo *et al.*, 2007), this step could already lead to a stress-induced increase of JA content in the cell. In addition, the protocol for the preparation of protoplasts includes further steps such as, for example, centrifugation but also pipetting, which are also mechanical actions. If the content of JA is increased for one of the reasons mentioned, JAZ1 would be degraded dependent on activities of the SCF^{COI1} ubiquitin ligase and the 26S proteasome (Katsir *et al.*, 2008; Fonseca *et al.*, 2009). While, in the case of BiFC, positive control showed a very strong YFP signal indicating interaction between MYC2 and JAZ1, a possible increased content of JA should not degrade all JAZ1 protein. There should be no marked difference between

the BiFC and transactivation assay with regard to the physiological events since the constructs were equally transformed into *A. thaliana* mesophyll protoplasts.

4.1.5 Overexpression of JAZ1 as a repressor in stable transformed *A. thaliana*

In some stable transformed lines such as lines expressing the construct containing full length MYC2 fused to C-terminus of intact TALE or MYC2 without bHLH fused to C-terminus of TALE without activation domain, the level of the GFP fluorescence was very high even as in positive control lines. In consideration of the results obtained from protein interaction experiments and transactivation assay, JAZ1 could bind to TALE: MYC2 and has tendency to reduce the activity of this MYC2-TALE fusion. This led to the conclusion that in plants showing high basal GFP fluorescence, there are not enough JAZ proteins binding to these TALE-MYC2 fusions. Therefore, the *GFP* expression would not be sufficiently suppressed in absence of JA. *JAZ1* coding sequence driven by *35S* promoter was transferred into the stable transformed lines showing high GFP fluorescence. Positive re-transformed lines were checked for GFP fluorescence in 7-days old seedlings. The results showed that over expression of *JAZ1* in these lines did not change the GFP fluorescence level neither before nor after MeJA or COR treatment (Fig. 32). On the one hand, a possible reduction of *GFP* expression in non-treated plants could not be visible since the basal GFP fluorescence was too strong. On the other hand, it is tempting to speculate that the high *GFP* expression in these lines was possibly not due to the lack of JAZ1, since JAZ1 repressed the activity of MYC2-TALE fusions only slightly.

So far, this system cannot indicate the occurrence of JA effectively. The key problem is that the transcriptional activity of fusion proteins cannot be controlled effectively by JAZ1. The MYC2-TALE fusions have to be optimized by adding a linker or modification of the critical sites to be equipped with the expected properties.

4.2 A system based on JAZ-CSY4 mediated controlling of the *GUS* expression

In this system, CSY4 was used as a repressor whose specificity is determined at the RNA level. Previous studies showed that the efficiency of CSY4-mediated processing on RNA depends on the position of the hairpin sequence. CSY4 has high efficiency when the hairpin is located in the 5' UTR or immediately after the start codon of the

target RNA, but is of low efficiency when the hairpin is located in the 3' UTR prior to the poly (A) signal (Borchardt *et al.*, 2017). The hairpin sequence for CSY4 recognition was cloned after the start codon of the *GUS* gene and expressed under the constitutive promoter *AtActin2*. At the same vector backbone, coding sequences for CSY4 or JAZ1-CSY4 fusion protein were cloned and expressed under a constitutive promoter *AtActin2* as well (the constructs were designed and tested already in *N. benthamiana* by T. Schreiber). The constructs were stably transformed into *A. thaliana*. JAZ1-CSY4 fusion protein was expected that could target the hairpin sequence and cleave the transcription product leading to repression of *GUS* expression. Active JA signaling should lead to degradation of the JAZ1-CSY4 fusion protein and therefore eliminate the repression effect on mRNA of the *GUS* gene, resulting in the expression of *GUS*.

The positive control leads to a high *GUS* expression driven by the constitutive promoter *AtActin2* since the absence of CSY4 assumes a successful expression of the reporter gene. The negative control did not show *GUS* expression, indicating that the *GUS* expression was repressed by constitutively expressed *CSY4* through binding to the mRNA of *GUS*. The results indicated that CSY4 successfully cleave the mRNA of *GUS* by targeting the specific hairpin sequence. CSY4 was identified as an endoribonuclease, and recognizes substrate RNA through a specific 28-nt hairpin sequence and cleaves it at the 3' end of the stem (Przybilski *et al.*, 2011; Haurwitz *et al.*, 2012), and the RNA processing functionality of CSY4 was also shown recently in plant system (Liang *et al.*, 2017).

The leaves expressing JAZ1-CSY4 fusion protein showed various *GUS* expression levels already without any treatment of the leaves. It is possible that JAZ1-CSY4 fusion protein has reduced RNA processing activity in comparison to CSY4 resulting in considerable amounts of *GUS*-mRNA out of the nucleus and accomplishing the translation step. Another possible reason might be that JAZ1-CSY4 fusion protein is not exclusively localized in nucleus where CSY4 carries out the processing of the mRNA. To trigger the *GUS* expression, the leaves were wounded, a treatment that should lead to degradation of JAZ1-CSY4 fusion proteins followed by release of the repression on *GUS* expression. The results showed that wounding does not change the *GUS* expression in positive control or negative control indicating that increased JA levels in the cell had no influence on the stability of CSY4 or expression of *GUS*. In

contrast, several T1 transformants showed higher *GUS* expression after wounding. This might indicate that wounding results in degradation of JAZ1-CSY4 thereby triggering the *GUS* expression. This promising result showed that JAZ1-CSY4 fusion protein exhibits the RNA processing function like CSY4 but can also be degraded upon JA treatment like JAZ1. The promising transformants were considered as candidate plants and the single insertion homozygous lines will be characterized in future works.

4.3 A system based on Jasmonate-Responsive Promoter Element (JARE)

Promoter *cis*-elements play important roles in global regulation of gene expression. Promoters contain two major domains, the region where the transcription machinery is recruited and initiates transcription, and the upstream elements, which are bound by TFs that can either activate or repress transcription (Hahn and Young, 2011). JARE, which was used in this system, is a novel identified jasmonate-responsive element in the *At JMT* promoter (Seo *et al.*, 2013) distinct from other JA-responsive elements, such as the G-box or the G-box-like motif (Dombrecht *et al.*, 2007). It regulates JA-responsive gene expression and contains a heptanucleotide sequence motif (G/C) TCCTGA. A multimerized JARE-containing construct could mediate JA-responsive induction of transcription showing that this element mediates response to JA (Seo *et al.*, 2013).

4.3.1 Infiltration experiments with the synthetic JARE promoter constructs

A region containing the putative JARE was duplicated 4 or 8 times and fused with the TATA-box sequence of the *CaMV 35S* promoter and a *HTA6-GFP* coding sequence that can surely localize the fluorescent protein into nucleus. Two different terminators *ocs* and *mas* were used since the same cloning cassette with different terminators showed different translation efficiency (pers. communication, S. Marillonnet, IPB). The negative control showed that the expression of *GFP* was absent and showed no response to any exogenous hormone or wounding, since a TATA-box (*35S*-minimal promoter) alone cannot trigger the recruitment of the core transcription machinery and is not able to activate transcription. The positive control showed strong basal fluorescence since the tetramer of JERE (Menke *et al.*, 1999) in the promoter is

enough to initiate transcription. Except of 4 repeats of JARE fused with 35S-minimal promoter and with the *Mas* terminator, other test promoters have a strong basal activity observed by GFP fluorescence but less than the positive control (Fig. 36). This result indicates that 4 or 8 repeats of JARE fused with 35S-minimal promoter consist a functional promoter, and the terminator is also a factor influencing gene expression (Chen *et al.*, 2013). The activity of these promoters was variably changed after hormone treatment or wounding. However, several promoters such as 4 repeat of JARE and 4 repeats of the complement sequences fused to 35S-minimal promoter showed slight inducibility by wounding.

4.3.2 Amplification of synthetic JARE activity using TALE

It was shown that the JARE-containing promoter is a very weak promoter and can only slightly induced by hormone treatment. To enhance the induction, TALE was cloned under the control of the JARE-containing promoter as well as the reporter *GFP* gene driven by the synthetic promoter, which TALE can bind (EBE002). TALE will be produced JA-dependently and will subsequently activate the expression of reporter gene, thereby acting as an amplifier. The constructs created were stable transformed into *A. thaliana* and the GFP fluorescence of the single-insertion homozygous plants was checked in 7-days old seedlings. The results showed that the GFP fluorescence of seedlings from some lines selected could be induced by MeJA or coronatine treatment (Fig. 38). Even though the basal activity of the JARE-containing promoter is varying in different lines, it can be induced leading to a visible higher GFP fluorescence. In order to verify whether JA signaling is occurring, delayed or diminished in transgenic lines, root growth inhibition effect of JA on transgenic lines was checked. The results showed that the root growth on MeJA-containing plates is inhibited significantly compared to non-treated controls. Most importantly, there was the same effect as in wild type *A. thaliana*. This supported the fact that JA signaling is not altered by expression of the respective constructs. The activity of JARE-containing promoter *in planta* allows the visualization of the spatial pattern of JA responses, thus being indicative for the occurrence of JA at cellular level. In future, the promising transgenic plants have to be crossed with mutants deficient in JA biosynthesis (*opr3*) and being JA-insensitive (*coil*) to confirm that *GFP* expression in transgenic plants is due to JA occurrence.

Summing up the insights gained in this study, the system based on JAZ protein-mediated repression of MYC2 failed due to the transcriptional activity of TALE-MYC2 fusions that could not be repressed by JAZ1 effectively. The synthetic transcription factor based on MYC2-TALE fusions will be optimized in future. The system based on CSY4-mediated degradation of mRNA of reporter gene in combination with JA-mediated degradation of JAZ1-CSY4 fusion protein seem to be a usable method to detect JA on cell specific level. The system based on JAREs combined with TALE also seems to be a usable method to detect JA on cell specific level. Furthermore, the specificity of these two promising systems based on CSY4 and JARE have still to be confirmed by crossing with JA-deficient or JA-insensitive mutants.

5 Summary

As sessile organisms, plants are constantly exposed to biotic and abiotic environmental factors. Phytohormones such as jasmonic acid (JA) and its derivatives, which are low molecular weight signal molecules, control the adaptation in plants to specific conditions. Cell- and tissue-specific detection of jasmonates is still quite difficult due to their low levels within plant cells or tissues. This study aims to develop and to use a new non-invasive detection system for active jasmonates. The envisaged system should be based on the rise of fluorescent proteins in living cells appearing after specific induction by jasmonates. Such a monitoring of JA will allow getting insights to the cell specific functions of JA in biotic interactions and developmental processes. Three approaches to develop a non-invasive system for the detection of JA were followed in this study.

Firstly, a system based on jasmonate ZIM-domain (JAZ) protein mediated repression of MYC2, the “master” regulator of JA-induced gene expression, should be employed. A synthetic promoter construct was used that contains binding sites for TALE and the encoding gene for GFP. Synthetic TFs were created based on MYC2-TALE fusion proteins which can bind synthetic promoter and exhibits functional similarity to MYC2. Therefore, the synthetic TFs should be repressed by JAZ proteins in cells with low JA levels. An active JA signaling resulting in JAZ degradation would lead to liberation of the transcription factor and therefore to expression of *GFP*. This will lead to a detectable fluorescence signal, thus being indicative for the occurrence of JA at cellular level. N- and C-terminus fusions of TALE containing or not the activation domain with MYC2 or parts of it were tested transiently in *Nicotiana benthamiana* leaves or stable transformed into *Arabidopsis thaliana* plants. The results revealed that TALE-MYC2 fusions bound to the synthetic promoter resulting in effective and specific expression of *GFP*. Interaction tests using yeast-2-hybrid assays and Bimolecular Fluorescence Complementation showed that only TALE: MYC2 and TALE: MYC2 Δ bHLH fusions interact with JAZ1. Additionally, JAZ1 tend to reduce the transcriptional activity of these fusions as tested in transactivation assays. However, upon JA treatment, no increase in GFP fluorescence was visible in plant systems, thus occurrence of JA cannot be detected effectively. Since the transcriptional activity of fusion proteins might be not controlled effectively

by JAZ1, the MYC2-TALE fusions have to be optimized in further work to retain the expected properties.

Secondly, a system based on Csy4-mediated degradation of mRNA encoding a reporter protein and the JA-mediated degradation of JAZ1-Csy4 fusion protein was employed. CSY4 was used as a repressor on mRNA level. JAZ1-CSY4 fusion protein was created which can target the hairpin sequence and cleave the transcription products leading to repression of *GUS* expression. An active JA signaling resulting in JAZ1-CSY4 fusion protein degradation would lead to elimination of the repression effect on mRNA and therefore to expression of *GUS*. The results showed that *GUS* expression was repressed in the lines expressing CSY4 or JAZ1-CSY4 fusion protein. Additionally, *GUS* expression can be induced upon JA treatment in several lines expressing JAZ1-CSY4 fusion protein. With these preliminary results, it looks like a quite promising system to detect JA at cell specific level. Single-insertion homozygous lines will be screened in future work and the specific induction by JA has to be confirmed.

Thirdly, a system based on jasmonate-responsive *cis*-elements (JARE) was employed. In previous studies, a promoter containing multimerized JAREs was identified to be specifically responsive to JA, but did not show sufficient strength resulting in reporter gene expression that was visible in plant cells. Here the synthetic promoter consisting of multimerized JARE fused to 35S-minimal promoter was combined with TALE which should act as an amplifier. TALE under control of the synthetic promoter should be expressed upon an active JA signaling and subsequently activate the expression of *GFP* which is driven by TALE binding promoter. Although the multimerized JARE-containing promoter led to a basal GFP fluorescence in stable transformed *A. thaliana* plants, the GFP fluorescence became much stronger upon JA treatment in several of the lines. The specificity of this response has still to be confirmed by crossing with JA-deficient or JA-insensitive mutants. Nevertheless, based on these results, a promising system to detect JA at cell specific level has been developed.

In conclusion, three systems were developed in this study. One of them, the system based on JAZ-mediated repression of TALE-MYC2 fusions failed due to the transcriptional activity of TALE-MYC2 fusions that could not be repressed by JAZ1 effectively. The synthetic transcription factor based on MYC2-TALE fusions will be

optimized in further. The other two approaches, the Csy4-mediated degradation of mRNA of reporter gene in combination with JA-mediated degradation of JAZ1-Csy4 fusion protein and the use of JAREs combined with TALE seem to be usable methods to detect JA on cell specific level.

References

- Abe, H., Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Hosokawa, D. and Shinozaki, K. (1997). Role of Arabidopsis MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. *The Plant Cell*, 9(10): 1859-1868.
- Acosta, I. F. and Farmer, E. E. (2009). Jasmonates. *The Arabidopsis Book* 0129.
- Amoutzias, G. D., Robertson, D. L., Van de Peer, Y. and Oliver, S. G. (2008). Choose your partners: dimerization in eukaryotic transcription factors. *Trends in biochemical sciences*, 33(5): 220-229.
- Arai, R., Ueda, H., Kitayama, A., Kamiya, N. and Nagamune, T. (2001). Design of the linkers which effectively separate domains of a bifunctional fusion protein. *Protein engineering*, 14(8): 529-532.
- Arnold, R., Brandmaier, S., Kleine, F., Tischler, P., Heinz, E., Behrens, S., Niinikoski, A., Mewes, H.-W., Horn, M. and Rattei, T. (2009). Sequence-based prediction of type III secreted proteins. *PLoS pathogens*, 5(4): e1000376.
- Bai, C., Sen, P., Hofmann, K., Ma, L., Goebel, M., Harper, J. W. and Elledge, S. J. (1996). SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell*, 86(2): 263-274.
- Balbi, V. and Devoto, A. (2008). Jasmonate signalling network in Arabidopsis thaliana: crucial regulatory nodes and new physiological scenarios. *New Phytologist*, 177(2): 301-318.
- Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A. and Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, 315(5819): 1709-1712.
- Berger, S. (2002). Jasmonate-related mutants of Arabidopsis as tools for studying stress signaling. *Planta*, 214(4): 497-504.
- Boch, J. and Bonas, U. (2010). Xanthomonas AvrBs3 family-type III effectors: discovery and function. *Annual review of phytopathology*, 48(
- Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A. and Bonas, U. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*, 326(5959): 1509-1512.

- Bogdanove, A. J., Schornack, S. and Lahaye, T. (2010). TAL effectors: finding plant genes for disease and defense. *Current opinion in plant biology*, 13(4): 394-401.
- Bolotin, A., Quinquis, B., Sorokin, A. and Ehrlich, S. D. (2005). Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology*, 151(8): 2551-2561.
- Borchardt, E. K., Meganck, R. M., Vincent, H. A., Ball, C. B., Ramos, S. B., Moorman, N. J., Marzluff, W. F. and Asokan, A. (2017). Inducing circular RNA formation using the CRISPR endoribonuclease Csy4. *RNA*, 23(5): 619-627.
- Boter, M., Ruíz-Rivero, O., Abdeen, A. and Prat, S. (2004). Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and Arabidopsis. *Genes & development*, 18(13): 1577-1591.
- Brown, S. A., Weirich, C. S., Newton, E. M. and Kingston, R. E. (1998). Transcriptional activation domains stimulate initiation and elongation at different times and via different residues. *The EMBO journal*, 17(11): 3146-3154.
- Browse, J. (2009). Jasmonate passes muster: a receptor and targets for the defense hormone. *Annual review of plant biology*, 60(183-205).
- Browse, J. (2009). The power of mutants for investigating jasmonate biosynthesis and signaling. *Phytochemistry*, 70(13): 1539-1546.
- Brückner, K., Schäfer, P., Weber, E., Grützner, R., Marillonnet, S. and Tissier, A. (2015). A library of synthetic transcription activator-like effector-activated promoters for coordinated orthogonal gene expression in plants. *The Plant Journal*, 82(4): 707-716.
- Büttner, D. and Bonas, U. (2010). Regulation and secretion of Xanthomonas virulence factors. *FEMS microbiology reviews*, 34(2): 107-133.
- Çevik, V., Kidd, B. N., Zhang, P., Hill, C., Kiddle, S., Denby, K. J., Holub, E. B., Cahill, D. M., Manners, J. M. and Schenk, P. M. (2012). MEDIATOR25 acts as an integrative hub for the regulation of jasmonate-responsive gene expression in Arabidopsis. *Plant Physiology*, 160(1): 541-555.
- Chen, R., Jiang, H., Li, L., Zhai, Q., Qi, L., Zhou, W., Liu, X., Li, H., Zheng, W. and Sun, J. (2012). The Arabidopsis mediator subunit MED25 differentially

- regulates jasmonate and abscisic acid signaling through interacting with the MYC2 and ABI5 transcription factors. *The Plant Cell*, 24(7): 2898-2916.
- Chen, X., Zaro, J. L. and Shen, W.-C. (2013). Fusion protein linkers: property, design and functionality. *Advanced drug delivery reviews*, 65(10): 1357-1369.
- Chen, Y.-J., Liu, P., Nielsen, A. A., Brophy, J. A., Clancy, K., Peterson, T. and Voigt, C. A. (2013). Characterization of 582 natural and synthetic terminators and quantification of their design constraints. *Nature methods*, 10(7): 659-664.
- Chini, A., Boter, M. and Solano, R. (2009). Plant oxylipins: COI1/JAZs/MYC2 as the core jasmonic acid-signalling module. *The FEBS journal*, 276(17): 4682-4692.
- Chini, A., Fonseca, S., Fernandez, G., Adie, B., Chico, J., Lorenzo, O., Garcia-Casado, G., Lopez-Vidriero, I., Lozano, F. and Ponce, M. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*, 448(7154): 666.
- Chung, H. S. and Howe, G. A. (2009). A critical role for the TIFY motif in repression of jasmonate signaling by a stabilized splice variant of the JASMONATE ZIM-domain protein JAZ10 in Arabidopsis. *The Plant Cell*, 21(1): 131-145.
- Chung, H. S., Niu, Y., Browse, J. and Howe, G. A. (2009). Top hits in contemporary JAZ: an update on jasmonate signaling. *Phytochemistry*, 70(13): 1547-1559.
- Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal*, 16(6): 735-743.
- Conconi, A., Smerdon, M. J., Howe, G. A. and Ryan, C. A. (1996). The octadecanoid signalling pathway in plants mediates a response to ultraviolet radiation. *Nature*, 383(6603): 826.
- Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W. and Marraffini, L. A. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science*, 339(6121): 819-823.
- Creelman, R. A. and Mullet, J. E. (1995). Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. *Proceedings of the National Academy of Sciences*, 92(10): 4114-4119.
- Creelman, R. A. and Mulpuri, R. (2002). The oxylipin pathway in Arabidopsis. *The Arabidopsis Book* 0012.

- Dathe, W., Rönsch, H., Preiss, A., Schade, W., Sembdner, G. and Schreiber, K. (1981). Endogenous plant hormones of the broad bean, *Vicia faba* L.(-)-jasmonic acid, a plant growth inhibitor in pericarp. *Planta*, 153(6): 530-535.
- Demole, E., Lederer, E., and Mercier, D. (1962). Isolement et détermination de la structure du jasmonate de méthyle, constituant odorant caractéristique de l'essence de jasmin. *Helvetica Chimica Acta*, 45(2): 675-685.
- Deveau, H., Garneau, J. E. and Moineau, S. (2010). CRISPR/Cas system and its role in phage-bacteria interactions. *Annual review of microbiology*, 64(475-493).
- Devoto, A., Nieto-Rostro, M., Xie, D., Ellis, C., Harmston, R., Patrick, E., Davis, J., Sherratt, L., Coleman, M. and Turner, J. G. (2002). COI1 links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in Arabidopsis. *The Plant Journal*, 32(4): 457-466.
- Dillen, W., Clercq, J., Kapila, J., Zambre, M., Montagu, M. and Angenon, G. (1997). The effect of temperature on *Agrobacterium tumefaciens*-mediated gene transfer to plants. *The Plant Journal*, 12(6): 1459-1463.
- Dombrecht, B., Xue, G. P., Sprague, S. J., Kirkegaard, J. A., Ross, J. J., Reid, J. B., Fitt, G. P., Sewelam, N., Schenk, P. M. and Manners, J. M. (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. *The Plant Cell*, 19(7): 2225-2245.
- Engler, C., Kandzia, R. and Marillonnet, S. (2008). A one pot, one step, precision cloning method with high throughput capability. *PLoS One*, 3(11): e3647.
- Estorellas, C., Otyepka, M., Koča, J., Banáš, P., Krepl, M. and Šponer, J. (2015). Molecular dynamic simulations of protein/RNA complexes: CRISPR/Csy4 endoribonuclease. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1850(5): 1072-1090.
- Ezer, D., Shepherd, S. J., Brestovitsky, A., Dickinson, P., Cortijo, S., Charoensawan, V., Box, M. S., Biswas, S. and Wigge, P. (2017). The G-box transcriptional regulatory code in Arabidopsis. *bioRxiv*128371.
- Fahad, S., Hussain, S., Matloob, A., Khan, F. A., Khaliq, A., Saud, S., Hassan, S., Shan, D., Khan, F. and Ullah, N. (2015). Phytohormones and plant responses to salinity stress: a review. *Plant growth regulation*, 75(2): 391-404.

- Farmer, E. E. and Ryan, C. A. (1990). Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences*, 87(19): 7713-7716.
- Fernández-Calvo, P., Chini, A., Fernández-Barbero, G., Chico, J.-M., Gimenez-Ibanez, S., Geerinck, J., Eeckhout, D., Schweizer, F., Godoy, M. and Franco-Zorrilla, J. M. (2011). The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *The Plant Cell*, 23(2): 701-715.
- Feys, B. J., Benedetti, C. E., Penfold, C. N. and Turner, J. G. (1994). Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *The Plant Cell Online*, 6(5): 751-759.
- Figueroa, P. and Browse, J. (2012). The Arabidopsis JAZ2 Promoter Contains a G-Box and Thymidine-Rich Module that are Necessary and Sufficient for Jasmonate-Dependent Activation by MYC Transcription Factors and Repression by JAZ Proteins. *Plant and Cell Physiology*, 53(2): 330-343.
- Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, C. and Solano, R. (2009). (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nature chemical biology*, 5(5): 344-350.
- Fonseca, S., Fernandez-Calvo, P., Fernandez, G. M., Díez-Díaz, M., Gimenez-Ibanez, S., Lopez-Vidriero, I., Godoy, M., Fernandez-Barbero, G., Van Leene, J. and De Jaeger, G. (2014). bHLH003, bHLH013 and bHLH017 are new targets of JAZ repressors negatively regulating JA responses. *PLoS One*, 9(1): e86182.
- Glauser, G., Grata, E., Dubugnon, L., Rudaz, S., Farmer, E. E. and Wolfender, J.-L. (2008). Spatial and temporal dynamics of jasmonate synthesis and accumulation in Arabidopsis in response to wounding. *Journal of Biological Chemistry*, 283(24): 16400-16407.
- Grant, S. G., Jessee, J., Bloom, F. R. and Hanahan, D. (1990). Differential plasmid rescue from transgenic mouse DNAs into Escherichia coli methylation-restriction mutants. *Proceedings of the National Academy of Sciences*, 87(12): 4645-4649.
- Grefen, C. and Blatt, M. R. (2012). Method summary. *Biotechniques*, 53(5): 311-314.

- Grissa, I., Vergnaud, G. and Pourcel, C. (2007). The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats. *BMC bioinformatics*, 8(1): 172.
- Hahn, S. and Young, E. T. (2011). Transcriptional regulation in *Saccharomyces cerevisiae*: transcription factor regulation and function, mechanisms of initiation, and roles of activators and coactivators. *Genetics*, 189(3): 705-736.
- Hale, C. R., Zhao, P., Olson, S., Duff, M. O., Graveley, B. R., Wells, L., Terns, R. M. and Terns, M. P. (2009). RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. *Cell*, 139(5): 945-956.
- Haurwitz, R. E., Sternberg, S. H. and Doudna, J. A. (2012). Csy4 relies on an unusual catalytic dyad to position and cleave CRISPR RNA. *The EMBO journal*, 31(12): 2824-2832.
- Heim, M. A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B. and Bailey, P. C. (2003). The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. *Molecular biology and evolution*, 20(5): 735-747.
- Hong, S. Y., Lee, J. S., Cho, K. M., Math, R. K., Kim, Y. H., Hong, S. J., Cho, Y. U., Kim, H. and Yun, H. D. (2006). Assembling a novel bifunctional cellulase-xylanase from *Thermotoga maritima* by end-to-end fusion. *Biotechnology letters*, 28(22): 1857-1862.
- Horvath, P. and Barrangou, R. (2010). CRISPR/Cas, the immune system of bacteria and archaea. *Science*, 327(5962): 167-170.
- Hsu, P. D., Lander, E. S. and Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. *Cell*, 157(6): 1262-1278.
- Hu, P., Zhou, W., Cheng, Z., Fan, M., Wang, L. and Xie, D. (2013). JAV1 controls jasmonate-regulated plant defense. *Molecular Cell*, 50(4): 504-515.
- Jefferson, R. A., Kavanagh, T. A. and Bevan, M. W. (1987). GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *The EMBO journal*, 6(13): 3901.
- Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A. and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096): 816-821.

- Jung, S. C., Martinez-Medina, A., Lopez-Raez, J. A. and Pozo, M. J. (2012). Mycorrhiza-induced resistance and priming of plant defenses. *Journal of chemical ecology*, 38(6): 651-664.
- Katsir, L., Schilmiller, A. L., Staswick, P. E., He, S. Y. and Howe, G. A. (2008). COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proceedings of the National Academy of Sciences*, 105(19): 7100-7105.
- Kay, S., Hahn, S., Marois, E., Hause, G. and Bonas, U. (2007). A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science*, 318(5850): 648-651.
- Kay, S., Hahn, S., Marois, E., Wieduwild, R. and Bonas, U. (2009). Detailed analysis of the DNA recognition motifs of the *Xanthomonas* type III effectors AvrBs3 and AvrBs3 Δ rep16. *The Plant Journal*, 59(6): 859-871.
- Kazan, K. and Manners, J. M. (2008). Jasmonate signaling: toward an integrated view. *Plant Physiology*, 146(4): 1459-1468.
- Kazan, K. and Manners, J. M. (2013). MYC2: the master in action. *Molecular plant*, 6(3): 686-703.
- Kim, S.-R., Choi, J.-L., Costa, M. A. and An, G. (1992). Identification of G-box sequence as an essential element for methyl jasmonate response of potato proteinase inhibitor II promoter. *Plant Physiology*, 99(2): 627-631.
- Kombrink, E. (2012). Chemical and genetic exploration of jasmonate biosynthesis and signaling paths. *Planta*, 236(5): 1351-1366.
- Koo, A. J. and Howe, G. A. (2009). The wound hormone jasmonate. *Phytochemistry*, 70(13): 1571-1580.
- Krumm, T., Bandemer, K. and Boland, W. (1995). Induction of volatile biosynthesis in the Lima bean (*Phaseolus lunatus*) by leucine- and isoleucine conjugates of 1-oxo- and 1-hydroxyindan-4-carboxylic acid: evidence for amino acid conjugates of jasmonic acid as intermediates in the octadecanoid signalling pathway. *FEBS letters*, 377(3): 523-529.
- Kudla, J. and Bock, R. (2016). Lighting the way to protein-protein interactions: recommendations on best practices for bimolecular fluorescence complementation analyses. *The Plant Cell Online*, 28(5): 1002-1008.
- Larrieu, A., Champion, A., Legrand, J., Lavenus, J., Mast, D., Brunoud, G., Oh, J., Guyomarc'h, S., Pizot, M. and Farmer, E. E. (2015). A fluorescent hormone

- biosensor reveals the dynamics of jasmonate signalling in plants. *Nature communications*, 6(6043).
- Li, C., Schillmiller, A. L., Liu, G., Lee, G. I., Jayanty, S., Sageman, C., Vrebalov, J., Giovannoni, J. J., Yagi, K. and Kobayashi, Y. (2005). Role of β -oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. *The Plant Cell*, 17(3): 971-986.
- Li, L., Zhao, Y., McCaig, B. C., Wingerd, B. A., Wang, J., Whalon, M. E., Pichersky, E. and Howe, G. A. (2004). The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell*, 16(1): 126-143.
- Liang, Y., Richardson, S., Yan, J., Benites, V. T., Cheng-Yue, C., Tran, T., Mortimer, J., Mukhopadhyay, A., Keasling, J. D. and Scheller, H. V. (2017). Endoribonuclease-based two-component repressor systems for tight gene expression control in plants. *ACS synthetic biology*
- Lorenzo, O., Chico, J. M., Sánchez-Serrano, J. J. and Solano, R. (2004). JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. *The Plant Cell*, 16(7): 1938-1950.
- Lu, P. and Feng, M.-G. (2008). Bifunctional enhancement of a β -glucanase-xylanase fusion enzyme by optimization of peptide linkers. *Applied microbiology and biotechnology*, 79(4): 579-587.
- Mahfouz, M. M., Li, L., Shamimuzzaman, M., Wibowo, A., Fang, X. and Zhu, J.-K. (2011). De novo-engineered transcription activator-like effector (TALE) hybrid nuclease with novel DNA binding specificity creates double-strand breaks. *Proceedings of the National Academy of Sciences*, 108(6): 2623-2628.
- Makarova, K. S., Grishin, N. V., Shabalina, S. A., Wolf, Y. I. and Koonin, E. V. (2006). A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. *Biology direct*, 1(1): 7.
- Mandaokar, A., Thines, B., Shin, B., Markus Lange, B., Choi, G., Koo, Y. J., Yoo, Y. J., Choi, Y. D., Choi, G. and Browse, J. (2006). Transcriptional regulators of

- stamen development in Arabidopsis identified by transcriptional profiling. *The Plant Journal*, 46(6): 984-1008.
- Marraffini, L. A. and Sontheimer, E. J. (2010). CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. *Nature reviews. Genetics*, 11(3): 181.
- Martin, C. and Paz-Ares, J. (1997). MYB transcription factors in plants. *Trends in Genetics*, 13(2): 67-73.
- McConn, M., Creelman, R. A., Bell, E. and Mullet, J. E. (1997). Jasmonate is essential for insect defense in Arabidopsis. *Proceedings of the National Academy of Sciences*, 94(10): 5473-5477.
- Memelink, J. (2005). The use of genetics to dissect plant secondary pathways. *Current opinion in plant biology*, 8(3): 230-235.
- Memelink, J. (2009). Regulation of gene expression by jasmonate hormones. *Phytochemistry*, 70(13): 1560-1570.
- Menke, F. L., Champion, A., Kijne, J. W. and Memelink, J. (1999). A novel jasmonate - and elicitor - responsive element in the periwinkle secondary metabolite biosynthetic gene Str interacts with a jasmonate -and elicitor -inducible AP2-domain transcription factor, ORCA2. *The EMBO journal*, 18(16): 4455-4463.
- Menkens, A. E., Schindler, U. and Cashmore, A. R. (1995). The G-box: a ubiquitous regulatory DNA element in plants bound by the GBF family of bZIP proteins. *Trends in biochemical sciences*, 20(12): 506-510.
- Mielke, K., Forner, S., Kramell, R., Conrad, U. and Hause, B. (2011). Cell-specific visualization of jasmonates in wounded tomato and Arabidopsis leaves using jasmonate-specific antibodies. *New Phytologist*, 190(4): 1069-1080.
- Miersch, O., Neumerkel, J., Dippe, M., Stenzel, I. and Wasternack, C. (2008). Hydroxylated jasmonates are commonly occurring metabolites of jasmonic acid and contribute to a partial switch-off in jasmonate signaling. *New Phytologist*, 177(1): 114-127.

- Mojica, F., Diez-Villasenor, C., Garcia-Martinez, J. and Almendros, C. (2009). Short motif sequences determine the targets of the prokaryotic CRISPR defence system. *Microbiology*, 155(3): 733-740.
- Mojica, F. J., García-Martínez, J. and Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *Journal of molecular evolution*, 60(2): 174-182.
- Morbitzer, R., Römer, P., Boch, J. and Lahaye, T. (2010). Regulation of selected genome loci using de novo-engineered transcription activator-like effector (TALE)-type transcription factors. *Proceedings of the National Academy of Sciences*, 107(50): 21617-21622.
- Moscou, M. J. and Bogdanove, A. J. (2009). A simple cipher governs DNA recognition by TAL effectors. *Science*, 326(5959): 1501-1501.
- Murre, C., Bain, G., van Dijk, M. A., Engel, I., Furnari, B. A., Massari, M. E., Matthews, J. R., Quong, M. W., Rivera, R. R. and Stuver, M. H. (1994). Structure and function of helix-loop-helix proteins. *Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression*, 1218(2): 129-135.
- Murre, C., McCaw, P. S. and Baltimore, D. (1989). A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell*, 56(5): 777-783.
- Pauwels, L., Barbero, G. F., Geerinck, J., Tilleman, S., Grunewald, W., Pérez, A. C., Chico, J. M., Bossche, R. V., Sewell, J. and Gil, E. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature*, 464(7289): 788.
- Pauwels, L. and Goossens, A. (2011). The JAZ proteins: a crucial interface in the jasmonate signaling cascade. *The Plant Cell*, 23(9): 3089-3100.
- Pauwels, L., Inzé, D. and Goossens, A. (2009). Jasmonate-inducible gene: what does it mean? *Trends in plant science*, 14(2): 87-91.
- Pauwels, L., Morreel, K., De Witte, E., Lammertyn, F., Van Montagu, M., Boerjan, W., Inzé, D. and Goossens, A. (2008). Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured Arabidopsis cells. *Proceedings of the National Academy of Sciences*, 105(4): 1380-1385.
- Payne, C. T., Zhang, F. and Lloyd, A. M. (2000). GL3 encodes a bHLH protein that regulates trichome development in Arabidopsis through interaction with GL1 and TTG1. *Genetics*, 156(3): 1349-1362.

- Peremarti, A., Twyman, R. M., Gómez-Galera, S., Naqvi, S., Farré, G., Sabalza, M., Miralpeix, B., Dashevskaya, S., Yuan, D. and Ramessar, K. (2010). Promoter diversity in multigene transformation. *Plant molecular biology*, 73(4-5): 363-378.
- Pourcel, C., Salvignol, G. and Vergnaud, G. (2005). CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology*, 151(3): 653-663.
- Przybilski, R., Richter, C., Gristwood, T., Clulow, J. S., Vercoe, R. B. and Fineran, P. C. (2011). Csy4 is responsible for CRISPR RNA processing in *Pectobacterium atrosepticum*. *RNA biology*, 8(3): 517-528.
- Qi, T., Song, S., Ren, Q., Wu, D., Huang, H., Chen, Y., Fan, M., Peng, W., Ren, C. and Xie, D. (2011). The Jasmonate-ZIM-domain proteins interact with the WD-Repeat/bHLH/MYB complexes to regulate Jasmonate-mediated anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*. *The Plant Cell Online*, 23(5): 1795-1814.
- Qin, W., Liang, F., Feng, Y., Bai, H., Yan, R., Li, S. and Lin, S. (2015). Expansion of CRISPR/Cas9 genome targeting sites in zebrafish by Csy4-based RNA processing. *Cell research*, 25(9): 1074.
- Rao, M. V., Lee, H.-i., Creelman, R. A., Mullet, J. E. and Davis, K. R. (2000). Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. *The Plant Cell*, 12(9): 1633-1646.
- Reymond, P., Weber, H., Damond, M. and Farmer, E. E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *The Plant Cell*, 12(5): 707-719.
- Römer, P., Hahn, S., Jordan, T., Strauß, T., Bonas, U. and Lahaye, T. (2007). Plant pathogen recognition mediated by promoter activation of the pepper Bs3 resistance gene. *Science*, 318(5850): 645-648.
- Sanjana, N. E., Le Cong, Y. Z., Cunniff, M. M., Feng, G. and Zhang, F. (2012). A transcription activator-like effector (TALE) toolbox for genome engineering. *Nature protocols*, 7(1): 171.
- Sasaki-Sekimoto, Y., Jikumaru, Y., Obayashi, T., Saito, H., Masuda, S., Kamiya, Y., Ohta, H. and Shirasu, K. (2013). Basic helix-loop-helix transcription factors JASMONATE-ASSOCIATED MYC2-LIKE1 (JAM1), JAM2, and JAM3 are

- negative regulators of jasmonate responses in Arabidopsis. *Plant Physiology*, 163(1): 291-304.
- Schaller, A. and Stintzi, A. (2009). Enzymes in jasmonate biosynthesis—structure, function, regulation. *Phytochemistry*, 70(13): 1532-1538.
- Schmelz, E. A., Engelberth, J., Alborn, H. T., O'donnell, P., Sammons, M., Toshima, H. and Tumlinson, J. H. (2003). Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. *Proceedings of the National Academy of Sciences*, 100(18): 10552-10557.
- Scholze, H. and Boch, J. (2011). TAL effectors are remote controls for gene activation. *Current opinion in microbiology*, 14(1): 47-53.
- Schutzendubel, A. and Polle, A. (2002). Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of experimental botany*, 53(372): 1351-1365.
- Seo, H. S., Song, J. T., Cheong, J.-J., Lee, Y.-H., Lee, Y.-W., Hwang, I., Lee, J. S. and Do Choi, Y. (2001). Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. *Proceedings of the National Academy of Sciences*, 98(8): 4788-4793.
- Seo, J. S., Koo, Y. J., Jung, C., Yeu, S. Y., Song, J. T., Kim, J.-K., Choi, Y., Lee, J. S. and Do Choi, Y. (2013). Identification of a novel jasmonate-responsive element in the AtJMT promoter and its binding protein for AtJMT repression. *PLoS One*, 8(2): e55482.
- Seo, S., Seto, H., Yamakawa, H. and Ohashi, Y. (2001). Transient accumulation of jasmonic acid during the synchronized hypersensitive cell death in tobacco mosaic virus-infected tobacco leaves. *Molecular plant-microbe interactions*, 14(2): 261-264.
- Shan. (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature biotechnology*, 31(8): 686-688.
- Shan, X., Wang, J., Chua, L., Jiang, D., Peng, W. and Xie, D. (2011). The role of Arabidopsis Rubisco activase in jasmonate-induced leaf senescence. *Plant Physiology*, 155(2): 751-764.
- Sheen, J. (2001). Signal transduction in maize and Arabidopsis mesophyll protoplasts. *Plant Physiology*, 127(4): 1466-1475.

- Smith, T. F., Gaitatzes, C., Saxena, K. and Neer, E. J. (1999). The WD repeat: a common architecture for diverse functions. *Trends in biochemical sciences*, 24(5): 181-185.
- Song, S., Qi, T., Fan, M., Zhang, X., Gao, H., Huang, H., Wu, D., Guo, H. and Xie, D. (2013). The bHLH subgroup IIIId factors negatively regulate jasmonate-mediated plant defense and development. *PLoS genetics*, 9(7): e1003653.
- Song, S., Qi, T., Huang, H., Ren, Q., Wu, D., Chang, C., Peng, W., Liu, Y., Peng, J. and Xie, D. (2011). The jasmonate-ZIM domain proteins interact with the R2R3-MYB transcription factors MYB21 and MYB24 to affect jasmonate-regulated stamen development in Arabidopsis. *The Plant Cell Online*, 23(3): 1000-1013.
- Staswick, P. (2009). Plant hormone conjugation: a signal decision. *Plant signaling & behavior*, 4(8): 757-759.
- Staswick, P. E. (2009). The tryptophan conjugates of jasmonic and indole-3-acetic acids are endogenous auxin inhibitors. *Plant Physiology*, 150(3): 1310-1321.
- Staswick, P. E., Su, W. and Howell, S. H. (1992). Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an Arabidopsis thaliana mutant. *Proceedings of the National Academy of Sciences*, 89(15): 6837-6840.
- Staswick, P. E. and Tiryaki, I. (2004). The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. *The Plant Cell*, 16(8): 2117-2127.
- Stella, S., Molina, R., Yefimenko, I., Prieto, J., Silva, G., Bertonati, C., Juillerat, A., Duchateau, P. and Montoya, G. (2013). Structure of the AvrBs3-DNA complex provides new insights into the initial thymine-recognition mechanism. *Acta Crystallographica Section D: Biological Crystallography*, 69(9): 1707-1716.
- Stitz, M., Gase, K., Baldwin, I. T. and Gaquerel, E. (2011). Ectopic expression of AtJMT in *Nicotiana attenuata*: creating a metabolic sink has tissue-specific consequences for the jasmonate metabolic network and silences downstream gene expression. *Plant Physiology*, 157(1): 341-354.
- Strassner, J., Schaller, F., Frick, U. B., Howe, G. A., Weiler, E. W., Amrhein, N., Macheroux, P. and Schaller, A. (2002). Characterization and cDNA - microarray expression analysis of 12-oxophytodienoate reductases reveals

- differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. *The Plant Journal*, 32(4): 585-601.
- Stringer, K. F., Ingles, C. J. and Greenblatt, J. (1990). Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. *Nature*, 345(6278): 783.
- Suza, W. P. and Staswick, P. E. (2008). The role of JAR1 in jasmonoyl-L-isoleucine production during Arabidopsis wound response. *Planta*, 227(6): 1221-1232.
- Szemenyei, H., Hannon, M. and Long, J. A. (2008). TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis. *Science*, 319(5868): 1384-1386.
- Szurek, B., Marois, E., Bonas, U. and Van den Ackerveken, G. (2001). Eukaryotic features of the Xanthomonas type III effector AvrBs3: protein domains involved in transcriptional activation and the interaction with nuclear import receptors from pepper. *The Plant Journal*, 26(5): 523-534.
- Tamogami, S., Rakwal, R. and Agrawal, G. K. (2008). Interplant communication: airborne methyl jasmonate is essentially converted into JA and JA-Ile activating jasmonate signaling pathway and VOCs emission. *Biochemical and biophysical research communications*, 376(4): 723-727.
- Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S. Y. and Howe, G. A. (2007). JAZ repressor proteins are targets of the SCFCO11 complex during jasmonate signalling. *Nature*, 448(7154): 661.
- Ueda, J. and Kato, J. (1980). Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). *Plant Physiology*, 66(2): 246-249.
- Wang, J. and Quake, S. R. (2014). RNA-guided endonuclease provides a therapeutic strategy to cure latent herpesviridae infection. *Proceedings of the National Academy of Sciences*, 111(36): 13157-13162.
- Wang, Z., Cao, G., Wang, X., Miao, J., Liu, X., Chen, Z., Qu, L.-J. and Gu, H. (2008). Identification and characterization of COI1-dependent transcription factor genes involved in JA-mediated response to wounding in Arabidopsis plants. *Plant cell reports*, 27(1): 125-135.
- Wasternack, C. (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of botany*, 100(4): 681-697.

- Wasternack, C. and Hause, B. (2013). Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *Annals of Botany*. *Annals of botany*, 111(6): 1021-1058.
- Wasternack, C., Stenzel, I., Hause, B., Hause, G., Kutter, C., Maucher, H., Neumerkel, J., Feussner, I. and Miersch, O. (2006). The wound response in tomato—role of jasmonic acid. *Journal of plant physiology*, 163(3): 297-306.
- Wroblewski, T., Tomczak, A. and Michelmore, R. (2005). Optimization of Agrobacterium-mediated transient assays of gene expression in lettuce, tomato and Arabidopsis. *Plant biotechnology journal*, 3(2): 259-273.
- Xie, D.-X., Feys, B. F., James, S., Nieto-Rostro, M. and Turner, J. G. (1998). COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. *Science*, 280(5366): 1091-1094.
- Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W. L., Ma, H., Peng, W., Huang, D. and Xie, D. (2002). The SCFCO11 ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. *The Plant Cell*, 14(8): 1919-1935.
- Yadav, V., Mallappa, C., Gangappa, S. N., Bhatia, S. and Chattopadhyay, S. (2005). A basic helix-loop-helix transcription factor in Arabidopsis, MYC2, acts as a repressor of blue light-mediated photomorphogenic growth. *The Plant Cell*, 17(7): 1953-1966.
- Yan, C. and Xie, D. (2015). Jasmonate in plant defence: sentinel or double agent? *Plant biotechnology journal*, 13(9): 1233-1240.
- Yan, J., Zhang, C., Gu, M., Bai, Z., Zhang, W., Qi, T., Cheng, Z., Peng, W., Luo, H. and Nan, F. (2009). The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. *The Plant Cell*, 21(8): 2220-2236.
- Yan, Y., Stolz, S., Chételat, A., Reymond, P., Pagni, M., Dubugnon, L. and Farmer, E. E. (2007). A downstream mediator in the growth repression limb of the jasmonate pathway. *The Plant Cell*, 19(8): 2470-2483.
- Yang, Y. and Gabriel, D. W. (1995). Xanthomonas avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. *MPMI-Molecular Plant Microbe Interactions*, 8(4): 627-631.

- Yoo, S.-D., Cho, Y.-H. and Sheen, J. (2007). Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. *Nature protocols*, 2(7): 1565.
- Zhang, F., Cong, L., Lodato, S., Kosuri, S., Church, G. M. and Arlotta, P. (2011). Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription. *Nature biotechnology*, 29(2): 149-153.
- Zhang, F., Gonzalez, A., Zhao, M., Payne, C. T. and Lloyd, A. (2003). A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis. *Development*, 130(20): 4859-4869.
- Zhao, H. L., Xue, C., Wang, Y., Li, X. Y., Xiong, X. H., Yao, X. Q. and Liu, Z. M. (2007). Circumventing the heterogeneity and instability of human serum albumin-interferon- α 2b fusion protein by altering its orientation. *Journal of biotechnology*, 131(3): 245-252.

6 Appendix

Table 7. List of level -1 vectors. Resistance: Kan

Construct Name/ID	Destination vector	Description
pAGT954-1	pAGM1311	MYC2 (fragment1)
pAGT954-2	pAGM1311	MYC2(fragment2)
pAGT960	pAGM1311	MYC2(JID Domain)
pAGT961	pAGM1311	MYC2(N-ter and JID Domain)
pAGT962	pAGM1311	MYC2(N-ter; JID and TAD Domain)
pAGT963	pAGM1311	MYC2(TAD Domain)
pAGT964	pAGM1311	MYC2 (JID and TAD Domain)
pAGT965	pAGM1311	MYC2(MYC2 without bHLH)
pAGT966	pAGM1311	MYC2 (full length)
pAGT974	pAGM1311	MYC2(JID Domain)
pAGT975	pAGM1311	MYC2(N-ter and JID Domain)
pAGT976	pAGM1311	MYC2(N-ter; JID and TAD Domain)
pAGT977	pAGM1311	MYC2(TAD Domain)
pAGT978	pAGM1311	MYC2 (JID and TAD Domain)
pAGT979	pAGM1311	MYC2(MYC2 without bHLH)
pAGT980	pAGM1311	MYC2 (full length)
pAGT1077	pAGM1311	truncated C-ter of TALE

Table 8. List of level 0 vectors. Resistance: Spec

Construct Name/ID	Destination vector	Description
pAGT700	pICH41308	TALE with truncated N-ter
pAGT701	pICH41308	TALE with truncated N-ter and without activation domain
pAGT702	pAGM1276	MYC2(JID Domain)
pAGT703	pAGM1276	MYC2(N-ter and JID Domain)
pAGT704	pAGM1276	MYC2(N-ter; JID and TAD Domain)
pAGT705	pAGM1276	MYC2(TAD Domain)
pAGT706	pAGM1276	MYC2 (JID and TAD Domain)
pAGT707	pAGM1263	TMV(Tobacco mosaic virus) Ω^+

pAGT799	pICH41308	New TALE with truncated N-ter
pAGT800	pICH41308	New TALE with truncated N-ter and without AD
pAGT811	pICH41308	TALE EBE002 with truncated N-ter and without AD
pAGT953	pAGM1276	MYC2(MYC2 without bHLH)
pAGT954	pAGM1276	MYC2 (full length)
pAGT967	pICH41308	TALE:MYC2(JID Domain)
pAGT968	pICH41308	TALE:MYC2(N-ter and JID Domain)
pAGT969	pICH41308	TALE:MYC2(N-ter; JID and TAD Domain)
pAGT970	pICH41308	TALE:MYC2(JID and TAD Domain)
pAGT971	pICH41308	TALE:MYC2(TAD Domain)
pAGT972	pICH41308	TALE:MYC2(MYC2 without bHLH)
pAGT973	pICH41308	TALE:MYC2 (full length)
pAGT981	pICH41308	TALE-AD:MYC2(JID Domain)
pAGT982	pICH41308	TALE-AD:MYC2(N-ter and JID Domain)
pAGT983	pICH41308	TALE-AD:MYC2(N-ter; JID and TAD Domain)
pAGT984	pICH41308	TALE-AD:MYC2(JID and TAD Domain)
pAGT985	pICH41308	TALE-AD:MYC2(TAD Domain)
pAGT986	pICH41308	TALE-AD:MYC2(MYC2 without bHLH)
pAGT987	pICH41308	TALE-AD:MYC2 (full length)
pAGT1078	pICH41308	TALE with truncated C-ter
pAGH108	pICH41295	Tetramer of JARE-JMT:35sMp
pAGH109	pICH41295	Octamer of JARE-JMT:35sMp
pAGH110	pICH41295	Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement)):35sMp

Table 9. List of level 1 vectors. Resisitance: Carb

Construct Name/ID	Destination vector	Description
pAGT754	pICH47751	Actin2pΩ ⁺ ::MYC2(JID Domain) :TALE
pAGT755	pICH47751	Actin2pΩ ⁺ ::MYC2(JID Domain) :TALE-AD
pAGT756	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter and JID Domain) :TALE
pAGT757	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter and JID Domain) :TALE-AD
pAGT758	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter; JID and TAD Domain):TALE
pAGT759	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter; JID and TAD Domain):TALE-AD

pAGT760	pICH47751	Actin2pΩ ⁺ ::MYC2(TAD Domain):TALE
pAGT761	pICH47751	Actin2pΩ ⁺ ::MYC2(TAD Domain):TALE-AD
pAGT762	pICH47751	Actin2pΩ ⁺ ::MYC2(JID and TAD Domain):TALE
pAGT763	pICH47751	Actin2pΩ ⁺ ::MYC2(JID and TAD Domain):TALE-AD
pAGT801	pICH47751	Actin2pΩ ⁺ ::MYC2(JID Domain) :TALE
pAGT802	pICH47751	Actin2pΩ ⁺ ::MYC2(JID Domain) :TALE-AD
pAGT803	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter and JID Domain) :TALE
pAGT804	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter and JID Domain) :TALE-AD
pAGT805	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter; JID and TAD Domain):TALE
pAGT806	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter; JID and TAD Domain):TALE-AD
pAGT807	pICH47751	Actin2pΩ ⁺ ::MYC2(TAD Domain):TALE
pAGT808	pICH47751	Actin2pΩ ⁺ ::MYC2(TAD Domain):TALE-AD
pAGT809	pICH47751	Actin2pΩ ⁺ ::MYC2(JID and TAD Domain):TALE
pAGT810	pICH47751	Actin2pΩ ⁺ ::MYC2(JID and TAD Domain):TALE-AD
pAGT812	pICH47751	Actin2pΩ ⁺ ::MYC2(JID Domain) :TALE
pAGT813	pICH47751	Actin2pΩ ⁺ ::MYC2(JID Domain) :TALE-AD
pAGT814	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter and JID Domain) :TALE
pAGT815	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter and JID Domain) :TALE-AD
pAGT816	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter; JID and TAD Domain):TALE
pAGT817	pICH47751	Actin2pΩ ⁺ ::MYC2(N-ter; JID and TAD Domain):TALE-AD
pAGT818	pICH47751	Actin2pΩ ⁺ ::MYC2(TAD Domain):TALE
pAGT819	pICH47751	Actin2pΩ ⁺ ::MYC2(TAD Domain):TALE-AD
pAGT820	pICH47751	Actin2pΩ ⁺ ::MYC2(JID and TAD Domain):TALE
pAGT821	pICH47751	Actin2pΩ ⁺ ::MYC2(JID and TAD Domain):TALE-AD
pAGT836	pICH47751	Actin2pΩ ⁺ ::TALE without AD
pAGT900	pICH47742	EBE2Tp::GFP
pAGT901	pICH47751	Actin2pΩ ⁺ : TALE
pAGT988	pICH47751	Actin2pΩ ⁺ ::TALE:MYC2(JID Domain)
pAGT989	pICH47751	Actin2pΩ ⁺ ::TALE: MYC2(N-ter and JID Domain)
pAGT990	pICH47751	Actin2pΩ ⁺ : TALE: MYC2(N-ter; JID and TAD Domain)
pAGT991	pICH47751	Actin2pΩ ⁺ ::TALE: MYC2(JID and TAD Domain)
pAGT992	pICH47751	Actin2pΩ ⁺ : TALE: MYC2(TAD Domain)
pAGT993	pICH47751	Actin2pΩ ⁺ : TALE: MYC2 (MYC2 without bHLH)

pAGT994	pICH47751	Actin2pΩ+:: TALE: MYC2(full length)
pAGT995	pICH47751	Actin2pΩ+::TALE-AD:MYC2(JID Domain)
pAGT996	pICH47751	Actin2pΩ+::TALE-AD: MYC2(N-ter and JID Domain)
pAGT997	pICH47751	Actin2pΩ+:: TALE-AD: MYC2(N-ter; JID and TAD Domain)
pAGT998	pICH47751	Actin2pΩ+::TALE-AD: MYC2(JID and TAD Domain)
pAGT999	pICH47751	Actin2pΩ+:: TALE-AD: MYC2(TAD Domain)
pAGT1000	pICH47751	Actin2pΩ+:: TALE-AD: MYC2 (MYC2 without bHLH)
pAGT1001	pICH47751	Actin2pΩ+:: TALE-AD: MYC2(full length)
pAGT1016	pICH47751	Actin2pΩ+::MYC2(MYC2 without bHLH):TALE
pAGT1017	pICH47751	Actin2pΩ+::MYC2(MYC2 without bHLH):TALE-AD
pAGT1018	pICH47751	Actin2pΩ+::MYC2(full length):TALE
pAGT1019	pICH47751	Actin2pΩ+::MYC2(J full length):TALE-AD
pAGT1079	pICH47751	Actin2pΩ+::TALE-AD
pAGH74	pICH47742	(Tetramer of JARE-JMT-35sM) p:: HTA6-GFP::mast
pAGH75	pICH47742	(Octamer of JARE-JMT-35sM)p::HTA6-GFP::mast
pAGH76	pICH47742	Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) -35sM)p::HTA6-GFP::mast
pAGH77	pICH47742	(Tetramer of JARE-JMT-35sM) p:: HTA6-GFP::ocst
pAGH78	pICH47742	(Octamer of JARE-JMT-35sM)p::HTA6-GFP::ocst
pAGH79	pICH47742	Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) -35sM)p::HTA6-GFP::ocst
pAGH111	pICH47781	LB-(Tetramer of JARE-JMT:35sM)p::TALE-RB
pAGH112	pICH47781	LB-(Octamer of JARE-JMT:35sM)p::TALE-RB
pAGH113	pICH47781	LB-(Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) :35sM)p::TALE-RB
pAGH114	pICH47852	RB-(Tetramer of JARE-JMT:35sM)p::TALE-LB
pAGH115	pICH47852	RB-(Octamer of JARE-JMT:35sM)p::TALE-LB
pAGH116	pICH47852	RB-(Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) :35sM)p::TALE-LB
pAGH301	pICH47742	EBE2Tp::JAZ1
pAGH302	pICH47742	Short 35spΩ::JAZ1
pAGH303	pICH47742	Short 35spΩ::GFP
pAGH324	pICH47742	Short 35spΩ::GUS

Table 10. List of level 2 vectors. Resistance: Kan

Construct Name/ID	Destination vector	Description
pAGT902	pAGM4673	Nosp::BAR-- EBE2Tp::GFP
pAGT903	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2p::MYC2(N-ter; JID and TAD Domain):TALE
pAGT904	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2p::TALE
pAGT1148	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(JID Domain):TALE
pAGT1149	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(JID Domain):TALE-AD
pAGT1150	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(N-ter and JID Domain):TALE
pAGT1151	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(N-ter and JID Domain):TALE-AD
pAGT1152	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(N-ter; JID and TAD Domain):TALE-AD
pAGT1153	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(TAD Domain):TALE
pAGT1154	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(TAD Domain):TALE-AD
pAGT1155	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(JID and TAD Domain):TALE
pAGT1156	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(JID and TAD Domain):TALE-AD
pAGT1157	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(MYC2 without bHLH):TALE
pAGT1158	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(MYC2 without bHLH):TALE-AD
pAGT1159	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(full length):TALE
pAGT1160	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ::MYC2(full length):TALE
pAGT1161	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE: MYC2(JID Domain)
pAGT1162	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE-AD: MYC2(JID Domain)
pAGT1163	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE: MYC2(N-ter and JID Domain)
pAGT1164	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE-AD: MYC2(N-ter and JID Domain)

pAGT1165	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE: MYC2(N-ter; JID and TAD Domain)
pAGT1166	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE-AD: MYC2(N-ter; JID and TAD Domain)
pAGT1167	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE: MYC2(JID and TAD Domain)
pAGT1168	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE-AD: MYC2(JID and TAD Domain)
pAGT1169	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE: MYC2(TAD Domain)
pAGT1170	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE-AD: MYC2(TAD Domain)
pAGT1171	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE: MYC2(MYC2 without bHLH):
pAGT1172	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE-AD: MYC2(MYC2 without bHLH)
pAGT1173	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE: MYC2(full length)
pAGT1174	pAGM4673	Nosp::BAR-- EBE2Tp::GFP--Actin2pΩ:: TALE-AD: MYC2(full length)
pAGH80	pAGM4673	Nosp::BAR-- (Tetramer of JARE-JMT-35sM) p:: HTA6-GFP::mast
pAGH81	pAGM4673	Nosp::BAR-- (Octamer of JARE-JMT-35sM)p::HTA6-GFP::mast
pAGH82	pAGM4673	Nosp::BAR--(Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) -35sM)p::HTA6-GFP::mast
pAGH83	pAGM4673	Nosp::BAR-- (Tetramer of JAHRE-JMT-35sM) p:: HTA6-GFP::ocst
pAGH84	pAGM4673	Nosp::BAR-- (Octamer of JAHRE-JMT-35sM)p::HTA6-GFP::ocst
pAGH85	pAGM4673	Nosp::BAR--(Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) -35sM)p::HTA6-GFP::ocst
pAGH144	pAGM4723	LB-Nosp:: BAR-- EBE2Tp::GFP --(Tetramer of JARE-JMT-35sM) p:: TALE-RB
pAGH145	pAGM4723	LB-Nosp:: BAR-- EBE2Tp::GFP-- (Octamer of JARE-JMT-35sM)p:: TALE-RB
pAGH146	pAGM4723	LB-Nosp::BAR-- EBE2Tp::GFP --(Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) -35sM)p:: TALE-RB
pAGH147	pAGM4723	LB-Nosp:: BAR-- EBE2Tp::GFP --TALE::(Tetramer of JARE-JMT-35sM) p-RB
pAGH148	pAGM4723	LB-Nosp:: BAR-- EBE2Tp::GFP--TALE::(Octamer of JARE-JMT-35sM)p-RB

pAGH149	pAGM4723	LB-Nosp::BAR-- EBE2Tp::GFP--TALE::(Octamer of JARE(4xJARE-JMT+4xJARE-JMT _(complement) -35sM)p-RB
---------	----------	---

Table 11. Sequences of oligonucleotides for PCR. The melting temperature (T_m) is based on a NEB T_m calculator.

Primer name	Sequence(5'-3')	Length (bp)	T _m [°C]	Description
omb1	TTGAAGACATTACTGTATAAGA GCTCATTTTTACAACAATTA	42	66	Fw PCR-primer for cloning of Tobacco mosaic virus (TMV) Ω enhancer Yellow =BpiI recognition site
omb5	TTGAAGACATATGGTATCGATA ATTGTAATGTAATTGTAATG	43	66	Rev PCR-primer for cloning of Tobacco mosaic virus (TMV) Ω enhancer Yellow =BpiI recognition site
myc2for1	TTTGAAGACATCCATGTATGATT TCTCCGGCGCCTC	36	75	Yellow =BpiI recognition site
myc2for2	TTTGAAGACATCCATGACTGAT TACCGGTACAACC	36	73	Yellow =BpiI recognition site ATG=start codon
myc2for3	TTTGAAGACATCCATGGTGACG GATACGGAATGGTTTTTC	40	74	Yellow =BpiI recognition site
myc2for5	TTTGGTCTCAACATCCATGACT GATTACCGGTACAACC	39	76	Yellow =BsaI recognition site ATG=start codon
myc2for6	TTTGGTCTCAACATTCGTGTTCT TCGATTAACCGGTGGGGA	42	77	Yellow =BsaI recognition site
myc2for7	TTTGGTCTCTACATGGAGCGCTI GCTGCCGCA TATGATTCTCCGG GCCTCC	53	85	Yellow =BsaI recognition site Red = linker between two fused proteins
myc2for8	TTTGGTCTCTACATGGAGCGCTI GCTGCCGCA ATGACTGATTACC GGCTACAACC	55	84	Yellow =BsaI recognition site Red = linker between two fused proteins ATG=start codon
myc2for9	TTTGGTCTCTACATGGAGCGCTI GCTGCCGCA GTGACGGATACGG AATGGTTTTTC	56	83	Yellow =BsaI recognition site Red = linker between two fused proteins
myc2for10	TTTGGTCTCATCGTGTCTTCGA TTAAACCGGTGGGG	37	76	Yellow =BsaI recognition site
myc2for11	TTTGGTCTCTACATGTTCGCTI GCTGCCGCA TATGATTCTCCGGC GCCTCC	52	84	Yellow =BsaI recognition site Red = linker between two fused proteins
myc2for12	TTTGGTCTCTACATGTTCGCTI GCTGCCGCA ATGACTGATTACCG GCTACAACC	54	82	Yellow =BsaI recognition site Red = linker between two fused proteins ATG=start codon

myc2rev1	TTTGAAGACTACATTGCGGCAG CAAGCTCCTCATCAACAGCGTC ATCC	48	81	Yellow=Bpil recognition site Red= linker between two fused proteins
myc2rev2	TTTGAAGACTACATTGCGGCAG CAAGTGCAAACGCTTTACCAGC TAATC	49	79	Yellow=Bpil recognition site Red= linker between two fused proteins
myc2rev3	TTTGAAGACTACATTGCGGCAG CAAGTCTTTGGTTTAGTTTCTCG CGTCTT	51	79	Yellow=Bpil recognition site Red= linker between two fused proteins
myc2rev4	TTTGGTCTCTACAAACGAGAC GACATATCTCCTCC	36	74	Yellow=Bsal recognition site Red= T by C change
myc2rev5	TTTGGTCTCTACAACATTGCGGC AGCAAGACCGATTTTGAATC AAACTTGCTCT	56	79	Yellow=Bsal recognition site Red= linker between two fused proteins
myc2rev6	TTTGGTCTCTACAAAAGCTTACT CCTCATCAACAGCGTCATCC	43	76	Yellow=Bsal recognition site
myc2rev7	TTTGGTCTCTACAAAAGCTTATG CAAACGCTTTACCAGCTAATC	44	74	Yellow=Bsal recognition site
myc2rev8	TTTGGTCTCTACAAAAGCTTATC TTTGGTTAGTTTCTCGCGTCTT	46	74	Yellow=Bsal recognition site
myc2rev9	TTTGGTCTCTACGAGACGACA TATCTCCTCC	32	73	Yellow=Bsal recognition site Red= T by C change
myc2rev10	TTTGGTCTCTACAAAAGCTTAAC CGATTTTGAATCAAACCTTGCT C	47	72	Yellow=Bsal recognition site
80373for1	TTTGGTCTCAACATTCGGCCCGA CCCTGCCTTA	33	78	Fw PCR-primer for cloning of truncated C-ter of TALE Yellow=Bsal recognition site
80373rev1	TTTGGTCTCAACAAAAGCTTAA CCGATTAAGGCCGAGCATGAG GT	46	79	Rev PCR-primer for cloning of truncated C-ter of TALE Yellow=Bsal recognition site
tetramer for1	TTTGGTCTCAGGAGATTATTAGT ATAACTCCTGAAAATGAAAAT TATTAGTATAACTC	59	69	Fw PCR-primer for cloning of JARE Yellow=Bsal recognition site
tetramer for2	TTTGGTCTCAATTATTAGTATAA CTCCTGAAAATGAAAATTAAT AGTATAACTC	55	67	Fw PCR-primer for cloning of JARE Yellow=Bsal recognition site
tetramer rev1	TTTGGTCTCATTTCATTTTCAG GAGTTATACTAATAATTTTCAT TTTCAG	52	68	Rev PCR-primer for cloning of JARE Yellow=Bsal recognition site
tetramer rev2	TTTGGTCTCATAATTTTCATTT TCAGGAGTTATACTAATAATTT TCATTTTCAG	56	68	Rev PCR-primer for cloning of JARE Yellow=Bsal recognition site
35sMP for1	TTTGGTCTCAAAAAGTCGACCG CAAGACCCTTC	33	75	Fw PCR-primer for cloning of 35s minimal promoter Yellow=Bsal recognition site

35sMP for2	TTTGGTCTCATTGTCGACCGC AAGACCCTTC	33	75	Fw PCR-primer for cloning of 35s minimal promoter Yellow =Bsal recognition site
35sMP rev1	TTTGGTCTCACATTGGTGGCCAC TCGAGCG	30	78	Rev PCR-primer for cloning of 35s minimal promoter Yellow =Bsal recognition site
HTA6-GFPfor1	TTTGGTCTCAAATGGAATCCAC CGGAAAAGTGAAG	35	73	Fw PCR-primer for cloning of HTA6:GFP fragment1 Yellow =Bsal recognition site ATG =start codon
HTA6-GFPfor2	TTTGGTCTCACAAATCCAGTGG GAAGAATCACTCG	36	75	Fw PCR-primer for cloning of HTA6:GFP fragment2 Yellow =Bsal recognition site
HTA6-GFPprev1	TTTGGTCTCAATTGAGACCGGC TTTCATCGATTTTC	36	72	Rev PCR-primer for cloning of HTA6:GFP fragment1 Yellow =Bsal recognition site Red = T by A change
HTA6-GFPprev2	TTTGGTCTCAAAGCCTAGAGGA TCCCCTGTACAGCTC	38	77	Rev PCR-primer for cloning of HTA6:GFP fragment2 Yellow =Bsal recognition site
JARE+35sMPfw	TTTGAAGACATGGAGATTATTA GTATAACTCTGAAAATG	40	67	Fw PCR-primer for cloning of JARE:35s MP promoter elements Yellow =Bpil recognition site
JARE+35sMPprev	TTTGAAGACTACATTGGTGGCC ACTCGAG	29	72	Rev PCR-primer for cloning of JARE:35s MP promoter elements Yellow =Bpil recognition site
For-JAZ1-Y2H	TTTGGTCTCAAATGTCGAGTTCT ATGGAATGTTCTGAGTTCGTCG	45	75	Fw PCR-primer for cloning of JAZ1 Yellow =Bsal recognition site ATG =start codon
Rev-JAZ1-Y2H	TTTGGTCTCAAAGCTCATATTTTC AGCTGCTAAACCGAGCC	40	76	Rev PCR-primer for cloning of JAZ1 Yellow =Bsal recognition site
For1-MYC2-Y2H	TTTGGTCTCAACATAATGACTG ATTACCGCTACAACCAACGAT G	45	75	Fw PCR-primer for cloning of MYC2 fragment1 Yellow =Bsal recognition site ATG =start codon
Rev1-MYC2-Y2H	TTTGGTCTCTACAAACGAGAC GACATATCTCTCC	36	74	Rev PCR-primer for cloning of MYC2 fragment1 Yellow =Bsal recognition site Red = T by C change
For2-MYC2-Y2H	TTTGGTCTCAACATTCGTGTTCT TCGATTAAACCGGTGGGGA	42	77	Fw PCR-primer for cloning of MYC2 fragment2 Yellow =Bsal recognition site

Rev2-MYC2-Y2H	TTTGGTCTCTACAAAAGCTTAAC CGATTTTTGAAATCAAACCTTGCT CTG	49	73	Rev PCR-primer for cloning of MYC2 fragment1 Yellow=Bsal recognition site
attB1-JAZ1-attB2 fw	GGGGACAAGTTTGTACAAAAA GCAGGCTCGATGTCGAGTTCTA TGGAAATGTTCTGAGTTC	61	79	Fw PCR-primer for cloning of JAZ1 for Gateway cloning Yellow=attB1 recombination site ATG=start codon
attB1-JAZ1-attB2 rev	GGGGACCACTTTGTACAAGAAA GCTGGGTC TCATATTTCACTGCTGC TAAACCGAGCC	56	81	Rev PCR-primer for cloning of JAZ1 for Gateway cloning Yellow=attB2 recombination site
KpnI-35s-XmaI fw	CGGGGTACC GGAGGTCAACATG GTG	25	76	Fw PCR-primer for cloning of short 35s promoter with TMV Ω + Yellow=KpnI restriction site
KpnI-35s-XmaI rev	TTCCCCCCCGGGAATTGTAAAT GTAATTGTAATGTTGTTGTTG	44	74	Rev PCR-primer for cloning of short 35s promoter with TMV Ω + Yellow=XmaI restriction site
M13 uni (-21)	TGTA AACGACGGCCAGT	18	64	Fw primer for sequencing
M13 rev (-29)	CAGGAAACAGCTATGACC	18	60	Rev primer for sequencing
moclof	AGCGAGGAAGCGGAAGAGCG	20	73	Fw primer for sequencing of Level 0 (pICH41308...) and Level -1 universal (pAGM1311)
moclor	GCCACCTGACGTCTAAGAAACC	22	68	Rev primer for sequencing of Level 0 (pICH41308...) and Level -1 universal (pAGM1311)

7 Acknowledgments

I would like to express my deep gratitude to all people who supported me during this work. Foremost I want say thanks to my supervisor **Prof. Dr. Bettina Hause**, for the interesting topic she offered me, for her advices, and her valuable suggestions. With pleasure I have worked on this interesting project and the time as a member of the AG Hause will always be a very good memory.

Furthermore I want to thank my co-supervisor **Prof. Dr. Alain Tissier** for his excellent ideas and advices. Thanks for the very pleasant working atmosphere in his department. Thanks **Dr. Sylvestre Marillonnet** and **Ramona Grützner** for their help in Golden Gate cloning system. Dr. Marillonnet helped me a lot in designing of cloning process and explaining principles of the Golden Gate cloning. Ramona helped me in supplying me the vectors and bacterial strains. Thanks to them.

I further want to thank **Dr. Tom Schreiber** for his ideas for optimizing the approach using in my work and his helpful discussions in my work. Thanks to **Petra Schäfer** for her help in infiltration experiment.

A special thanks is directed to the team of our lab. Thanks to **Carolin Alfs**, my Master student, now a Ph.D.student in our institute for her work which is also included in this thesis and her helpful discussion. Thanks to **Hagen Stellmach** for his help in using epifluorescence microscope. Thanks to **Ulrike Huth** for her help in preparation of mediums and buffers. They helped me solve many problems in the lab with their long practical experience. It was a great pleasure to have **Kati Mielke**, **Dorothee Dreher**, **Ramona Schubert**, **Heena Yadav**, and **Chandan Chiniga Kemparaju** as colleagues. I had a lot fun working with you. As an overseas student here, I felt warm and sweet like in a big family due to their warm heart and happiness they give me.

Thanks **Chinese Scholarship Council** for funding me. Without funding from CSC, Working on this project would not have been possible.

Thanks my family, I give my deepest gratitude to my **parents** and my wife **Qiong Yu** who always supported me in my life. I would also say sorry to them since I cannot accompany them in these four years.

8 Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende wissenschaftliche Arbeit selbstständig und ohne fremde Hilfe angefertigt habe. Ich erkläre weiterhin, dass ich keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe und die den Werken wörtliche und inhaltlich entnommene Stellen als solche kenntlich gemacht habe.

Mit dieser Arbeit bewerbe ich mich erstmals um die Erlangung des Doktorgrades. Diese Arbeit wurde an keiner anderen Fakultät oder Universität zur Begutachtung eingereicht.

Halle (Saale), den 17. Januar 2018

Yulong Li

Curriculum Vitae

Yulong Li

Mail liyulongcas@163.com

Phone +49 (0) 176 374605984

Address Ernst-Abbe-Str. 33 06122 Halle, Germany



Personal Details

Born 9th May 1989, in Zoucheng, Shandong, China

Citizenship Chinese

Marital status married

Gender Male

Education

Since 09/2013	Doctoral studies at the Leibniz- Institute for Plant Biochemistry in Halle (Saale) in the group of Prof. Bettina Hause: Jasmonate function & Mycorrhiza with the topic “Development and use of molecular reporters for cell-specific visualization of jasmonates”
09/2010-06/2013	Major in Biotechnology, Master studies direction: physiology and molecular biology of plant stress, Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences; Degree: M. Sc.;
09/2006-06/2010	Bachelor studies of biology science, Shandong University of Technology; Degree: B. Sc.
09/2003-06/2006	Zoucheng first senior high school
09/2000-06/2003	Guoli junior high school
09/1994-06/2000	Gaolicun elementary school

Scientific Activities

- 02/2017 Poster: “New tools for cell-specific detection of jasmonates” at the 30th Tagung Molekularbiologie der Pflanzen in Dabringhausen, Germany
- 07/2016 Talk: “Development and use of a molecular reporter for cell-specific visualization of jasmonates” at the 12th Plant Science Student Conference (PSSC) in Gatersleben, Germany

Publications

Dongqing Shi, Yuan Zhang, Jihu Ma, **Yulong Li**, Jin Xu. (2013) Identification of zinc deficiency-responsive MicroRNAs in *Brassica juncea* roots by small RNA sequencing. *Journal of Integrative Agriculture*, 12(11).

Yulong Li, Yuan Zhang, Dongqing Shi, Xiaojing Liu, Jun Qin, Qing Ge, Longhua Xu, Xiangliang Pan, Wei Li, Yiyong Zhu, Jin Xu. (2013). Spatial-temporal analysis of zinc homeostasis reveals the response mechanisms to acute zinc deficiency in *Sorghum bicolor*. *New Phytologist*, 200(4): 1102-1115.

Jin Xu, **Yulong Li**, Jianhang Sun, Liguang Du, Yuan Zhang, Qiong Yu, Xiaojing Liu. (2012) Comparative physiological and proteomic response to abrupt low temperature stress between two winter wheat cultivars differing in low temperature tolerance. *Plant Biology*, doi:10.1111/j.1438-8677.2012.00639.x

Jin Xu, Yiyong Zhu, Qing Ge, **Yulong Li**, Jianhang Sun, Yuan Zhang, Xiaojing Liu. (2012) Comparative physiological responses of *Solanum nigrum* and *Solanum torvum* to cadmium stress. *New Phytologist*, 2012, 196: 124-138.

Jin Xu, Hengxia Yin, **Yulong Li**, Xiaojing Liu. (2010) Nitric Oxide Is Associated with Long-Term Zinc Tolerance in *Solanum nigrum*. *Plant Physiology*, 154: 1319-1334

Yulong Li, Feng Gao, Jianhang Sun, Qiong Yu, Xiaoyu Hou, Xiaojing Liu, Jin Xu. (2012) Effects of Fe supplementation on *Solanum nigrum* seedling growth under Zn toxicity. *Chinese Journal of Eco-Agriculture*. 2012, DOI: 10.3724/SP.J.1011.2012.0

000