Transcriptional Regulation of the Jasmonate Signal Pathway in *Arabidopsis thaliana*

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Abstract

In response to a biologically active JA signal (JA-Ile), JAZ proteins bind to COI1 and are then degraded by ubiquitin and destroyed by the 26S proteasome. MYC2 is therefore relieved from the JAZ suppression and activates the transcription of JA responsive genes. The purpose of this research was to identify new components of the JA signal pathway.

JA-inducible transcription factors were investigated from publically available data. The expression of a bHLH transcription factor (At2g46510) was induced by MeJA and wounding treatments. This transcription factor, therefore, was named as Jasmonate Up-regulated Transcription factor (JUT). Gene expression analyses verified that *AtMYC2* and *JUT* were rapidly induced by MeJA and wounding, and that COI1 was required for the MeJA-induced expression of *JUT*. A T-DNA insertion mutant *jut* had a reduced responsiveness to MeJA-induced root growth inhibition and wound-induced shoot growth inhibition. This result indicated that JUT is required for the regulation of plant growth in response to JA. The MYC2 C-terminal deletion mutant *jin1*-1 had a reduced responsiveness to MeJA-induced root growth inhibition than that was *jut.* The double mutant *jin1*-1/*jut* had a reduced responsiveness to MeJA than *jut*, but an increased responsiveness to MeJA than *jin1*-1. This indicated that both MYC2 and JUT are required for MeJA-induced root growth inhibition. In agreement, lines with constitutive expression of either MYC2 or JUT displayed increased responsiveness to MeJA-induced growth inhibition compared to wild type Col-0. Additionally, *jin1*-1 and *jut* both flowered earlier than wild type. Moreover, lines with constitutive expression of either MYC2 or JUT flowered later than wild type. This indicated that MYC2 and JUT negatively regulate the flowering time. The results of microarray assays and qRT-PCR demonstrated that JUT co-operates in different ways with MYC2 to regulate the transcription of a group of JA responsive genes. Taken together, the results in this thesis establish that JUT functions as a novel transcription regulator in the JA signal pathway.

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Originality Statement

I certify that this PhD thesis is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at University of East Anglia or any other educational institution, except where due acknowledgement or reference is made in the thesis.

Any contribution made from other members of the joint research project, which I have worked with, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this PhD thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

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List of Abbreviations

ABA	Abscisic acid
cDNA	complementary DNA
dCTP	Deoxycytidine triphoshate
DEPC	diethypyrocarbonate
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide
dsDNA	double strand DNA
EDTA	Ethylenediaminetetraacetic acid
ET	ethylene
GA	Gibberellin acids
HCl	hydrochloric acid
JA	Jasmonic acid
JAs	Jasmonates
KAc	potassium acetate
KOH	potassium hydrate solution
LB	T-DNA left border primer
MeJA	Methyl jasmonate
$MgCl_2$	Magnesium chloride
MOPS	3-(N-morpholino) propanesulfonic acid
MS	Murashige & Skoog
NaAc	sodium acetate
NaCl	sodium chloride
NaOH	sodium hydroxide
OPDA	12-oxophyto-dienoic acid
RNA	Ribonucleic acid
SA	salicylic acid
SDS	sodium dodecyl sulphate
SE	standard error
ssDNA	salmon sperm DNA
Tris	tris(hydroxymethyl) amino methane
Tris-Cl	Tris Hydrochlordie
v/v	volume/volume
w/v	weight/volume

Chapter 1

General Introduction

1.1 Jasmonates Regulate Many Functions

The phytohormone jasmonic acid (JA) is biosynthesised from the fatty acid α-linolenic acid (α-LeA). The jasmonates, which include JA and its biologically active derivatives, regulate both plant development and defence (Avanci et al., 2010). For example, the growth of roots and shoots, the development of stamens and pollen, the processes of tendril coiling, fruit ripening and leaf senscence, are all regulated by jasmonates (Farmer et al., 2003; Baena-Gonzalez and Sheen, 2008; Balbi and Devoto, 2008).

Jasmonates also regulate plant responses to environmental stresses, such as salinity, dehydration and drought, as well as the biotic challenges from pathogens and insects (Devoto and Turner, 2003). These responses include growth inhibition, secondary product formation, and defence against pathogen and chewing insects. Wound-induced endogenous JA biosynthesis regulates plant systemic wounding responses (Koo and Howe, 2009). Additionally, wound-induced jasmonates negatively regulate mitosis and therefore repress plant growth (Zhang and Turner, 2008), and in this respect it may be relevant that jasmonates repress the activation of M-phase genes during the cell cycle (Pauwels et al., 2008). Jasmonates also participate in the regulatory process of sex determination of tassel in maize, which prevents the physical touching between female and male flowers and therefore positively regulates the biological selection of cross-fertilization (Browse, 2009a). Considering many functions of jasmonates, an understanding of the JA signal pathway at the molecular level may allow us to improve the crop field productivity and plant protection in the future (Browse and Howe, 2008; Browse, 2009b).

1.2 JA Biosynthesis

The process of JA biosynthesis occurs in two cellular compartments: the chloroplast and the peroxisome (**Figure 1.1**). The initial step of JA biosynthesis is from α-linolenic acid (α-LeA), which is released from the chloroplast membrane by phospholipase (PL) (Wasternack, 2007). An enzyme of linoleate oxygen oxidoreductase (LOX), belongs to the lipoxygenase family and catalyses the first step of oxylipin biosynthesis by adding an oxygen molecule the position C-13 of linolenic acid (Holkova et al., 2009). Allene oxide synthase (AOS) and allene oxide cyclase (AOC) catalyse the conversion of linear allene oxide 13S-hydroperoxy-(9Z, 11E, 15Z)-octadeatrienoic acid (13-HPOT) into cis-(+)-12-oxophytodienoic acid (OPDA) in the chloroplast (Turner et al., 2002; Devoto and Turner, 2003; Wasternack, 2007). In parallel, the enzymes LOX, AOS and AOC together catalyse hexadecatrienonic acid (C16:3) to form dinor-OPDA (dnOPDA). The transportation of OPDA and dnOPDA from the chloroplast into peroxisome is dependent on a transporter COMATOSE (CTS1), or an ion trapping system (Theodoulou et al., 2005). In the peroxisome, OPDA reductase 3 (OPR3) reduces the OPDA and dnOPDA to form 12-oxophytoenic acid (OPC-8) and 12-oxophytoenic acid (OPC-6), respectively. OPDA, dnOPDA, OPC-8 and OPC6 are activated by the acyl-coenzyme A synthetases to form CoA esters, so that the carboxylic acid side chains can be shorted by the three or two rounds of β-oxidation by acyl-CoA oxidase (ACX), a multifunctional protein (MFP), and L-3-ketoacyl CoA thiolase (KAT) (Schneider et al., 2005; Kienow et al., 2008). Jasmonoyl-CoA, the final product of the β-oxidation reactions, is cleaved by thioesterase (TE) to form cis-7-iso-jasmonic acid ((+)-7-iso-JA), which converts spontaneously to the stable form of (-)-JA.

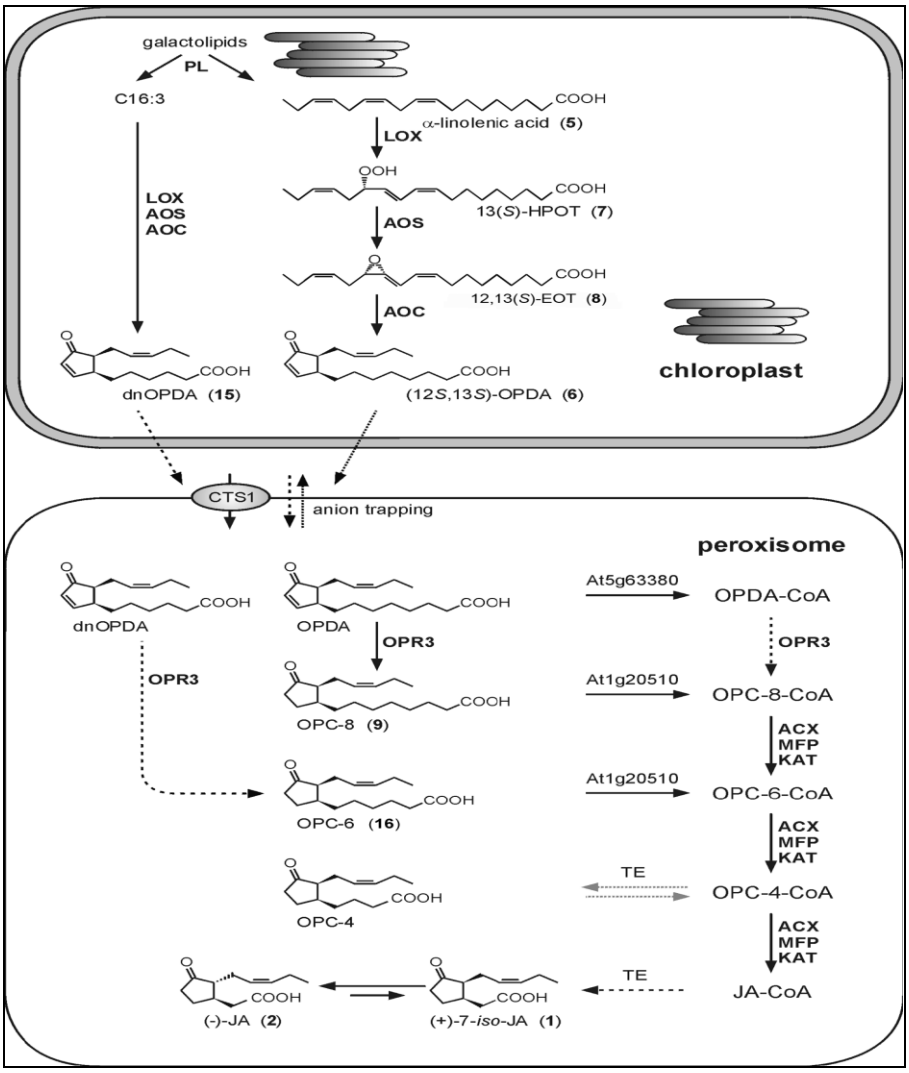


Figure 1.1: **JA biosynthesis in the chloroplast and the peroxisome** (Wasternack and Kombrink, 2010). PL, phospholipase; LOX, linoleate oxygen oxidoreductase; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPDA, cis-(+)-12-oxophytodienoic acid; dnOPDA, dinor-OPDA; OPR3, OPDA reductase 3; OPC-8, 12-oxophytoenic acid (C-8); TE, thioesterase; At5g63380 encodes a peroxisomal protein in the activation of fatty acids through esterification with CoA; At1g20510 encodes OPC-8:CoA ligase (OPCL1). Dashed arrows show the putative reactions.

Chapter 1: General Introduction

Knowledge of the JA biosynthesis pathway has been advanced by the identification and characterisation of mutants selected for their inability to display what has emerged as key jasmonate responses. **Table 1.1** displays a number of Arabidopsis mutants defective in JA biosynthesis. For instance, the triple mutant *fad3*-2*fad7*-1*fad8* lacks three fatty acid desaturases required to produce linolenic acid, the precursor for OPDA, dnOPDA and JA. The endoplasmic reticulum localised FAD3 (Browse et al., 1993) and the chloroplast localised FAD7 and FAD8 (McConn et al., 1994) are involved in the biosynthesis of 16:3 and 18:3 fatty acids, and the triple mutant *fad3*-2*fad7*-1*fad8*, lacks 16:3 and 18:3 fatty acids, and is deficient for JA biosynthesis. The mutant *dad1* (defective anther dehiscence 1) has a mutation in the chloroplastic phospholipase A1 and displays a male sterile phenotype (Ishiguro et al., 2001). This gene is expressed only during flower development in Arabidopsis, and does not contribute to wound-induced JA synthesis. The mutants *dde2* (delayed dehiscence 2) and *aos* have mutations in the AOS gene, and are deficient in JA biosynthesis and are also male sterile. (Von Malek et al., 2002; Park et al., 2002). In another male sterile mutant *opr3*, the conversion from OPDA into OPC is deficient, identifying the key function of OPR3 in JA biosynthesis (Stintzi and Browse, 2000).

Table 1.1: Arabidopsis JA biosynthesis mutants. (This table was edited from Devoto and Turner, 2005).

JA biosynthesis mutant	Gene	Screen	Reference
fad3-2*fad7*-1*fad8*	Fatty acid desaturases *FAD3, 7* and *8*	Altered fatty acid composition of single leaf by Gas Chromatograpy	(Browse et al., 1993)
dad1	chloroplastic phospholipase A1(*DAD1*)	Altered male fertility	(Ishiguro et al., 2001)
dde2	Allene oxide synthase (*AOS*)	Altered male fertility	(von Malek et al., 2002)
aos	CYP74A (cytochrome P450-allene oxide synthase (*AOS*)	T-DNA insertion (TJ1180)	(Park et al., 2002)
opr3	12-oxophytodienoic acid reductase (*OPR3*)	Altered male fertility	(Stintzi and Browse, 2000)

1.3 JA-Ile and MeJA

1.3.1 The Biosynthesis of JA-Ile and MeJA

JA methylation by JA carboxyl methyltransferase (JMT) produces methyl jasmonate (MeJA). MeJA esterase (MJE) in turn converts MeJA back to JA. The reversible conversion between JA and Jasmonoyl-isoleucine (JA-Ile) is catalysed by a jasmonate-amino acid synthetase (JAR1). **Figure 1.2** illustrates the structures and biosynthetic reactions of OPDA, JA, MeJA and JA-Ile.

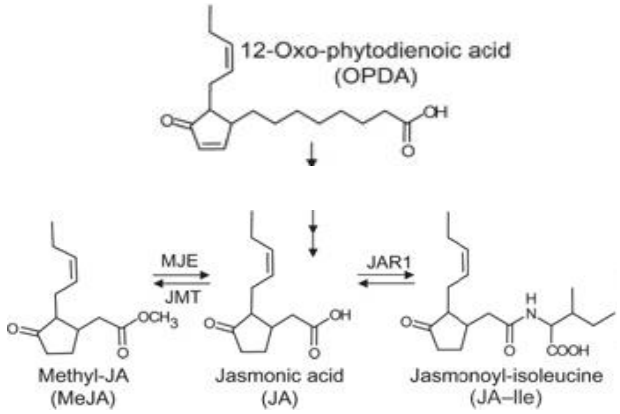


Figure 1.2: Biosynthetic reactions between OPDA, JA, MeJA and JA-Ile (Thines et al., 2007). JMT, JA carboxyl methyltransferase; MJE, MeJA esterase; JAR1, jasmonate resistant 1.

1.3.2 JA-Ile and Its Biological Activity

Jasmonoyl-isoleucine (JA-Ile) is the conjugate between JA and isoleucine. JA-Ile promotes systemic JA biosynthesis and regulates the expression of wound- and JA- responsive genes (Koo et al., 2009). As indicated in **Figure 1.2**, the conjugation of jasmonate and isoleucine is catalysed by a jasmonate-amino acid synthetase, which is encoded by the jasmonate resistant locus 1 (JAR1) (Staswick et al., 1992; Fujiki et al., 2001; Staswick et al., 2002). This locus was defined by mapping and cloning the jasmonate-insensitive mutant *jar1*. JAR1 plays an important role in JA-Ile accumulation (Staswick et al., 2002). The molecular level of JA-Ile was 60-fold higher than other jasmonoyl amino-acid conjugates in leaf

tissues. However, the JA-Ile level was dramatically reduced by 90% in the mutant *jar1* in comparison with the wild type background. In wild type plants, the transcription of *JAR1* and the accumulation of JA-Ile were increased by wounding (Suza and Staswick, 2008). Stereo-chemical and bioactivity analyses revealed that the epimer of (+)-7-iso-Jasmonoyl-L-isoleucine ((+)-7-iso-JA-L-Ile) expresses the biological activity to promote the JA signal pathway (Fonseca et al., 2009b).

1.3.3 MeJA and Its Biological Activity

JA methylation by JMT produces MeJA as shown in **Figure 1.2**. Exogenous application of MeJA significantly reversed susceptibility to root infection by *Pythium mastophorum*, in the triple mutant *fad3*-2/*fad7*-2/*fad8*. The description of *fad3*-2/*fad7*-2/*fad8* is shown in **Table 1.1**. This jasmonate deficient mutant also displayed 80% mortality after challenge by chewing insects. After applying MeJA, the mortality of this mutant was dramatically reduced to 12% (McConn et al., 1997). Presumably in these cases, MeJA is being converted to the biologically active JA-Ile. By contrast, in some cases JA signalling makes plants less resistant to certain pathogens. For example, the tomato mutant *def1* is defective in jasmonate biosynthesis but has enhanced resistance to *Alternaria alternata*, and application of MeJA to this mutant makes it more susceptible to the pathogen (Egusa et al., 2009). Presumably, here the MeJA is converted to the biologically active form, JA-Ile, and the JA signal pathway antagonises salicylic acid dependent defences.

MeJA is reported to activate a range of seemingly unrelated responses in plants. For example, MeJA initiates the process of plant programmed death (PCD) by affecting the movement and distribution of mitochondria, and inducing the distortion in chloroplast morphology. Reactive oxygen species (ROS) production was initially localised in the mitochondria 1h after MeJA treatment, while the ROS was formed in the chloroplast 3h after MeJA treatment (Zhang and Xing, 2008). A recent research discovered that 100μM of exogenous MeJA stimulated flower development on the early flowering cultivars of rape (*Brassica napus L.*). Moreover, the transcription of a group of flower-specific genes was

altered after MeJA treatment (Pak et al., 2009). A microarray analysis of transcription profiles indicated that many systemic defensive genes, which containing the G-box motif in their promoter region, were significantly regulated by MeJA (Delessert et al., 2004).

1.4 JA Signal Pathway

The JA signal pathway includes JA perception and JA signal transduction. These important biological events are precisely programmed in plant cells and coordinated by both endogenous signals and environmental stresses (Shan et al., 2007; Wasternack, 2007).

The JA signal pathway is executed by the core components COI1, JAZs and the transcription factor MYC2 (Staswick, 2008; Chini et al., 2009a; Fonseca et al., 2009a; Frankowski et al., 2009; Gfeller et al., 2010). COI1 associates with SKP1, Cullin and other proteins to form the SCF^{COI1} complex, which forms the main receptor of the biologically active form of JA (Chini et al., 2009a). JAZ proteins apparently form hetro-dimers which act as repressors of the transcription activity of MYC2. In this state MYC2 is unable to activate the transcription of JA responsive genes. When JA-Ile is formed, it binds to COI1, and promotes the binding of JAZ proteins to the SCF^{COI1}. The JAZ proteins are then (predicted to be) ubiquitinated and destroyed in the 26S proteasome. MYC2 is thereby relieved from suppression and is free to activate transcriptional events in the JA signal pathway (Staswick, 2008; Chini et al., 2009a; Fonseca et al., 2009a; Frankowski et al., 2009; Gfeller et al., 2010). **Figure 1.3** illustrates a model of the JA signal pathway (edited from Chini et al., 2009a). COI1, JAZs, MYC2 and JA responsive genes are introduced in this section, respectively. **Table 1.2** gives Arabidopsis JA perception and signal transduction mutants that have played key roles in deciphering the JA signal pathway.

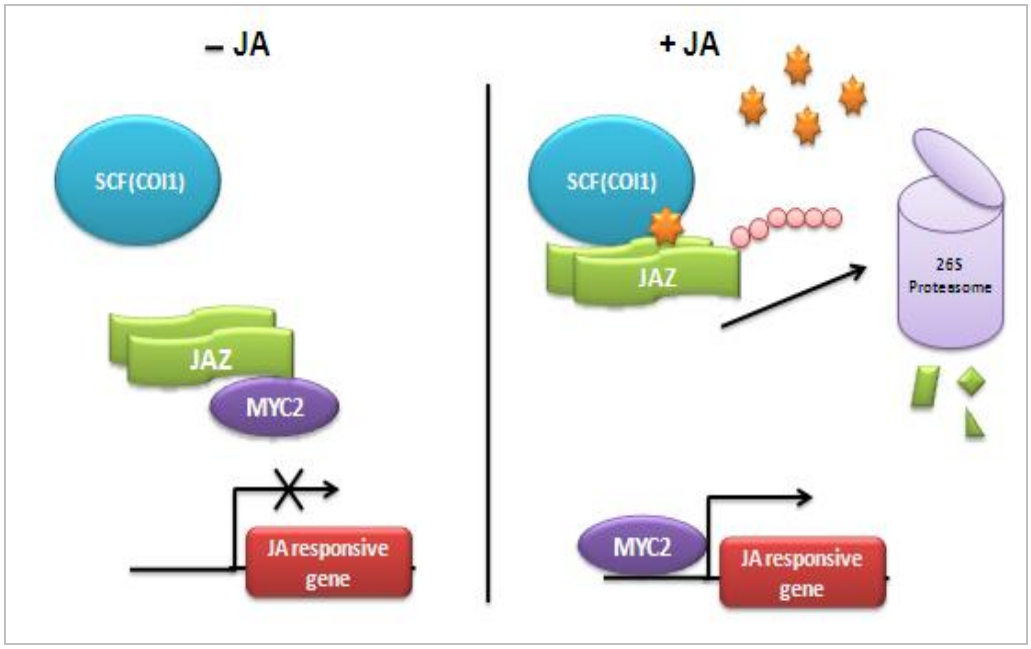


Figure 1.3: A model of the JA signal pathway.

JA-Ile is shown in orange, and ubiquitin is shown in pink.

Table 1.2: JA perception and signal transduction mutants. (This table is edited from Devoto and Turner, 2005).

Mutant	Gene	Screen	Reference
coi1-1	*COI1*-Fbox-LRR	Insensitive to coronatine	(Feys et al., 1994; Xie et al., 1998)
coi1-16	*COI1*-Fbox-LRR	Lacks *pVSP1-luc* transgene expression in presence of JA	(Ellis and Turner. 2002)
coi1-20	*COI1*-Fbox-LRR	Enhanced resistance to *Pseudomons.syringae*	(Kloek et al., 2001)
jai/jin1	*AtMYC2*-nuclear localized bHLHzip transcription factor	Insensitive to MeJA	(Lorenzo et al., 2004)
jin1-9	*AtMYC2*-nuclear localized bHLHzip transcription factor	Insensitive to MeJA	(Dombrecht et al.,2007)
jai3-1	*JAZ3*	Insensitive to MeJA	(Lorenzo et al., 2004)

1.4.1 COI1

Many F-box proteins modulate different hormone signal-perception and signal-transduction pathways (Santner et al., 2009). COI1 is an F-box protein and contains sixteen leucine-rich repeat domains (Xie et al., 1998). On the basis of a yeast-two hybrid library screen, the interaction between COI1 and homologues of SKP1 was identified. Western blot analysis showed that SKP1 and CULLIN were associated in a protein complex forming the SCF^{COI1} -E3-type ubiquitin ligase (Devoto et al., 2002). COI1 is the jasmonate receptor, responsible for transducing the JA signal (Thines et al, 2007) to activate gene expression in the JA signal pathway (Devoto and Turner, 2003).

1.4.1.1 Discovery of COI1

The molecular functions of COI1 have been defined by the properties of the *coronatine insensitive 1* (*coi1*) mutant, which is male sterile and insensitive to growth inhibition by the bacterial toxin coronatine that is a structural and functional homologue of JA. A screen of mutagenised Col-gl seeds for insensitivity to growth inhibition by the toxin coronatine led to the isolation of the *coi1*-1 mutant, which was found to be insensitive also to MeJA (Feys et al., 1994). Vegetative storage protein 1 (*VSP1*), thionin 2.1 (*thi2.1),* and plant defensin 1.2 (*PDF1.2*) were not expressed in *coi1*-1. Another screen of mutagenised Col-gl seeds carrying a JA responsive reporter *VSP1*::luciferase leads to the identification of the *coi1-16* mutant (Ellis and Turner, 2002).

1.4.1.2 COI1-dependent Plant Defence and Development

COI1 is essential for plant defence and development. The phytotoxin coronatine insensitive mutant *coi1*-20 was originally isolated because it displayed high resistance to bacterial pathogen *Pseudomonas syringae* DC3000. This resistance was due to up regulation of the salicylic acid (SA) dependent signal pathway, which is suppressed by coronatine and its structural homologue, jasmonate (Barbara N . Kunkel1 2000; Kloek et al., 2001).

The mutant *coi1*-1 is male sterile (Fays et al., 1994) but the mutant *coi1*-16 is conditionally fertile under 16°C (Ellis and Turner, 2002). COI1 was required for JA-inducible anthocyanin accumulation (Fays et al., 1994) and the expression of the anthocyanin biosynthesis gene for dihydroflavonol reductase (Chen et al., 2007). A flower-specific proteomics profile analysis discovered that sixteen proteins differentially expressed in the wild type and the mutant *coi1*-1. The gene AtGSTF9, which encodes a glutathione transferase, was significantly reduced in the flower of *coi1*-1 in comparison with wild type. (Chua et al., 2010).

1.4.1.3 COI1-dependent Regulation on JA Responsive Genes

COI1 regulates the expression of a considerable number of genes in the JA signal pathways. According to gene transcription profiling studies, about 84% of 212 JA-induced genes and 44% of 153 wound-induced genes are COI1-dependent. On the other hand, about 53% of 104 JA-suppressed genes and 46% of 83 wound-suppressed genes are repressed by COI1 (Devoto et al., 2005). So far, at least fifteen COI1-dependent transcription factors have been identified. Most of these are also JA responsive genes and have significant gene expression changes after wounding. For instance, the JA & wounding responsive and COI1-dependent transcription factors WRKY18 (At4g31800), DREB (At1g74930) and a zinc finger protein (At3g53600) function as positive transcriptional regulators in the JA signal pathway (Wang et al., 2008). These findings suggest that the COI1-dependent transcription factors might be potential regulators that reprogram the transcriptional events in the JA signal pathway.

1.4.1.4 JA Receptor, COI1

The first *COI1* mutation was reported in 1994 (Feys et al, 1994), the gene was cloned in 1998 (Xie et al, 1998), and in 2002 it was predicted that COI1 was the JA receptor (Turner et al, 2002). In 2007, it was demonstrated that JA-Ile initiates the interaction between COI1 and JAZ1 (Thines et al., 2007). Finally in 2009, it was demonstrated that COI1 directly binds to JA-Ile/coronatine and contributes as a receptor of biologically active jasmonate signal (Yan et al., 2009). These authors demonstrated that COI1 bound not to JA but to an amino acid conjugate, JA-Ile, which is structurally related to coronatine, a compound used to identify the original mutant *coi1*-1. JA-Ile is formed by the enzyme JAR1 (**Figure 1.2 & section 1.3**). The binding of COI1 and JAZ1 occurred in the presence of JA-Ile but not in the presence of JA, MeJA or OPDA (Thines et al., 2007). The results of a yeast-two hybrid assay illustrate that only JA-Ile initiates the protein interaction between COI1 and JAZ1 from both *Solanum lycopersicum* and *Arabidopsis thaliana* (**Figure1.4**). The introductions of JAZ proteins are given in the **section 1.4.2**.

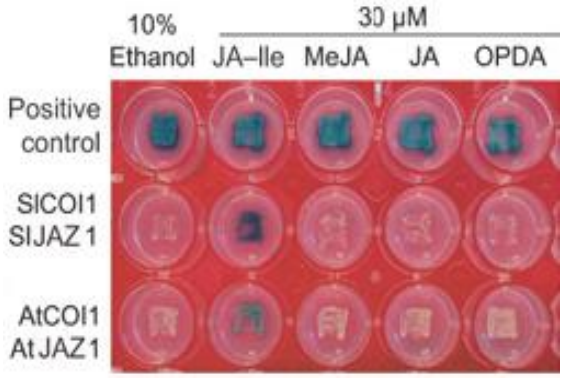


Figure 1.4: JA-Ile initiates the interaction between COI1 and JAZ1 (Thines et al., 2007). The strains of pLexA-53/pB42AD-T are used for the positive controls, displaying as blue colonies in all top row. The results of the yeast-two hybrid assay illustrate that only JA-Ile initiates the protein interaction between COI1 and JAZ1 from both *Solanum lycopersicum* (Sl) and *Arabidopsis thaliana*(At), displaying as blue colonies on the second and third row, respectively.

COI1 alone is not sufficient for the JA-Ile receptor in the JA signal pathway. The minimal sequence from Glu200 to Val220, designated as JAZ1 degron, is required for high affinity interaction between JAZ1 and COI1. The complex of COI1 with JAZ proteins in the presence of JA-Ile requires, in addition, inositol pentakisphosphate as a co-receptor of JA-Ile or coronatine (Sheard et al., 2010). **Figure 1.5** displays a surface representation of COI1, molecular JA-Ile, JAZ1 degron and JA-Ile binding pocket.

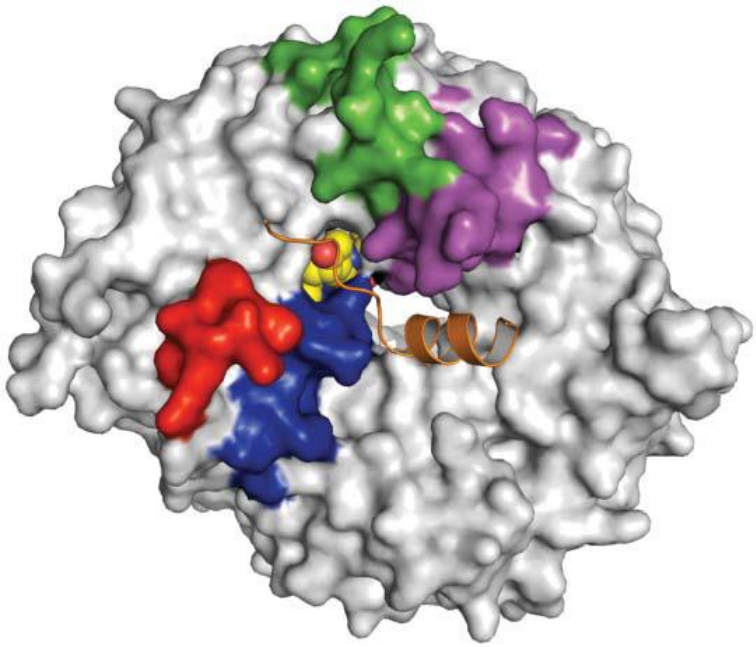


Figure 1.5: Protein structure of COI1, JA-Ile and JAZ1 degron (Sheard et al., 2010).

The JA-Ile binding specific structure is formed by four loops, shown as loop-C (red), loop-2(blue), loop-12 (purple) and loop-14 (green), on the surface of COI1 protein (gray). The molecular JA-Ile (yellow & red ball) is binding in the pocket of the COI1 structure. The JAZ1 degron (orange) is co-binding with JA-Ile and fits inside the COI1 protein pocket. The degron is defined as a specific amino acid sequence in a protein which directs the degradation starting place.

1.4.2 JAZ Proteins

JAZ proteins belong to one large group of Zinc-finger Inflorescence Meristem (ZIM) proteins. Two significant motifs of ZIM and Jas localised on the N-terminal and C-terminal of JAZ proteins, contain 28 conserved amino acids and 26 conserved amino acids, respectively (Nishii et al., 2000; Vanholme et al., 2007; Yan et al., 2007). **Figure 1.6** Shows a phylogenetic analysis of twelve JAZ proteins.

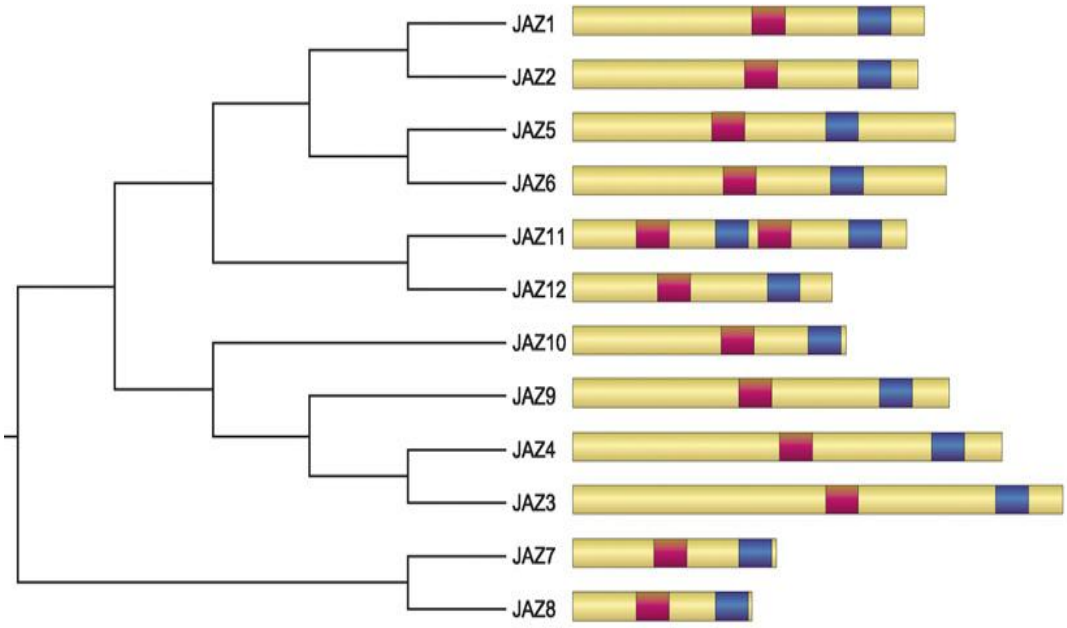


Figure 1.6: Phylogenetic analysis of twelve JAZ proteins (Staswick, 2008).

The conserved ZIM and Jas motif in JAZ proteins are shown as red bar and blue bar, respectively.

Chapter 1: General Introduction

The ZIM domain of JAZ proteins has been defined as the essential element to mediate the physical interactions among JAZ proteins for forming the homodimers and heterodimers. The JA-dependent interaction between JAZ3 and COI1 was mediated by the Jas motif on JAZ proteins. According to yeast two-hybrid assays and pull-down analyses, each member of JAZ proteins displayed the capacity to interact with MYC2, which is specifically mediated by the C-terminal Jas motif of JAZ proteins (Chini et al., 2009b).

The jasmonate-insensitive 3 mutant (*jai3*) defines a gene *JAI3*, which encodes the JAI3/JAZ3 protein that repressed the transcription activity of MYC2. The JAZ3 protein was specifically degraded via the SCF^{COI1} ubiquitin-proteasome system. In the *jai3*-1 mutant, the truncated JAZ3 protein lacks the C-terminal and another fourteen amino acids. Pull down results indicated that in *jai3*-1, JAZ1 and JAZ9 are not degraded, and as a consequence the mutant has a dominant JA insensitive phenotype. One possible way to interpret this result is that the JAI3-1 truncated protein blocks the activity of the SCFCOI1 complex and represses the degradation of other JAZ proteins in the cell, which continue to repress the activity of MYC2. Apparently, in the *jai3*-1 mutant the truncated protein JAI3-1 is not degraded, and this blocks JA-induced degradation of other JAZ proteins (Chini et al., 2007).

JAZ1, which encodes a repressor of the JA signal pathway, was rapidly induced by both JA and auxin. The JA-independent and auxin-inducible expression of *JAZ1* was mediated via the auxin responsive transcriptional regulation. These findings suggest that *JAZ1* crosslinks the JA and auxin signal pathways (Grunewald et al., 2009).

1.4.3 MYC2

The transcription factor MYC2 has been well-defined as an important transcription regulator of JA signal pathway. MYC2 was defined by the jasmonate-insensitive mutant *jin1*. The expression of *AtMYC2* was rapidly induced by JA and was COI1-dependent (Lorenzo et al., 2004). Genomic microarray analyses of wild type Col-0 and the mutant *myc2/jin1* revealed that MYC2 positively regulates genes for JA-dependent resistance to *Helicoverpa armigera*, and negatively regulates genes for indole glucosinalate biosynthesis. The expression of a sub-group of JA-inducible oxidative stress tolerance genes, such as tyraminotransferase (*TAT3*), dehydroascorbate reductase (*DHAR*) and mono-dehydroascorbate reductase (*MDHAR*) was reduced in *jin1*-9, a T-DNA insertion mutation in *AtMYC2*. (Dombrecht et al., 2007).

Promoter analyses of MYC2 regulated genes revealed that MYC2 may bind to the G-box motif (5'-CACGTG-3'). Moreover, the MYC2-specific binding motif G-box has been identified in the promoter region of the *AtMYC2* gene. MYC2 also negatively regulates its own transcription (Dombrecht et al., 2007). **Figure 1.7** displays genes containing a MYC2-binding motif in their promoter region. Grey bars show the relative DNA binding activity.

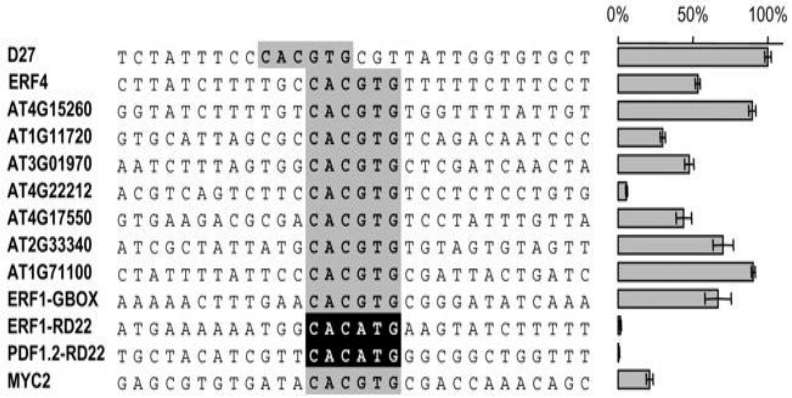


Figure 1.7: Genes containing G-box in their promoter region (Dombrecht et al., 2007).

MYC2 binding activities of motifs have been identified in the promoter region of these Arabidopsis genes. Gray boxes denote for the G-box ("5-CACGTG-3"), and black boxes denote for the sequence of "5-CACGTG-3".The values denote the mean value of three replicates, and error bars denote for the value of SD.

1.4.4 Examples of JA Responsive Genes

The transcription of *AtMYC2* and *JAZ* genes is increased in response to both endogenous and exdogenous JA signals. The transcription of *COI1* appears not to be regulated by JA (Genevestigator database). However, most JA responsive genes are COI1-dependent as described in **section 1.4.1.3**. The transcription of plant alkaloid nicotine biosynthesis genes are rapidly induced by MeJA and are COI1-dependent (Shoji et al., 2008). Transcriptional profiling indicates that the JA-responsive transcription factors MYB21 and MYB24 regulate stamen development (Mandaokar et al., 2006). The transcription of *MYB108* and *MYB24* are largely dependent on MYB21. These three TFs co-operate to modulate stamen maturation in Arabidopsis (Mandaokar and Browse, 2009).

The JA responsive element has been identified in the promoter region of JA responsive genes. The JA responsive gene *VSP1* contains a significant JA responsive element in its promoter region. This jasmonate responsive element has at least 41 base pairs and is located upstream of the TATA box (Guerineau et al., 2003). However, this JA-responsive element has not been precisely defined. The JA-responsive elements JASE1 ("5-CGTCAATGAA-3") and JASE2 ("5-CATACGTCGTCAA-3") have been identified in the promoter region of the *OPR1* gene, which encodes the enzyme of 12-oxophytodienoate. A rice JA responsive gene *OsOPR1*, which contains a 19bp JA responsive element, was rapidly induced by wounding rather than other abiotic stresses (Agrawal et al., 2003; Jang et al., 2009).

1.5 JA Interlinking with Other Plant Hormones

The cross-talk among different plant hormone signal pathways appears to interlink the integration of environmental and developmental signals, to modulate plant growth and development. The transcriptional interactions between JA and other plant hormones are briefly introduced in this section.

1.5.1 JA & ABA

Abscisic acid (ABA) positively regulates plant responses to environmental stresses, such as salinity, dehydration and drought. The JA signal transcription regulator MYC2 has been previously identified as a positive transcription regulator of ABA-induced genes. Promoter analyses indicated that MYB2 and MYC2, specifically recognise the promoter region of the gene *rd22* (responsive to dehydration 22), which is induced by both ABA and drought. Over-expression of *AtMYB2* increased ABA-sensitivity and up-regulated the expression of *rd22* and alcohol dehydrogenase 1 (*AtADH1*). Apparently therefore, MYC2 will serve to integrate response to ABA and JA. Microarray analysis also indicated that the expression of ABA-inducible genes were up-regulated in lines constitutively expressing of *AtMYB2* and *AtMYC2* (Abe et al., 2003). Exogenous ABA represses the transcription of JA-induced genes, and the transcripts of JA-induced genes were increased in an ABA biosynthesis mutant (Anderson et al., 2004). Clearly, ABA regulates JA responsive genes, and *vice versa*.

1.5.2 JA & Auxin

Auxin promotes plant growth and development. Auxin Response Factors (ARFs) and Auxin/Indole-3-Acetic Acid (Aux/IAA) proteins function as transcription regulators in the auxin signal pathway. The auxin signal pathway shares a similar regulatory pattern to the JA signal pathway. The Aux/IAA proteins, similar to JAZs in the JA signal pathway, repress the activity of transcription factor ARFs, and then block the transcription of a number of auxin responsive genes (Santner et al., 2009). As described in **section 1.4.2**, the expression of *JAZ1* was rapidly induced by both JA and auxin. Auxin-induced *JAZ1* expression depends on the auxin signal pathway rather than the JA signal pathway (Grunewald et al., 2009). These findings suggest that *JAZ1* is independently regulated by JA and auxin signal pathways.

1.5.3 JA & ET

Ethylene (ET) regulates seed germination, plant growth, fruits ripening and plant senescence (Yang and Hoffman, 1984; Van Der Straeten and Van Montagu, 1991). Increased JA and ET promote the process of leaf senescence under biotic stresses (Wingler and Roitsch, 2008). The ethylene responsive transcription factor ERF1 was induced by both ET and JA. Over-expression of *ERF1* caused the constitutive expression of one-third of ET- and JA-induced genes and two-thirds of defence responsive genes (Lorenzo et al., 2003). These results suggest that the transcription factor ERF1 plays a vital function in both JA and ET signal pathways. The expression of *PDF1.2* was induced by both JA and ET, and regulated by the ethylene responsive transcription factor Octadecanoid-Responsive Arabidopsis AP2/ERF 59 (*ORA59*) (Leon-Reyes et al., 2010).

1.5.4 JA & SA

Salicylic acid (SA) regulates photosynthesis, transpiration, plant systemic acquired resistance (SAR), and plant defence under environmental stresses (Hayat et al., 2010). JA and SA cooperate to enhance the plant defence to herbivores and pathogens, and also co-regulate the resistance of host plants to parasitic plants (Smith et al., 2009). Pharmacological studies found that the expression of JA responsive gene *PDF1.2* was significantly repressed by SA. This means that SA functions as a repressor of JA signal pathway, and then primarily regulates plant defence rather than JA (Koornneef et al., 2008). Over-expression of *WRKY70* significantly reduced the expression of JA responsive genes and caused the insensitivity to root growth inhibition by JA. Moreover, WRKY70 positively regulates SA-inducible genes, including pathogenesis related gene 1 (*PR1*). The transcription regulation of WRKY70 is essential but not sufficient for interlinking JA and SA pathways (Ren et al., 2008). The transcription factor WRKY41 functions as interlink between the SA and JA signal pathways. Gene expression analyses found that over-expression of *WRKY41* resulted in the constitutive expression of SA-inducible gene *PR5* and reduced the expression of JA-inducible gene *PDF1.2* (Higashi et al., 2008). These

results indicated that the transcriptional regulation cascade might be sufficient for mediating the transcription events in JA and SA induced plant response pathways. Further investigation on rice WRKY transcription factors identified that over-expression of *OsWRKY13* positively regulates the expression of SA biosynthesis genes and SA-inducible genes, and negatively regulates the expression of JA biosynthesis genes and JA responsive genes. Moreover, OsWRKY13 can regulate its own transcription via binding the promoter region of its coding gene (Qiu et al., 2007). Apparently therefore OsWRKY13 activates the SA defence pathway, and suppresses the JA defence pathway. These evidences indicate that the WRKY transcription factors function as the potential activator of SA signal pathway and also the repressor of JA signal pathway in the modulation of plant defence response.

1.5.5 JA & GA

Gibberellic acids (GAs) are dihydroxylated tetracyclic diterpene acids, and regulate seed germination, stem elongation, flowering time and fruit patterning (Richards et al., 2001; Yamaguchi, 2008; Arnaud et al., 2010). In GA signal pathway, the protein gibberellin insenstive dwarf 1 (GID1) functions as a receptor of active GA and binds to DELLA proteins in a GA-dependent pattern. DELLA proteins, which contain one N-terminal DELLA domain and one C-terminal GRAS domain, repress the GA signal pathway and plant growth. In response to a bioactive GA signal, DELLA proteins bind to GID1 and are degraded by the E3 ubiquitin & 26S proteasome protein degradation pathway (Itoh et al., 2003; Schwechheimer, 2008; Santner et al., 2009). Exogenous application of GA3 activates endogenous SA synthesis and rescues the plant growth and development inhibition of diverse adverse abiotic stresses, such as salt and oxidative (Alonso-Ramirez et al., 2009).

1.6 bHLH Transcription Factors

Transcription factors (TFs) are proteins containing DNA-binding domains. They regulate gene transcription by binding the conserved cis-regulatory elements of their downstream genes (Dare et al., 2008). Transcription factors exist extensively in all eukaryotic organisms. There are 2,016 transcription factors in *Arabidopsis thaliana* that have been classified into 58 families depending on the conservation of DNA-binding domain, and reported in the PlantTFDB database (Guo et al., 2008). The basic helix-loop-helix (bHLH) superfamily transcription factors contain one N-terminal DNA binding domain and one C-terminal bHLH domain (Murre et al., 1989; Ferredamare et al., 1994; Abe et al., 1997; Abe et al., 2003).

1.6.1 bHLH Transcription Factors in Plants

The basic helix-loop-helix transcription factors have been extensively characterised in plants. 147 bHLH transcription factors have been classified into 21 subgroups in Arabidopsis (Toledo-Ortiz et al., 2003). Phylogenetic analysis in *Arabidopsis thaliana* and other plants (*Physcomitrella patens*, *Populus trichocarpa*, and *Oryza sativa*) and algae revealed that over 32 subfamilies of 638 bHLH genes confer diverse evolutionary functions (Carretero-Paulet et al., 2010). For example, 167 bHLH genes in rice (*Oryza sativa*) have been analysed with phylogenetic tree which indicating the diverse sub-clusters of biological functions. Bioinformatics characterisation revealed that closely related members of bHLH transcription factors share a similar expression profile and the equivalent regulatory function in rice (Li et al., 2006). It has been well-characterised that the gene transcriptional regulation of pollen development shares high similarity between Arabidopsis and rice (Wilson and Zhang, 2009).

1.6.2 Transcriptional Regulation by bHLH TFs

The bHLH super-family TFs regulate many transcription events for seed germination, root development, flower development, as well as plants responses to abiotic stresses, hormone signals and light signals. Here are some examples of the transcriptional regulation by bHLH TFs in these processes.

1.6.2.1 bHLH TFs Regulate Seed Germination

A bHLH TF SPATULA regulates seed germination, carpel development and lateral plant architecture events. The expression of *SPATULA* was regulated by another bHLH TF INDEHISCENT (Groszmann et al.). SPATULA cooperates with DELLA proteins to regulate the cell size of cotyledons after GA application (Josse et al., 2009). It has been reported that seed germination was co-regulated by environmental conditions and a gibberellin biosynthesis gene for GA3 oxidase (*GA3OX*). SPATULA and PHYTOCHROME INTERACTING FACTOR 3-LIKE 5 (PIL5) co-repress the gene expression of *GA3OX* and then mediate the seed germination under controlled light and temperature conditions (Penfield et al., 2005).

1.6.2.2 bHLH TFs Regulate Root Development

Root Hair Defective 6-like-4 protein (RSL4) positively regulates root hair cell growth and size in response to endogenous and exogenous cell growth signals. The mutant *rsl4* displayed very short root hairs, however, over-expression of *RSL4* promoted the growth extension of root hair cells (Yi et al., 2010). ROOTHAIRLESS1 in *Lotus japonicas* (LjRHL1) putatively regulates the development of root hair in *Lotus japonicas*. Homology analysis revealed that three Arabidopsis bHLH genes At2g24260, At4g30980 and At5g58010 shared the equivalent function with LjRHL1 (Karas et al., 2009).

1.6.2.3 bHLH TFs Regulate Flower Development

The processes of flower initiation, flower development and flower architecture are regulated by many transcriptional regulators in *Arabidopsis* (Irish, 2010). For example, ABORTED MICROSPORES (AMS) regulates the development of tapetal cell and microspore in Arabidopsis. Early pollen degeneration, microspore degeneration and shorter stamen filaments contribute to the complete male sterile phenotype in the T-DNA insertion mutant *ams* (Sorensen et al., 2003). Another example, a petal-specific bHLH TF of BIGPETAL (BPE) has been characterised as a regulator controlling the size of plant petals. The ubiquitous transcript BPEub and the petal-specific transcript BPEp have been identified in Arabidopsis. BPEp positively regulates the petal-specific genes *APETALA1*, *APETALA3, PISTILLATA* and *PISTILLATA3,* but negatively regulates cell size (Szecsi et al., 2006). It has been reported that JA regulates the expression of *BPEp*. A significant reduction of *BPEp* expression was detected in the flowers of jasmonate synthesis deficient mutant *opr3*. Exogenous application of JA rescued the expression level of *BPEp* in *opr3* (Brioudes et al., 2009).

1.6.2.4 bHLH TFs Regulate Plants in Response to Abiotic Stresses

Abiotic stresses, such as drought and low temperature, greatly affect plant growth, development, survival and geographical distribution. They have become the critical factors which limit crop quality and productivity. The resistance to these stresses is integrative reactions regulated by multi-genes. Many bHLH TFs have been characterised as the major transcriptional regulators for plant response to various abiotic stresses. Some examples are introduced in this section.

The bHLH92 TF functions as the transcriptional regulator to mediate plant response to abiotic stresses. The expression of *bHLH92* (At5g43650) was significantly up-regulated after NaCl treatment. Microarray analysis discovered that nineteen genes were regulated by bHLH92. Over-expression of *bHLH92* resulted in the increased tolerance to salt and osmotic stresses (Jiang et al., 2009).

Chapter 1: General Introduction

High-light and heat-shock induced expression of *ANAC078* has been identified as a positive regulator in the pathway of flavonoid biosynthesis. Over-expression of *ANAC078* caused the increased expression of 166 genes after high-light and heat-shock treatments. Additionally, some of the flavonoid biosynthesis genes displayed expression reduction in the knockout mutant of *ANAC078* gene. These consistent results indicate that ANAC078 acts as a transcription activator in the flavonoid biosynthesis pathway under high-light and high-temperature conditions (Morishita et al., 2009).

A number of TFs that regulate plant response to cold have been identified. For instance, Inducer of CBF Expression 1 (ICE1) positively regulates the transcription of *CBF* to enhance plant freezing tolerance (Chinnusamy et al., 2003). The transcription factor ICE1 has been previously identified that it specifically binds the Myc sequence in the promoter region of the cold-inducible *CBF3* gene. A cold-inducible MYB TF ICE2 interacts with the ICE1 to co-regulate the transcription of *CBF* under cold stress (Agarwal et al., 2006). A cold-inducible *OsbHLH1* gene, which encodes one nucleus localised bHLH-ZIP protein in rice, regulates plants in response to lower temperature adversity (Wang et al., 2003). OrbHLH001, primarily identified in Dongxiang Wild Rice (*Oryza rufipogen*), regulates the plants in response to cold and salt stresses. The expression of *OrbHLH001* was significantly up-regulated under freezing and salt. Moreover, Over-expression of *OrbHLH001* endowed the plants displaying high tolerance to freezing and salt stresses. The regulation of OrbHLH001 did not depend on the CBF/DREB1(Li et al., 2010). OrbHLH2, which shares high identity with ICE1, has been cloned and characterised in wild rice (*Oryza rufipogon*). Over-expression of *OrbHLH2* enhanced the salt tolerance and up-regulated a group of salt responsive genes, such as *DREB1A*, *CBF3* and *RD29A* in Arabidopsis. The transcriptional regulation of OrbHLH2 to salt responsive genes is independent on the ABA signal pathway (Zhou et al., 2009).

1.6.2.5 bHLH TFs Regulate Plants in Response to Hormone Signals

The transcription factors regulating transcriptional interactions between JA and other plant hormones have been previously introduced in **section 1.5**. The bHLH TFs regulating the transcription events in the plant hormone signals are briefly introduced in this section.

AtAIG1, encoding a bHLH TF, is a positive transcription regulator in the ABA signal pathway. The ABA inducible AIG1 appears to prefer to bind the E-box (5'-CANNTG-3') in the promoter region of ABA responsive genes. The knockout mutant *ataig1* displayed ABA-high-sensitivity and decreased the expression of dehydration responsive genes *RD29A* and *RD22* (Kim and Kim, 2006). *AtAIB* (At2g46510) encodes a bHLH TF which has been demonstrated to be a positive regulator of the ABA signal pathway in Arabidopsis. Phenotype analyses found that the knockdown expression of *AtAIB* reduced ABA-sensitivity, however, the constitutive expression of *AtAIB* resulted in higher ABA-sensitivity and drought tolerance (Li et al., 2007). Their results indicate that AtAIB acts as one component in the ABA signal pathways. However, the upstream and downstream targets of AtAIB have not yet been well-defined quantitatively and functionally. *GhBHLH1*, which encodes a bHLH TF in *Gossypium hirsutum*, has been well-defined as the positive regulator interlinking the ABA and dehydration signal pathway. The amino acid sequence of GhbHLH1 has high similarity with MYC2, and the expression of *GhbHLH1* was significantly up-regulated by ABA and PEG treatments (Meng et al., 2009).

RIM1 functions as the transcription regulator of the JA signal pathway in rice. The JA biosynthesis related genes *LOX*, *AOS2* and *OPDR7* were up regulated in the *rim* mutant in comparison with the wild type background. The mutant *rim1* displayed root growth inhibition (Yoshii et al., 2010). NbbHLH1 and NbbHLH2, which were induced by MeJA in *Nicotiana benthamiana*, positively regulate the nicotine biosynthesis genes in the JA signal pathway (Todd et al., 2010).

1.6.2.6 bHLH TFs Regulate Plants in Response to Light

Phytochromes (Phys) are photoreceptors and regulate plants respond to light. Phytochrome phyA, phyB, phyC, phyD and phyE function as the red and far-red light receptors in the light signal pathway. The phytochrome-mediated light signal largely depends on the transcription regulation of phy-interacting transcription factors (PIFs), which belong to the bHLH transcription factors (Kim et al., 2002; Duek and Fankhauser, 2005).

The phytochrome-interacting factors (PIFs) confer negative regulation to the phytochrome signal transduction (Duek and Fankhauser, 2005). PIF1 negatively regulates plant photomorphogenesis, and was degraded via the ubiquitin-26S proteasome system (Shen et al., 2005). PIF3 cooperates with PIF1 to control chloroplast development under different light and circadian conditions (Stephenson et al., 2009). PIF4**,** a negative regulator of the phyB signal pathway, has been identified as the transcriptional regulator of cell expension genes (Huq and Quail, 2002). PIF4 also regulates plant architectural events under high temperature (Koini et al., 2009). The transcription of light responsive TFs was significantly altered in the quadruple mutant *pifq*, which lacks the protein of PIF1, PIF3, PIF4 and PIF5, (Leivar et al., 2009). PIF7, interacting with the active phyB binding motif, cooperates with PIF3 to regulate seedling deetiolation (Leivar et al., 2008). It has been reported that the transcription factor long Hypocotyls in Far-red light Reduced phytochrome signaling 1 (HFR1) cooperates with PIFs to regulate the phytochrome signal pathway (Fairchild et al., 2000).

The JA signal pathway interacts with the phytochrome-dependent light signal pathway in Arabidopsis. The JA biosynthesis and the expression of JA responsive genes are significantly increased in the phytochrome chromophore biosynthesis mutants, such as *hy1*-101, *hy1*-102 and *hy2*. These results indicated that the phytochrome signal pathway suppresses the JA biosynthesis and the transcription of JA responsive genes. Moreover, the light-inducible genes and the phytosynthesis associated genes were repressed after JA treatment (Zhai et al., 2007).

Far-red light significantly reduces the plant sensitivity to JA (Moreno et al., 2009). PhyA was required for the COI1-dependent JAZ1-β-glucuronidase degradation after JA and wounding treatments. COI1 was required for the expression of far-red light induced bHLH TFs (*HFR1* and *PIL1*) and for plant response to far-red for early seedling development (Robson et al., 2010).

1.7 Aim and Objective

Considerable molecular events in the JA signal pathway are precisely reprogrammed via the transcriptional regulation of JA-responsive transcription factors. In this thesis, I present original research on identifying and characterising a novel transcriptional regulator of the JA signal pathway, investigating the downstream regulation by this transcription factor, and developing a transcription regulatory model for the JA signal pathway.

Chapter 2

Materials and Methods

2.1 Investigating JA Responsive TFs Using Publically Available Database

2.1.1 Investigating JA and Wounding Responsive TFs

2.1.1.1 Search for Arabidopsis TFs via the Plant TFs Database

The Arabidopsis gene list of 135 bHLH (**Appendix Table A.1**), 143 MYB (**Appendix Table A.2**) and 145 AP2-EREBP transcription factors (**Appendix Table A.3**) were downloaded from the plant TF Database (Guo et al., 2008) and selected as a target subset for the analysis on Genevestigator.

2.1.1.2 Using Genevestigator to Analyse Gene Expression Profile

Genevestigator is an integrative database for gene expression analyses (Tomas et al., 2008). A subset of the 4069 Arabidopsis thaliana arrays (ATH1 22K array) on Genevestigator were used to analyse gene expression profiles following treatments (MeJA and wounding) and in a specific genetic background (eg. *coi1*). The Gene IDs of 135 bHLH TF, 143 MYB TF and 146 AP2-EREBP TF were entered into a gene selection window to generate a gene expression database. A dataset on gene expression level was collected from the MeJA and wounding treatment, and downloaded from ArrayExpress Database (http://www.ebi.ac.uk/microarray-as/ae/) for the investigation of MeJA and wounding responsive genes.

2.1.2 Search for T-DNA Insertion Mutations in At3g50060, At2g46510 and At2g23290

By utilizing the Arabidopsis gene mapping tool, T-DNA Express (http://signal.salk.edu/cgi-bin/tdanexpress) on the SALK database (Alonso et al., 2003), MeJA and wounding responsive genes identified in **section 2.1.1** were searched for suitable T-DNA insertions. The Gene IDs of At3g50060, At2g46510 and At2g23290

were individually entered into the "Query" selection box. This action lists the T-DNA insertion lines of each target gene. By clicking a T-DNA insertion line, more detailed information was presented, including the insertion type, flanking sequence, and insertion site, as well as the information of the target gene. These T-DNA insertion lines were then ordered from NASC-European Stock Centre (http://arabidopsis.info/).

2.1.3 Gene Co-expression Analyses

The platform GeneCAT (http://genecat.mpg.de/), which combines gene co-expression analyses and gene cluster tools (Mutwil et al., 2008), was used to identify genes co-expressed with two transcription factors identified in the JA signal pathway. The gene IDs of At1g32640 and At2g46510, were entered into the search blank and 0.7 was set as the r-value for their gene co-expression analysis. Two input genes (At1g32640 and At2g46510) and the closely co-expressed genes (At1g19180, At1g74950, At1g17380 and At1g80840) were then submitted to generate a Gene Expression Tree via the Gene Expression Tree database on GeneCAT.

2.1.4 G-box Motif Analysis for Nine JA Responsive Genes

G-box motifs were analysed via SCOPE (http://genie.dartmouth.edu/scope/), which is available for computational identification of regulatory elements in the promoter region of candidate genes (Carlson et al., 2007; Chakravarty et al., 2007). In order to identify G-box motif in the promoter region of nine JA-responsive genes, *Arabidopsis thaliana* was firstly selected as background database. Next, the upstream sequence (before transcription start site) was then fixed as 2500bp. Then the gene list was entered as At1g32640, At2g46510, At2g24850, At1g19180, At5g13220, At3g49620, At1g66690, At1g53885 and At2g26020. And then the G-box motif of "5-CACGTG-3" was identified as the must included target motif. The target motif displays on the corresponding position in the promoter region of the candidate gene (**Figure 5.4**).

2.1.5 StarNet for Gene Regulatory Network Analyses

StarNet was designed by the VanBuren group as a web-based tool for investigating gene regulatory networks. This program produces hypotheses for correlated networks and supplementary information on gene function (Jupiter and VanBuren, 2008; Jupiter et al., 2009). StarNet analyses are based upon the original microarray data. The original microarray data was collected from the Gene Expression Omnibus of NCBI (National Centre for Biotechnology Information: http://www.ncbi.nlm.nih.gov/) and then normalised by Robust Multichip Average (RMA: http://rmaexpress.bmbolstad.com/) in Bioconductor. This database can be integrated via a StarNet query, which uses Octave (http://www.gnu.org/software/octave/) to generate Pearson Correlation coefficients between the input query gene expression, and the most highly correlated output genes, in all combinations (Jupiter et al., 2009).

The input process has two main steps. Firstly, an original cohort of microarray data is chosen from a specific species list, eg. *Arabidopsis thaliana*. Next the specific Entrez ID of a gene or protein is typed into the Query page. The Entrez ID can be obtained from the tool of Gene ID lookup (http://vanburenlab.tamhsc.edu/gene_lookup.html). Other parameters are set as default values.

2.1.6 Other Bioinformatics Databases for Consulted Supplementary Information

1) Primer3 http://biotools.umassmed.edu/bioapps/primer3_www.cgi

2) PLACE http://www.dna.affrc.go.jp/PLACE/

3) TF database http://plntfdb.bio.uni-potsdam.de/v2.0/downloads.php

4) PED http://bioinfo.ucr.edu/projects/Unknowns/external/express.html

5) CressExpress http://www.cressexpress.org/

6) eFP browser http://bbc.botany.utoronto.ca/efp/cgi-bin/efpWeb.cgi

7) Textpresso for Arabidopsis: http://www.textpresso.org/arabidopsis/

8) NASCArrays: http://affymetrix.arabidopsis.info/narrays/experimentbrowse.pl

Chapter 2: Materials and Methods

2.2 Plant Growth and Plant Treatment

2.2.1 Arabidopsis Plants Used in This Research

Fourteen Arabidopsis lines have been used in this research. **Table 2.1** lists the source and description of each line. Col-0, *jin1*-1, *jai3*-1, *coi1*-16 and T-DNA insertion lines (*jut*, N662814, N662523 and N876044) were used for root growth inhibition assay. Col-0, Col-gl, 2M, *jin1*-1, *jut* and JUTOE were used for gene expression analyses in Chapter 4. Wild type Col-0, the single mutants of *jin1*-1, *jut* and the double mutant *jin1*-1/*jut* were used for microarray experiments in Chapter 5. Wild type Col-0, the single mutants of *jin1*-1, *jut*, *coi1*-16, *jai3*-1, the double mutant *jin1*-1/*jut* and JUTOE were used for qRT-PCR in Chapter 6.

Table 2.1: Arabidopsis plants used in this research.

Seeds	Source	Description
Col-0	lab stock	Arabidopsis wild type Columbia
Col-gl	lab stock	Ecotype, Columbia glabrous
2M	lab stock	Col-gl carrying *VSP1*::luciferase reporter
***coi1*-16**	lab stock	COI1 mutation in Col-gl background
***jin1*-1**	gift from Prof. Susnne Berger	*AtMYC2* C-terminal deletion mutant
***jin1*-2**	lab stock	*AtMYC2* T-DNA insertion mutant
***jai3*-1**	gift from Prof. Roberto Solano	JAZ3 protein truncated mutant
***jut*(N874647)**	ordered from SALK centre (SAIL_536_F09)	T-DNA insertion mutant in At2g46510 (*JUT*), generated by vacuum infiltration of Columbia (Col-0) plant with *Agrobacterium tumefaciens* vector pDAP101.
jin1*-1/*jut	generated for this research	Double mutant on *AtMYC2* and *JUT*
MYC2OE	gift from Dr. Roberto.Solano	*35S*::*AtMYC2* over-expression line
JUTOE	gift from Dr. Hongmei Li	No.1 of *35S*::*JUT* over-expression line
N662814	ordered from NASC centre (SALK_067655.56.00.x)	T-DNA insertion mutant in At3g50060, generated by vacuum infiltration of Columbia (Col-0) plant with *Agrobacterium tumefaciens* vector pROK2.
N662523	ordered from NASC centre (SALK_055373.34.15.X)	T-DNA insertion mutant in At3g50060, generated by vacuum infiltration of Columbia (Col-0) plant with *Agrobacterium tumefaciens* vector pROK2.
N876044	ordered from NASC centre (SAIL_700_C03)	T-DNA insertion mutant in At2g23290, generated by vacuum infiltration of Columbia (Col-0) plant with *Agrobacterium tumefaciens* vector pDAP101.

2.2.2 Media Preparation

1/2 MS media:

1/2 MS Murashige & Skoog media was prepared as follows:

MS salt (Duchefa Biochemie®, Haarlem, Netherlands)	2.215g
MES (SIGMA-ALDRICH CHEME GmbH™)	0.5g
Sucrose (Fisher Scientific®)	5g

The above reagents were dissolved in 1 litre distilled water and the pH value was adjusted to 5.9 by adding potassium hydroxide solution (KOH). Plant agar (Duchefa Biochemie®) (1.75g) was added to every 250ml of liquid media and this was then autoclaved at 121°C for 15min. The autoclaved media was then cooled to 50°C and, where appropriate, supplements were added before pouring into square Petri dishes.

2.2.3 Reagent Preparation

1) 100mM MeJA stock solution

In a flow-hood, MeJA (Bedoukian Research®) (20µl, 4.59M) was diluted in ethanol (898µl, 100%), and stored in a 1.5ml eppendorf tube at 4°C.

2) 50µM MeJA media

The autoclaved 1/2 MS media (250ml) was melted in a microwave oven at 100°C for 5mins and then cooled in a flow-hood to 50°C. In a flow-hood, 50ml molten 1/2 MS media was transferred into one 50ml fresh centrifuge tube. The MeJA stock solution (25µl, 100mM) was added to each 50ml of the molten 1/2 MS media in one 50ml centrifuge tube, and then the molten media was gently mixed and poured into a square Petri dish. The cooled Petri dish with solid media was either used immediately or sealed with Micropore™ tape and stored at 4°C.

3) 20µM MeJA medium

As described before, the MeJA stock solution (10µl, 100mM) was added to each 50ml of the molten 1/2 MS media in one 50ml centrifuge tube, and then the molten media was gently mixed and poured into a square Petri dish.

2.2.4 Seed Surface-sterilisation

Arabidopsis seeds were put into a 1.5ml eppendorf tube, and then were washed with ethanol (500µl, v/v:100%) for 5min at room temperature. The seeds were surface-sterilised with bleach (500µl, v/v: 20%) for 15min and then washed with 500µl of sterilised water for three five-minute washes. The sterilised seeds were then stored in a 1.5ml eppendorf tube with sterilised water for two days at 4 °C to synchronise germination.

2.2.5 Plant Growth Conditions

Half strength MS agar plates were sown with the surface-sterilised seeds and placed in a growth chamber (SANYO® Illinois, USA) at 22°C with 100-120 µmol/m^2/sec light intensity in long day (LD) growth conditions (16 hours light and 8 hours dark). To avoid the seedling roots growing down into the media, the plates were placed vertically in the growth chamber.

2.2.6 Arabidopsis Crossing

Arabidopsis is a self-pollinating plant. To make a genetic cross between two genotypes, an unopened flower on the primary inflorescence was chosen. The sepals were opened and the immature anthers were removed with fine forceps. The mature anthers from the other genetic background were picked, and then touched onto the stigma of the recipient flower leaving visible pollen. Cling film was used to make a small protective chamber for covering the crossed flowers for a few days and removed after the styles elongated. The F1 seeds were collected after the siliques became mature.

2.2.7 MeJA Treatment

To test the effect of MeJA on Arabidopsis root length growth inhibition in **Chapter 3**, more than fifteen Arabidopsis seeds of each line were germinated and grown on 1/2 MS media in a LD growth chamber. After one week, more than fifteen seedlings of each line were carefully transferred with sterilised fine forceps onto a 50μM MeJA MS plate for 48h treatment.

To test the effect of MeJA on Arabidopsis root growth in **Chapter 4**, two groups of ten Arabidopsis seeds of each line were directly germinated and grown on both 1/2 MS media and 1/2 MS media containing 20μM MeJA in a LD growth room for seven days. To quantitatively measure the MeJA effect on different lines, the root length of ten seedlings of each line were analysed by ImageJ software and the fresh weight of ten seedlings of each line were measured with an electronic balance.

To test the effect of MeJA on Arabidopsis shoot growth in **Chapter 4**, an equal number Arabidopsis seeds of each line were directly germinated and grown on both 1/2 MS media and 1/2 MS media containing 20μM MeJA in a LD growth room for twelve days.

For the gene expression analyses after treatment with MeJA (**section 4.2.1** in **Chapter 4**), six groups of fourteen twelve-day-old Col-0 seedlings were carefully transferred with sterilised fine forceps onto 1/2 MS medium containing 50μM MeJA. These groups of fourteen MeJA-treated seedlings were harvested by snap freezing in liquid nitrogen at 15mins, 30mins, 45mins, 1h, 3h and 6h after transfer. Fourteen untreated seedlings were harvested (as above) for the control sample.

For the gene expression analyses after treatment with MeJA (**section 4.2.2** in **Chapter 4**), two groups of fourteen Arabidopsis seeds of each line were germinated and grown

on 1/2 MS media in a LD growth chamber. Fourteen twelve-day-old seedlings of each line were carefully transferred with sterilised fine forceps onto 1/2 MS medium containing 50μM MeJA. These MeJA-treated seedlings of each line were harvested at 15mins after transfer, and snap froze in liquid nitrogen. Fourteen untreated seedlings were harvested (as above) for the control sample.

For the microarray assay in **Chapter 5**, two groups of fourteen Arabidopsis seeds of each line were germinated and grown on 1/2 MS media in a LD growth chamber. After two-weeks, fourteen seedlings of each line were carefully transferred with sterilised fine forceps onto 1/2 MS medium containing 20μM MeJA for 30mins treatment, and snap froze in liquid nitrogen. Fourteen untreated Arabidopsis seedlings were transferred onto 1/2 MS media and harvested at 30mins after transfer (as above) for the control samples.

For the qRT-PCR experiments in **Chapter 6**, nine groups of fourteen Arabidopsis seeds of each line were germinated and grown on 1/2 MS media in a LD growth chamber. After two-weeks, four groups of fourteen seedlings of each line were carefully transferred with sterilised fine forceps onto 1/2 MS containing 20μM MeJA. These groups of fourteen MeJA-treated seedlings were harvested by snap freezing in liquid nitrogen at 30mins, 1h, 3h and 6h after transfer. Five groups of Arabidopsis seedlings were transferred onto 1/2 MS media and then were harvested by snap freezing in liquid nitrogen at 0, 30mins, 1h, 3h and 6h after transfer for the control samples.

2.2.8 Wounding Treatment

To test the effect of wounding on Arabidopsis shoot growth in **Chapter 3**, two groups of eight Arabidopsis seeds of each line were germinated and grown on soil in two square pots (eight seeds per pot) in a SD (8h light/16h dark) growth room. Eight 21-day-old seedlings for each line were selected for wounding treatment. An equal number of same age unwounded seedlings of each line were selected as the control

group. For wounding treatment, one leaf of each seedling was lightly squeezed with forceps once per day, and this was then repeated for ten consecutive days. Pictures of wounded and unwounded seedlings of each line were recorded each day at the same time point. The Arabidopsis leaf area in both wounded and control groups were measured with the software (http://www.comp.leeds.ac.uk/yanong/alm/index.html).

For gene expression analyses following wounding (**section 4.2.1** in **Chapter 4**), six groups of fourteen twelve-day-old Col-0 seedlings were selected for wounding treatment. For wounding treatment, one leaf of each seedling was lightly squeezed with sterilised fine forceps. Fourteen wounded-seedlings were harvested by snap freezing in liquid nitrogen at 15mins, 30mins, 45mins, 1h, 3h and 6h after wounding. Fourteen unwounded seedlings were harvested for the control sample.

2.3 Molecular Biological Methods

2.3.1 Arabidopsis Genomic DNA Extraction

1) Fresh Arabidopsis leaf samples (200mg) of each line were harvested from four-week-old Arabidopsis seedlings, placed in each 1.5ml eppendorf tube, and then snap-frozen in liquid nitrogen.
2) A small amount of liquid nitrogen was added to each tube, and the leaves were ground to a fine powder with the aid of a motorised pestle.
3) DNA extraction buffer (700µl) was immediately added to each 1.5ml eppendorf tube. The tubes were incubated at 65°C for 12mins with occasional inversions.
4) KAc (200µl, 5M) was added into each tube. The tubes were put on ice for 10 minutes and then were centrifuged at the max speed for 8mins. The supernatant was transferred into fresh tube.
5) An equal volume of phenol/chloroform was added into the tubes and mixed by vortexing. The tubes were centrifuged at the max speed for 8mins. The aqueous phase (containing the nucleic acid) was transferred into a clean centrifuge tube.

6) Iso-propanol (500µl) and NaAc (60µl, 3M) were added into the tubes. The tubes were mixed by vortex and placed at -20°C for at least 30mins, and then centrifuged at the max speed for 15mins. The supernatant was carefully removed from the tubes.
7) The pellet was washed with ethanol (500µl, v/v: 75%). The tube was centrifuged at 13,000 rpm for 2mins. Any trace of ethanol was carefully removed.
8) The pellet was dried at room temperature for 20mins and re-suspended with 50µl of TE solution.
9) To eliminate RNA, RNaseA (3µl, 10mg/ml) was added into 50µl of DNA solution, mixed by flicking the tube and spun down for a few seconds. The DNA/RNaseA mixture was then incubated at 37°C for 30min. DNA samples were stored at -20°C.

2.3.2 Arabidopsis Total RNA Extraction

This followed the protocol of RNeasy Plant Mini Kit of QIAGEN®.

1) Fresh whole Arabidopsis seedlings (100mg) of each line were harvested and placed in a 1.5ml eppendorf tube and then snap-frozen in liquid nitrogen.
2) A small amount of liquid nitrogen was added to the tube, and the whole seedlings were ground to a fine powder with the aid of a motorised pestle.
3) The antioxidant β-Mercaptoethanol (100µl) was added into 10ml RNeasy Lysis Buffer (RLT) and then incubated at 65°C before using.
4) The preheated Buffer RLT containing β-Mercaptoethanol (450µl) was then immediately added into each 1.5ml eppendorf tube with the maximum of 100mg tissue powder. Each sample was vortexed vigorously and then incubated at 65°C for 2mins with occasional inversions.
5) The cleared lysate was transferred into QIAshredder spin column and spun at 13,000 rpm for 2mins. The supernatant was transferred into a new micro-centrifuge tube.
6) Ethanol (225µl, 100%) was immediately mixed with the clear lysate by careful pipetting.

7) Then each sample (675µl) was transferred into RNeasy Spin column and the lid was closed gently, followed by spinning at the speed of 13,000 rpm for 15 seconds. The flow-through was discarded.
8) RNeasy Wash Buffer RW1 (700µl) was added into the RNeasy Spin column. Then the column was spun at 13,000 rpm for 15 seconds. The flow-through was discarded.
9) RNeasy Wash Buffer RPE (500µl) was added into RNeasy Spin column, and spun at the speed of 13,000 rpm for 15 second. The flow-through was discarded.
10) Buffer RPE (500µl) was added to the RNeasy Spin column. This was then spun at 13,000 rpm for 2mins. The RNeasy Spin column was carefully placed in a fresh 1.5ml collection tube.
11) RNase-free water (40µl) was directly added to the spin column membrane. After one minute, the tubes were spun at 13,000 rpm for one minute. The total RNA samples were collected in the 1.5ml collection tubes and then stored at -20°C or -80°C.

2.3.3 Agarose Gel Electrophoresis

Electrophoresis grade Agarose (Invitrogen™ life technologies) was melted in 1×TAE buffer with a microwave oven and then cooled to 50°C in a fume-hood. Ethidium Bromide (Sigma®) (V/V: 0.001%) was added into the molten agarose, and then the molten agarose was poured in a gel casting tray with comb and allowed to set. Loading buffer was mixed with samples as well as DNA ladder marker. The samples and markers were loaded into the wells on the gel and run at 100 volts for 1 hour in an electrophoresis tank (BIO-RAD®). The electrophoresis images were then analysed with Quantity One 1-D analysis Software (BIO-RAD®).

2.3.4 DNA Quantification

DNA concentration control λ-Hind III DNA (100ng or 200ng) was loaded for calculating the concentration of the DNA samples.

2.3.5 RNA Quantification

One micro-litre of each RNA sample was loaded onto a Nanodrop ND-1000 Spectrophotometer machine (NanoDrop Technologies, Inc) to measure the RNA qualification and concentration.

2.3.6 Polymerase Chain Reaction

The PCR reaction mixture (20µl) was prepared as follows: 1µl of DNA template, 1µl of 10×NH4 reaction buffer, 2.5µl of 2mM dNTPs (Roche®), 0.6µl of 50mM $MgCl_2$, 2µl of 10µM Forward Primer, and 2µl of 10µM Reverse Primer, 0.3µl of Taq DNA polymerase (Bioline®) and 10.6µl of sterilised water.

2.3.6.1 PCR-based Confirmation of the Presence of the T-DNA Insertion in the Target Gene

The PCR reaction mixture was prepared as describes before, and the reaction was processed in Peltier Thermal Cycler PCR machine using the following temperature and time conditions: 94°C for 2 minutes, 35 cycles of three reactions (94°C for 30 seconds, 63°C for 30 seconds and 72°C for 1 minute), 72°C for 5 minutes.

2.3.6.2 PCR-based Confirmation of the *JUT* Gene Over-expression Line

The PCR reaction mixture was prepared as described before, and the reaction was processed in Peltier Thermal Cycler PCR machine using the following temperature and time conditions: 94°C for 2 minutes, 35 cycles of three reactions (94°C for 30 seconds, 63°C for 30 seconds and 72°C for 1 minute), 72°C for 5 minutes.

2.3.6.3 Production of Probes for Northern Blotting and Hybridisation

The PCR reaction mixture was prepared as described before, and the reaction was processed in Peltier Thermal Cycler PCR machine using the following temperature and time conditions: 94°C for 2 minutes, 35 cycles of three reactions (94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1 minute), 72°C for 5 minutes.

2.3.6.4 PCR-based Identification for the Double Mutant *jin1*-1/*jut*

The PCR reaction mixture was prepared as described before, and the reaction was processed in Peltier Thermal Cycler PCR machine using the following temperature and time conditions: 94°C for 2 minutes, 35 cycles of three reactions (94°C for 30 seconds, Δ°C for 30 seconds and 72°C for 1 minute), 72°C for 5 minutes. **Notes:** Δ°C is dependent on the primers being used in the specific PCR reaction. Δ°C was set as 60°C when using primers of group A and B, Δ°C was set as 63°C when using primers of group C and D.

2.3.7 PCR Product Purification

This followed the protocol of QIAquick Kit QIAGEN®.

1) The band of PCR product was carefully cut off from the gel and weighed in one 1.5ml centrifuge tube.
2) To solubilise the agarose gel, three volume of Buffer QG (solubilisation and binding buffer) was added into the tube containing one volume of PCR band gel. The tube was then incubated at 50°C for 10mins with occasional inversions.
3) One volume of iso-propanol was added into the tube and then mixed well. The QIAquick spin column was placed in a fresh 2ml collection tube.
4) The sample mixture (800μl) was transferred into the QIAquick column and then centrifuged at 13,000 rpm for one minute. The flow-through was discarded.
5) To bind DNA onto the membrane, Buffer QG (0.5ml) was added into the QIAquick column and centrifuged at 13,000 rpm for 1 minute.

6) Wash Buffer PE (750µl) was added into QIAquick column and centrifuged at 13,000 rpm for one minute. The flow-through was discarded. The QIAquick column was centrifuged at 13,000 rpm for 1 minute. Then the QIAquick column was placed into a fresh 1.5ml centrifuge tube.
7) Elution Buffer EB (30µl) was added onto the column membrane. The column was then left for one minute at room temperature and then centrifuged at 13,000 rpm for one minute. The purified PCR product was then collected at the bottom of the collection tube.

2.3.8 DNA Sequencing Reaction

The sequencing ready reaction (10µl) was set up as follows: purified PCR product (1µl, 50ng/µl), 1µl of Primer, 1µl of ½ BD solution, 1µl of Big Dye 3.1(Applied Biosystems®), 1µl of Buffer (Applied Biosystems®) and 5µl of sterilised water. The reaction mixture was processed using the following temperature and time conditions: 96°C for 1 minute, 28 cycles of three reactions (96°C for 30 seconds, 46°C for 15 seconds and 60°C for 4 minutes). The product of the sequencing ready reaction was then submitted to the John Innes Centre for sequencing analyses.

2.3.9 Northern Blotting and Hybridisation

Northern blotting analysis is the traditional method to measure gene expression/mRNA level, and was developed in 1977. Its name followed the Southern blotting (Alwine et al., 1977; Trayhurn, 1996). **Figure 2.1** illustrates the procedure of Northern blotting. The total RNA sample is separated by eletrophoresis, and then was transferred onto a nylon membrane. Radioactive-^{32}P labelled single-stranded DNA probe of the target gene is then hybridised with its complementary RNA targets on the membrane. Hybridisation was then detected by phosphor imaging. There are many advantages using Northern blotting analysis to investigate gene expression/mRNA level. For instance, it allows expression of one specific gene to be analysed. Additionally, spatial

and temporal gene expression can be studied by Northern blotting analysis in different organs and time series. Experimentally, Northern blotting analysis requires large amounts of RNA and has less sensitivity in comparison with quantitative real-time RT-PCR.

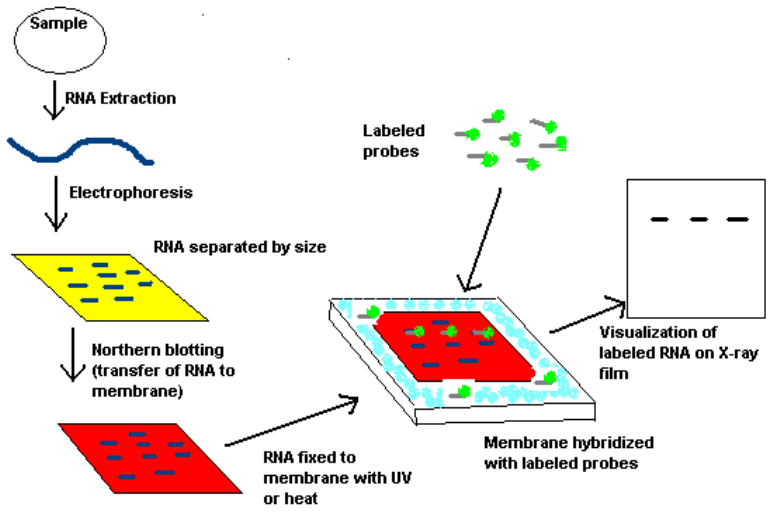


Figure 2.1: The procedure of Northern blotting analyses.

(en.wikipedia.org/wiki/Northern_blot).

2.3.9.1 RNA Gel Electrophoresis

1) Preparation of RNA running buffer

One hundred millilitres of 10×MOPS buffer were mixed with 900ml sterilised 0.1% DEPC water.

2) Preparation of RNA running gel (100ml)

Electrophoresis grade RNA agarose (Helena BioSciences®) (1.5g) was melted into 82ml sterilised 0.1%DEPC water in a flask using a microwave oven and then cooled to 50°C in a fume-hood. One hundred millilitres of 10×MOPS buffer were sterilised with a micro-pore membrane filter, and then added into this flask. The denaturant formaldehyde (8ml, 36.5%) was added into this flask. This mixture was then poured into a gel casting tray with comb and allowed to set.

3) RNA loading dye:

Ethidium Bromide (Sigma, Dorset, UK) (v/v: 0.25%) was mixed into 5×RNA loading Dye.

4) RNA gel electrophoresis

Eight micrograms of total RNA of individual samples were mixed with RNA loading dye and loaded per lane in the RNA agarose gel, and run at 80 volts over 2 hours in an electrophoresis tank (BIO-RAD®). The electrophoresis images were then analysed with Quantity One 1-D analysis Software (BIO-RAD®).

2.3.9.2 Transfer RNA from Gel to Hybond^{TM}-XL Membrane

The gel containing Arabidopsis total RNA was washed in sterilised water for 10mins. Two pieces of filter paper were wet with 10×SSC buffer and set on a glass bridge over a tray containing 10×SSC buffer. The gel was then placed onto the filter paper bridge. One piece of Amersham Hybond^{TM}-XL membrane (GE Healthcare Life Sciences®) and three other pieces of filter paper were cut to the same size of the RNA gel and then carefully layered above the RNA gel. Cling film was placed to surround the gel to stop the buffer short-circuiting. At least two boxes of tissues and one heavy book were put onto the filter paper. The gel was allowed to blot overnight. The next day, the membrane was washed in 10×SSC buffer for 1min then cross-linked by a UV Stratalinker 2400 machine (STRATAGENE®).

2.3.9.3 Pre-hybridisation

PerfectHyb^{TM} Plus Hybridisation Buffer (1×, Sigma Life Science®) was pre-heated in 65°C water bath. The blotted membranes were washed with 2×SSC solution and rolled into the dry hybridisation tube. The ssDNA was denatured in boiling water for 5mins. Hybridisation buffer (5ml) and denatured ssDNA (150µl) were added into the hybridisation tube and then incubated at 65°C for least one hour.

2.3.9.4 Label the ^{32}P-dCTPs Probes

Each hybridisation reaction was set up in a 2ml centrifuge tube using the Amersham Megaprime™ DNA Labelling Systems Kit (RPN1607). DNA probe (100ng) was added with sterilised water up to 30µl, and then denatured in boiling water for 5mins. Buffer (10µl), primer (5µl) and Polymerase (2µl) were then added into this 2ml centrifuge tube. The above mixture was then centrifuged immediately. The radioactive ^{32}P-dCTP (3µl) was added into mixture carefully. The mixture was incubated at 37°C for half hour.

2.3.9.5 Hybridisation

TE buffer (400µl) was added into each ^{32}P-dCTPs labelled probe solution. Each ^{32}P-dCTPs labelled probe was denatured at 95°C for 5mins and then carefully added into each hybridisation tube. The hybridisation tube was incubated at 65°C overnight.

2.3.9.6 Blot Washing

A solution of 2×SSC/0.1% SDS was preheated to 65°C before using. After overnight hybridisation, each piece of Amersham Hybond™-XL membrane (GE Healthcare Life Sciences®) was washed with the 2×SSC/0.1% SDS solution in a plastic tray until no radioactive counts remained in the washing buffer. Each membrane was carefully wrapped in cling film and fixed into a cassette.

2.3.9.7 Phosphor Imaging

A phosphor-imaging screen was erased by light for 15mins and placed on the wrapped membrane in the cassette for phosphor imaging. After a few days, the phosphor imaging screen was scanned in a Molecular Imager FX machine (BIO-RAD®). The images were then analysed with Quantity One 1-D analysis Software (BIO-RAD®).

2.3.10 Global Transcription Profiling Analyses

The principle of the microarray assay is to hybridise a labelled cDNA library from a particular treatment against an array of probes which may represent each gene in the geneome, printed or spotted at high densities (e.g. 6,000 spots per cm^2). The arrays are then scanned optically to identify which genes have become labelled by the hybridisation. Because the optical scan can be quantitative, the results can reveal quantitatively the patterns of gene expression following that treatment. A variety of different methods have been used to produce microarrays. The two main methods are to spot cDNA's or oligonucleotides onto the slide, or to synthesise in situ on the slide oligonucleotides that represent the genes in the genome. The latter technology developed by a company named Affymetrix has been widely adopted by the Arabidopsis community. The basic Affymetrix technology is briefly introduced in this section.

GeneChip® Arabidopsis ATH1 Genome array, one kind of commercial product from Affymetrix company, covers the entire Arabidopsis genome and contains more than 225,000 specific probe-sets for approximately 24,000 Arabidopsis genes. **Table 2.2** displays the specificity of Arabidopsis ATH1 Genome Array.

Table 2.2: Specifications of Arabidopsis ATH1 Genome Array.
(www.affymetrix.com/products_services/arrays/specific/arab.affx)

Information	Description
Number of arrays	one
Number of sequence represented	> 24,000 gene sequences
Feature size	18μm
Oligo-nucleotide probe length	25-mer
Probe pairs	11
Control Sequences	Arabidopsis maintenance genes GAPDH, Ubituitin and Actin

The syntheses of oligonucleotides on Affymetrix chip are based on photolithography technology. Each probe set on Affymetrix chip is consisted with 22 different oligonucleotides for both perfect match oligo and mismatch oligo. Each oligonucletides consists of 25 bases (**Figure 2.2.A**). The light goes through the openings of the mask on the top of strand and then removes the protective group of strands. Next, the free nucleotides are able to combine with the non-protective strand. After replication of this reaction each probe is synthesized *in situ* till there are 25 nucleotides (**Figure 2.2.B**).

The labelling procedure of Affymetrix technology is illustrated in **Figure 2.2.C**. First of all, the total RNA is extracted from the tissues, for instance, fresh seedlings of Arabidopsis. Next, the double-stranded cDNA is synthesized by reverse transcription. After the *in vitro* transcription by T7 RNA polymerase, the double-stranded cDNA transform to the single strand of cRNA (the complementary RNA of cDNA). At the same time, Biotin is tagged onto all uracil bases of cRNA. Next, the Biotin-labeled cRNA is randomly fragmented into 30 to 400 base pairs and added onto the Affymetrix array for hybridisation. After washing to remove the non-hybridised cRNA, the hybridised Biotin-labelled cRNA is incubated with a streptavidin dye conjugate which binds specially to Biotin and gives a fluorescent signal. Finally, the whole Affymetrix array is scanned and quantitatively analysed by the computer. The raw data of fluorescent signal is then transferred to give the gene expression level.

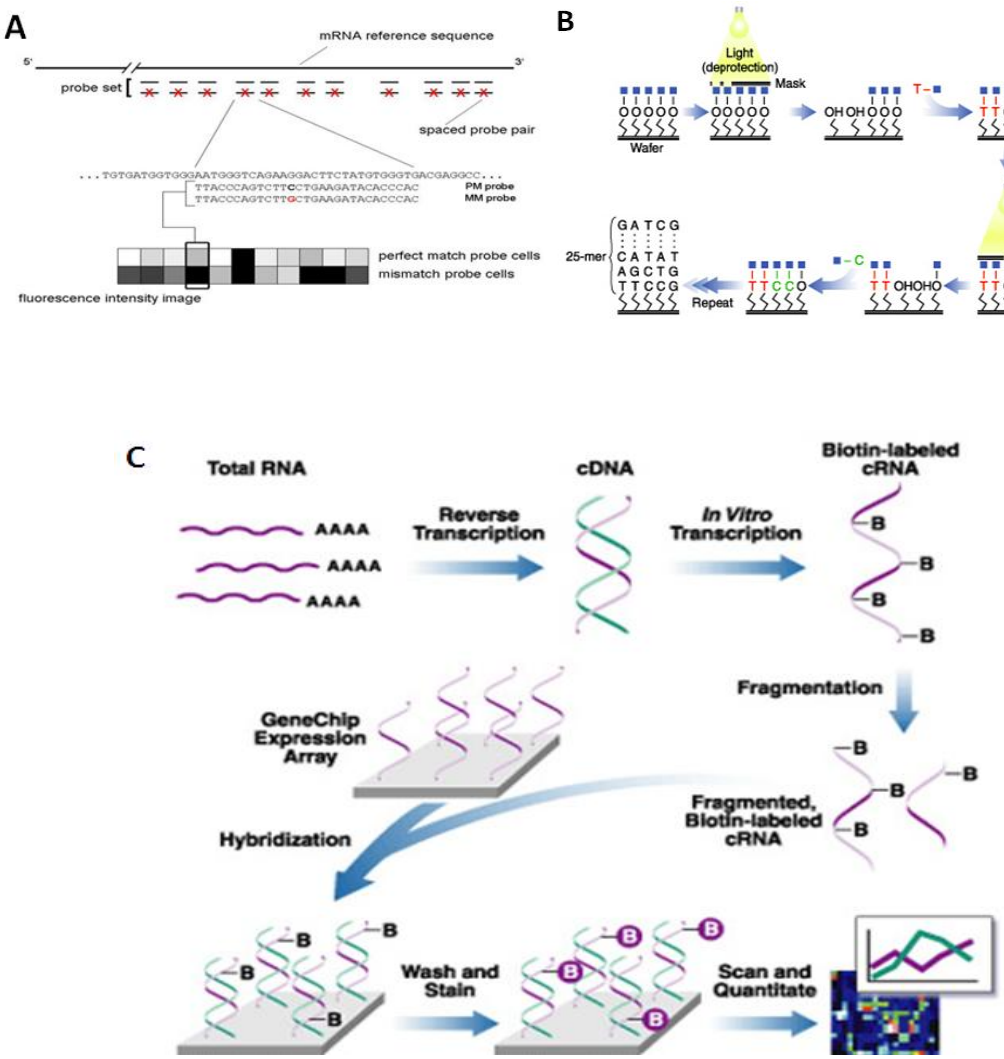


Figure 2.2: The principle of Affymetrix technology.
(http://www.dkfz.de/gpcf/24.html)

A. Each probe set on the Affymetrix chip consists of 22 different oligocleotides;

B. The *in situ* syntheses of oligonucleotides on Affymetrix chip by photolithography;

C. The labelling procedure of Affymetrix technology.

2.3.11Quantitative Real-time RT-PCR

In general, TaqMan® and SYBR®-Green are two kinds of method to detect the PCR-products of quantitative real time RT-PCR. **Figure 2.2** displays the Principle of TaqMan® and SYBR®-Green based RT-PCR.

TaqMan® based qRT-PCR requires one pair of target gene-specific primers, and one fluorescent probe which hybridises to the target sequence between the primers. The fluorescent oligonucleotide probe contains a reporter fluorescent dye and a quencher dye. On the intact probe, the quencher dye reduces the reporter fluorescence by Fluorescence Resonance Energy Transfer (FRET). As a consequence of each PCR cycle, the fluorescent probe is cleaved by the 5' nuclease activity of TaqDNA polymerase, releasing the fluorescent dye from the quencher. Therefore, the fluorescent signal increases with each cycle, corresponding to the amount of cDNA (**Figure 2.3**). TaqMan® based detection is therefore quantitative, and has very high specificity for targeting the gene. Because of the high costs for synthesis of the unique probe for each target gene, the TaqMan® based method is not the best suited for repeated assays of selected genes.

The SYBR-Green based method has been extensively applied for real time PCR amplification. The asymmetrical cyanine dye SYBR-Green preferentially binds to the double-stranded cDNA and becomes fluorescent (Zipper et al., 2004). The increased fluorescence is detected at the end of each PCR cycle when the products anneal to form double stranded cDNA. Highly specific primers for the target genes are essential for increasing the specificity of the SYBER-Green method (Udvardi et al., 2008).

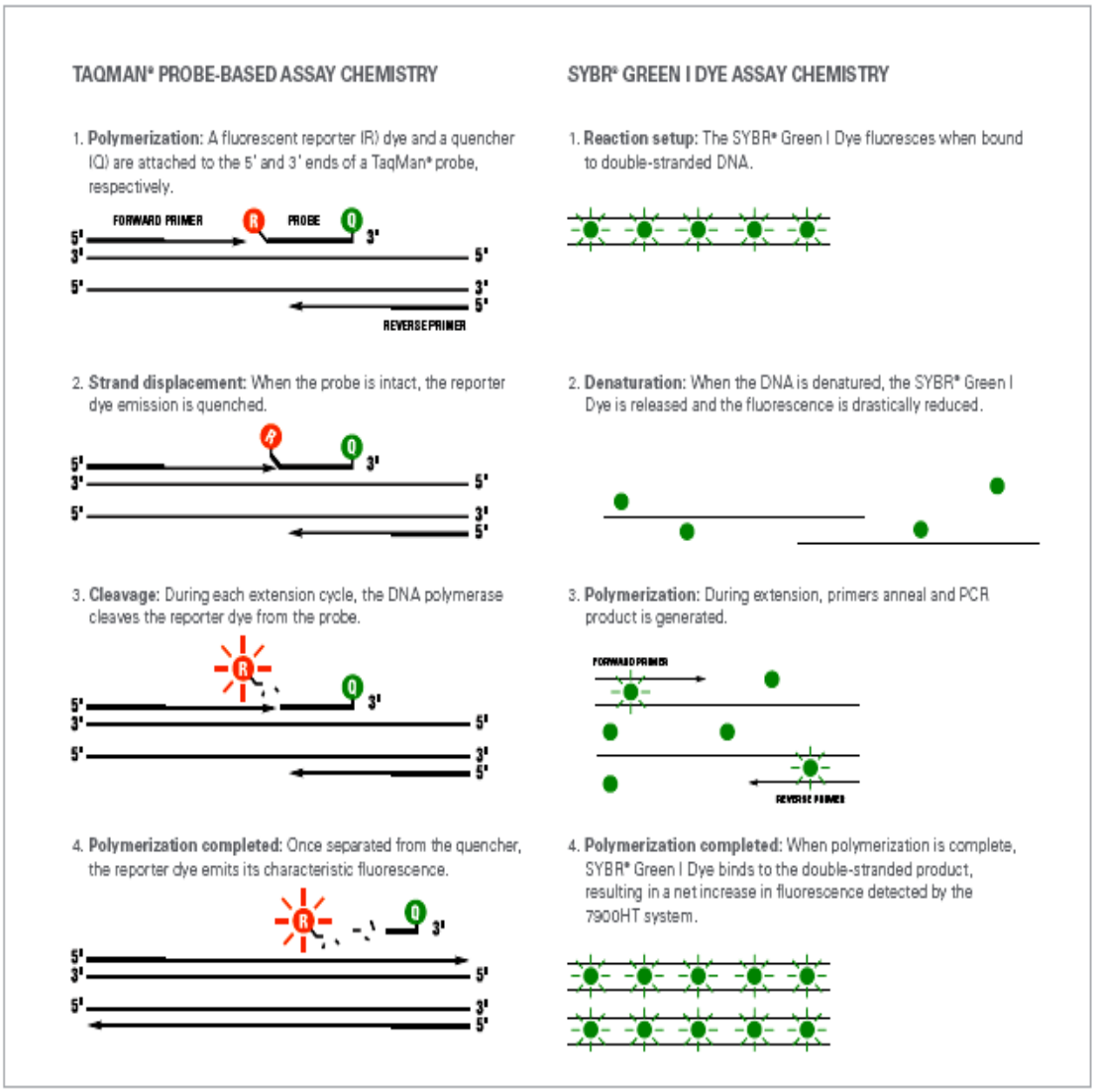


Figure 2.3: Principle of TaqMan® and SYBR®-Green based RT-PCR.

(http://www.appliedbiosystems.com)

2.3.11.1 Plant Preparation for qRT-PCR

For the qRT-PCR experiments, Arabidopsis seeds of *Col-0*, the single mutant *jin1*-1, *jut*, *coi1*-16, *jai3*-1, the double mutant *jin1*-1/*jut* and one constitutive expression line JUTOE were germinated and grown on 1/2 MS media in a LD growth chamber. After two weeks, fourteen seedlings of each line were carefully transferred with sterilised fine forceps onto 20µM MeJA MS plate for each treatment, such as 30mins, 1h, 3h and 6h. An equal number of Arabidopsis seedlings were transferred onto 1/2 MS media and

then collected at the indicated times as the control sample. Fourteen whole seedlings were collected at the indicated times and pooled for each biological replicate. Three biological replicates were individually harvested for quantitative real time RT-PCR.

2.3.11.2 RNA Samples for qRT-PCR

The Arabidopsis total RNA extraction for qRT-PCR was performed following the protocol of **section 2.3.2**. The RNA Quantification and concentration were checked with the protocol of **section 2.3.5**.

2.3.11.3 DNaseI Treat RNA Samples

The genomic DNA was removed following the protocol of Deoxyribonuclease I kit (Amplification Grade, Invitrogen ®). RNase-free water and 0.5ml micro-centrifuge tubes were put on ice in advance. The total RNA (1μg), 10×DNase I reaction buffer (1μl) and DNase I (1μl) were added into each 0.5ml micro-centrifuge tube on ice. The RNase-free water was then added up to 10μl solution. All tubes were incubated at room temperature for 15mins. (**Note:** Mg^{2+}-dependent hydrolysis of RNA could be formed under higher temperature and longer time, so the incubation time was exactly controlled no longer than 15mins.) And then EDTA solution (1μl, 25mM) was added into reaction before heat incubation in order to inactivate the DNase I. The reaction mixture was then incubated at 65 °C for 10mins. The DNase I-treated RNA samples were stored at -20 °C and ready for the reaction of reverse transcription.

2.3.11.4 Reverse Transcription Reaction

Each DNase I-treated RNA sample (1μg, 0.1μg/μl) was transferred into a PCR reaction tube. Random Hexamers (QIAGEN®) (1μl) were added into one PCR tube. The PCR tube was then incubated in Peltier Thermal Cycler PCR machine at 70°C for 10mins. DTT buffer (in Superscript Kit) and 5×buffer (in Superscript Kit) were defrosted at 4°C

before using. The Superscript II (Invitrogen®) and RNase inhibitor (Promega®) were kept in a freezer before using. Each PCR tube was transferred onto ice and then shortly spun at 10,000rpm at 4 °C. Four micro-litre (µl) of 5×buffer, 2µl of DTT, 1µl of dNTPs (Roche®), 1µl of Superscript II and 1µl of RNase inhibitor were added into the reaction tube. (**Note:** The superscript and RNase inhibitor were added in the last step and then mixed very well.) Each reaction tube was incubated in Peltier Therma Cycler PCR machine at 42 °C for 1 hour. Then the stable cDNA products were stored at -20°C and ready for quantitative real time PCR reaction. The estimated concentration of cDNA is 50ng/µl.

2.3.11.5 Quantitative Real-time PCR Using SYBR®-Green Detection Chemistry

1) Make the solutions for the standard curve

All cDNA samples (50ng/µl) were defrosted at room temperature for 10mins with gentle flicking and spinning. Each cDNA sample (2µl, 50ng/µl) was pooled into one 0.5ml tube. The pooled cDNA samples (50ng/µl) were diluted in six tubes for one standard curve with six samples as 50ng, 25ng, 12.5ng, 6.25ng, 3.125ng and 1.5625ng, respectively.

2) Loading the samples into a 96-well plate

Each cDNA sample (4µl, 50ng/µl) was diluted with 396µl of RNAse/DNAse free water (Primerdesign®) to the concentration of 0.5ng/µl. The cDNA template (10µl, 0.5ng/ul) was loaded into the sample wells. The cDNA samples of 50ng, 25ng, 12.5ng, 6.25ng, 3.125ng and 1.5625ng were loaded into six wells for generating one standard curve. Another 10µl of RNAse/DNAse free water (Primerdesign®) was loaded for no cDNA template control (NTC) in order to check the foreign cDNA contaminations.

3) Primer preparation for qRT-PCR

The lyophilised primer mix (Primerdesign®) was quickly centrifuged before opening. RNAse/DNAse free water (Primerdesign®) (660µl) was added into each lyophilised primer mix for a volume about 600 reactions. Each tube was thoroughly vortexed and then left at room temperature for 5mins. These re-suspended primer mixtures were quickly vortexed before using.

4) Reaction mixture preparation for qRT-PCR

The Reaction mixture for each qRT-PCR reaction (15µl) was prepared as follows: 1µl of Re-suspended Primer mix (Primerdesign®), 10µl of 2× Precision^{TM} Master Mix (Primerdesign®) and 4µl RNAse/DNAse free water (Primerdesign®). And then 15µl of reaction mix (without cDNA template) was carefully transferred into each well on one 96-well plate.

5) qRT-PCR reactions

The reaction was processed in a TaqMan machine (ABI®7500 Fast Real-Time PCR System) using the following temperature and time conditions: 95°C for 10 minutes, and 40 cycles for reactions as 95°C for 15 seconds and 60°C for 60 seconds. The dissociation curves for checking the specific product from high specific primers were generated as 95°C for 15 seconds, 60°C for 60 seconds and 95°C for 15 seconds.

2.3.12 Reagent Preparation

1) DNA extraction buffer

DNA extraction buffer (50mls) was prepared as follows: 5mls of 1M Tris-Cl (pH= 8.0), 5mls of 0.5M EDTA (pH= 8.0), 5mls of 5M NaCl and 7.5mls of 10% SDS and 27.5ml

sterilised water. The antioxidant β-Mercaptoethanol (50μl) was added into 50ml of DNA extraction buffer before using.

2) RNA extraction buffer

The antioxidant β-Mercaptoethanol (100μl) was added into 10ml RLT Buffer (RNeasy Plant Mini Kit of QIAGEN®).

3) 0.1%DEPC water

DEPC (Sigma®) 0.9ml was carefully added into the 900ml water. The solution was mixed well by shaking. The solution was covered with foil left overnight and autoclaved the next day.

4) 10×MOPS buffer

One litre of 10×MOPS Buffer was prepared as follows: 92.48g MOPS, 100mM NaAc (8.2g NaAc in 0.1%DEPC water), 20ml of 0.5M EDTA (pH=8.0, 0.1%DEPC water) and 0.1% DEPC water was added up to 1 litre and stored at 4 °C in dark condition.

5) 20×SSC buffer

One litre of 20×SSC buffer was prepared as follows: 175.32g NaCl and 88.23g NaAc were dissolved in 1 litre of sterilised water.

6) 5×RNA loading dye

5×RNA loading dye was prepared as follows: 0.75ml of Dimethyl fomamide NN, 0.15ml of 10×MOPS buffer and 0.5ml glycerol were mixed together. Bromophenol Blue (80μl, 0.01g/ml) was added into the mixture. The 5×RNA loading dye was then stored at -20°C before using.

7) 100bp DNA ladder

Two-hundred microlitres of 100bp DNA ladder were prepared as follows: 100bp DNA ladder stock solution (20µl, 1µg/µl), 6×DNA loading dye (30µl) and Tris (150µl, 10mM) were mixed well and then stored at -20°C before using.

8) 50×TAE

Tris-acetate (252g), glacial acetic acid (57.1ml) and EDTA (18.6g) were added to make 1 litre of 50×TAE stock solution. The pH value was adjusted to 8.0 before using.

9) 0.5M EDTA (pH 8.0)

Disodium EDTA-2 H_2O (186.1g) was added into 800mls of distilled water and stirred by a magnetic stirrer. NaOH pellets (20g) were used to adjust the pH to 8.0.

10) TE pH 8.0

Tris.Cl (10mM) and EDTA (1mM) were mixed together. Concentrated HCl was used to adjust the pH to 8.0.

2.3.13Primers Used in This Research

2.3.13.1 Primers Used for Confirming the T-DNA Insertion on Target Genes

Forward and reverse primers used for PCR in this research were designed by T-DNA primer design tool (http://signal.salk.edu/tdnaprimers.2.html). Primer LP was designed on the 5' end of T-DNA insertion, and primer RP was designed on the 3'end of T-DNA insertion in the region of flanking sequence. T-DNA left border primers were downloaded from the SALK database (http://signal.salk.edu/). Primers used for confirming the T-DNA insertion on target genes were shown in **Table 2.3**.

Table 2.3: Primers used for confirming the T-DNA insertion on target genes.

Gene	Primer sequences (from 5'to 3')
At3g50060	**LP(1):** GATGAGCAGCTACGAAGGATG
	RP(1): TGGTTATGAATCACCAAAACAAG
	LP(2): ATCCTCCAATATTTCCCGACC
	RP(2): ACAGATCTCCTCTTCTTCGGC
At2g46510	**LP(3):** TGGTTTCAAGCATCGTTGAGT
	RP(3): GGCAAAGACCAAACAGAACC
At2g23290	**LP (4):** AATGAGTCAACAACTCCACCG
	RP (4): TTGCAGAGCCACAGTATTGG
T-DNA left border	**LBa1:** GCGTGGACCGCTTGCTGCAACT
	LBb1: TGGTTCACGTAGTGGGCCATCG
	LB1: GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC

2.3.13.2 Primers Used for Identifying the *JUT* Gene Over-expression Line

Forward and reverse primers using for PCR in this section were designed by Primer3 online tool (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). The *JUT* gene over-expression line (JUTOE) was donated from Dr. Hongmei Li. As shown in **Figure 2.4**, primer CF1 was designed on the 35S promoter sequence of *Cauliflower mosaic virus*, and primer R1 was designed on the coding sequence of At2g46510 (JUT). The information of Primer CF1 and R1 was shown in **Table 2.4**.

Table 2.4: Primers used for identifying the *JUT* gene over-expression line.

Primer location	Primer sequences (from 5'to 3')
35S promoter	**CF1:** GATGTGATATCTCCACTGACG
JUT gene	**R1:** TCTCCGAGTTTGGCCAATCAA

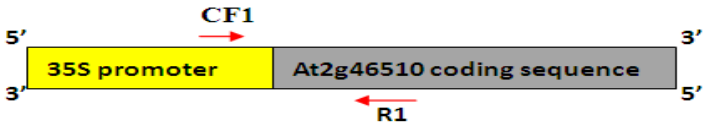


Figure 2.4: Schematic diagram of primer CF1 and R1.
The 35S promoter sequence of *Cauliflower mosaic virus* is shown in yellow. The At2g46510 coding sequence is shown in gray. The primers of CF1 and R1 are shown as red arrows.

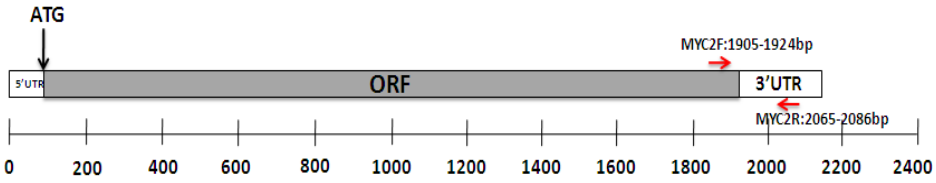
2.3.13.3 Primers for Target Gene Probes in Northern Blotting & Hybridisation

Forward and reverse primers used for PCR in this section were designed by Primer3 online tool (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Primers of MYC2F and MYC2R were designed for amplifying the *AtMYC2* probe and locate on the C-terminal deletion of MYC2 (**Figure 2.5.A**), and primers of JUTNF and JUTNR probe were designed for amplifying the *JUT* probe and locate on the 3' end of the At2g46510 gene (**Figure 2.5.B**). Primers used for MYC2 probe and JUT probe were shown in **Table 2.5**.

Table 2.5: Primers used for target gene probes in Northern blotting and hybridisation.

Gene	Primer sequences (from 5'to 3')
***AtMYC2* (At1g32640)**	**MYC2F:** GCAAGAACAGCTCAGAGCAA
	MYC2R: GAGCAAAAGAAGAAACTTGTCG
***JUT* (At2g46510)**	**JUTNF:** GGACAAGGCTTCTCTACTTGGA
	JUTNR: GCTGGATGTGAATCCAAAGG

A. Primers for *AtMYC2* probe



B. Primers for *JUT* probe

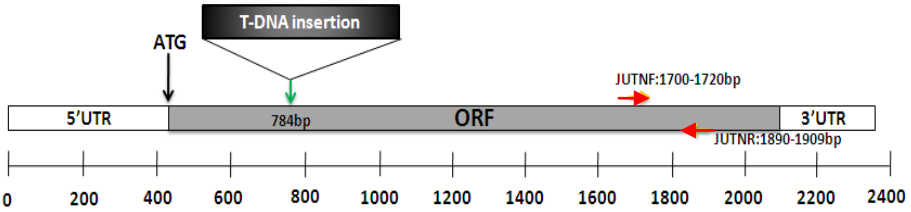


Figure 2.5: Schematic diagrams of primers for target gene probe used in Northern blotting & hybridisation.

The primers for the *AtMYC2* probe in the *AtMYC2* gene sequence are shown in diagram **A**. The primers for the *JUT* probe in the *JUT* gene are shown in diagram **B**. The white bars denote the 5'UTR and 3'UTR; gray bars denote the ORF region; Black bar denotes the T-DNA insertion. Start code of the gene is shown as ATG. Red arrows show the primer locations. The green arrow shows the T-DNA insertion site. The numbers of nucleotides are shown below each diagram.

2.3.13.4 Primers Used in PCR Identification of Double Mutant *jin1*-1/*jut*

Primers used for PCR identification of the double mutant *jin1*-1/*jut* were shown in **Table 2.6**. Primers in group A (**MYC2F** and **MYC2R** in **Figure 2.5.A**) were designed to amplify a *AtMYC2* product from wild type, but not from *jin1*-1 mutant. Primers in group B (**LP(3)** and **RP(3)**, **F(1)** and **R(1)** in **Figure 2.6**) were designed to amplify two *JUT* specific products from wild type, but not from the *jut* mutant, where the presence of the T-DNA would make the fragment too large to be amplified. Primers in group C (**LBb1** and **RP(1)** in **Figure 2.6**) were designed to amplify the T-DNA insertion specific product from *jut*, but not from wild type. Primers in group D (**CF1** and **R1** in **Figure 2.4**) were designed to amplify a 35S::*JUT* product.

Table 2.6: Primers used in PCR identification of the double mutant *jin1*-1/*jut*.

Primers Group	Primer sequences (from 5'to 3')
Group A (for *AtMYC2* gene)	**MYC2F**: GCAAGAACAGCTCAGAGCAA **MYC2R:** GAGCAAAAGAAGAAACTTGTCG
Group B (for *JUT* gene)	**LP(3):** TGGTTTCAAGCATCGTTGAGT **RP(3):** GGCAAAGACCAAACAGAACC **(F1):** TGTCGAGAGCCTAATGAGGAA **(R1):** CCACCAAACAACCTATGAAGC
Group C (for T-DNA left border)	**(R1):** CCACCAAACAACCTATGAAGC **LBb1:** TGGTTCACGTAGTGGGCCATCG
Group D (for over-expressed JUT)	**CF1:** GATGTGATATCTCCACTGACG **R1:** TCTCCGAGTTTGGCCAATCAA

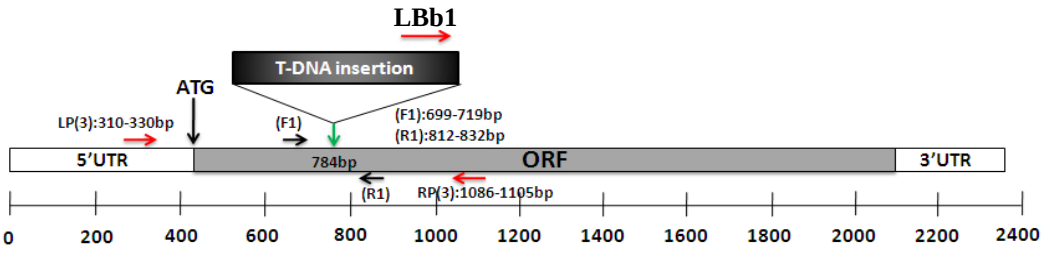


Figure 2.6: Schematic diagrams of primers in group B and group C.
The white bars denote the 5'UTR and 3'UTR; gray bars denote the ORF region; Black bar denotes the T-DNA insertion. Start code of the gene is shown as ATG. Red and black arrows show the primer locations. The green arrow shows the T-DNA insertion site. The numbers of nucleotides are shown below the diagram.

2.3.13.5 Primers Used in Quantitative Real-time RT-PCR

Quantitative real-time RT-PCR primers for a panel of candidate genes were designed by Primerdesign® and shown in **Table 2.7**. Blast analyses (from page 59 to page 68) indicated that the primers used for qRT-PCR are highly specific primers to individual target genes.

Table 2.7: Quantitative Real-time RT-PCR primers for the candidate genes.

Gene	Primer sequences (from 5'to 3')
At1g32640 (*AtMYC2*)	Sense primer: CGTCGTGAAAGAAGTAGCAGTA
	Anti-sense primer: CGTGGTTTAGTGGCTCTTCTC
At2g46510 (*JUT*)	Sense primer: GCCAAACTCGGAGAATTTCA
	Anti-sense primer: GCTCTCGACAACACCCATCT
At2g24850 (*TAT3*)	Sense primer: CGCCATGAACGATCCTAATG
	Anti-sense primer: GAAGTGCTTCCTGGAGAATAAAT
At3g18780 (*ACTIN2*)	Sense primer: GGTGGTTCCATTCTTGCTTCC
	Anti-sense primer:CCTTTGATCTTGAGAGCTTAGAAAC
At1g19180 (*JAZ1*)	Sense primer: CAACATCGCTACTATCGCAAAC
	Anti-sense primer: GCAATAGGAAGTTCTGTCAATGG
At5g13220 (*JAZ10*)	Sense primer: CTTTATTGTCATTGATAGCTTGATTTA
	Anti-sense primer: GCGTGTTATAATTTTCTTTACCATATA
At3g49620 (*DIN11*)	Sense primer: ACTATGGCTTGTTGACGCTTAT
	Anti-sense primer: CACCGATGTTGCAGATAAATGAT
At1g66690	Sense primer: AACCTCGACCTAGACTTGATTTC
	Anti-sense primer: ACTTCTCTTCCACGGCATCT
At2g26020 (*PDF1.2b*)	Sense primer: AGAAGTTGTGCGAGAAGCC
	Anti-sense primer: AGATCCATGTTTTGCTCCTTCA
At1g53885	Sense primer: ACAACTCCATCAAGACAAAGAT
	Anti-sense primer: AAGCCAACATCATCTTCGTC

1) The primers for candidate gene of At1g32640

A. Primer information for At1g32640 (*AtMYC2*):

Official gene symbol	MYC2
Sequence length	2,179 bp
Product length	86bp
Sense primer	5'CGTCGTGAAAGAAGTAGCAGTA3'
Anti-sense primer	5'CGTGGTTTAGTGGCTCTTCTC3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5'CGTCGTGAAAGAAGTAGCAGTA3'

```
>>AT1G32640.1 | Symbols: RD22BP1, JAI1, JIN1, MYC2, ZBF1  (2179 nt)
 initn: 110 init1: 110 opt: 110  Z-score: 160.3  bits: 36.8 E(): 0.011
banded Smith-Waterman score: 110;  100.000% identity (100.000% similar) in 22 nt
overlap (1-22:1350-1371)
                                          10        20

                                    CGTCGTGAAAGAAGTAGCAGTA

                                    ::::::::::::::::::::::

AT1G32 AGAATCAGATCACTCCGATCTAGAAGCTTCCGTCGTGAAAGAAGTAGCAGTAGAGAAACG

    1320      1330      1340      1350      1360      1370
```

b. Anti-sense Primer: 5'CGTGGTTTAGTGGCTCTTCTC3'

```
>>AT1G32640.1 | Symbols: RD22BP1, JAI1, JIN1, MYC2, ZBF1  (2179 nt)
rev-comp initn: 105 init1: 105 opt: 105  Z-score: 161.8  bits: 37.0 E(): 0.0091
banded Smith-Waterman score: 105;  100.000% identity (100.000% similar) in 21 nt
overlap (21-1:1415-1435)
                                    20        10

-                                   GAGAAGAGCCACTAAACCACG

                                    :::::::::::::::::::::

AT1G32 AGAAACGAGGAAGAAAGCCAGCAAACGGTAGAGAAGAGCCACTAAACCACGTCGAAGCAG

         1390      1400      1410      1420      1430      1440
```

2) The primers for candidate gene of At2g46510

A. Primer information for At2g46510 (*AtAIB*):

Official gene symbol	At2g46510
Sequence length	2,363bp
Product length	107bp
Sense primer	5’ GCCAAACTCGGAGAATTTCA 3’
Anti-sense primer	5’ GCTCTCGACAACACCCATCT 3’

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5’ GCCAAACTCGGAGAATTTCA 3’

```
>>AT2G46510.1 | Symbols: ATAIB, AIB | ABA-inducible BHLH  (2363 nt)

 initn: 152 init1: 152 opt: 152  Z-score: 131.2  bits: 31.4 E():  0.7

Smith-Waterman score: 152;  100.000% identity (100.000% similar) in 20 aa overlap
(1-20:602-621)

                                          10        20
                                    GCCAAACTCGGAGAATTTCA

                                    ::::::::::::::::::::

AT2G46 GAATAAGAAGCTCTCTAGTCTCGTTGATTGGCCAAACTCGGAGAATTTCAGCTGGAACTA

             580       590       600       610       620       630
```

b. Anti-sense Primer: 5’ GCTCTCGACAACACCCATCT 3’

```
>>AT2G46510.1 | Symbols: ATAIB, AIB | ABA-inducible BHLH  (2363 nt)

 initn: 143 init1: 143 opt: 143  Z-score: 139.5  bits: 32.9 E(): 0.24

Smith-Waterman score: 143;  100.000% identity (100.000% similar) in 20 aa overlap
(1-20:689-708)

                                            10        20

                                      AGATGGGTGTTGTCGAGAGC

                                      ::::::::::::::::::::

AT2G46 TAGATCCGGACAACAAGTCTTAGGTTGGGGAGATGGGTGTTGTCGAGAGCCTAATGAGGA

      660       670       680       690       700       710
```

3) The primers for candidate gene of At2g24850

A. Primer information for gene of At2g24850 (*AtTAT3*):

Official gene symbol	TAT3
Sequence length	1,642bp
Product length	110bp
Sense primer	5'CGCCATGAACGATCCTAATG3'
Anti-sense primer	5'GAAGTGCTTCCTGGAGAATAAAT3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5'CGCCATGAACGATCCTAATG3'

```
>>AT2G24850.1 | Symbols: TAT, TAT3 | TAT3 (1642 nt)
 initn: 160 init1: 160 opt: 160  Z-score: 134.1  bits: 31.4 E(): 0.45
Smith-Waterman score: 160;  100.000% identity (100.000% similar) in 20 aa overlap
(1-20:880-899)
                                              10        20

                                     CGCCATGAACGATCCTAATG

                                     ::::::::::::::::::::

AT2G24 GGTCAACCCAGGCTGGAGAGTTGGCTGGATCGCCATGAACGATCCTAATGGTATCTTTGT

     850       860       870       880       890       900
```

b. Anti-sense Primer: 5'GAAGTGCTTCCTGGAGAATAAAT3'

```
>>AT2G24850.1 | Symbols: TAT, TAT3 | TAT3 (1642 nt)
rev-comp initn: 115 init1: 115 opt: 115  Z-score: 172.2  bits: 38.7 E(): 0.0032
banded Smith-Waterman score: 115;  100.000% identity (100.000% similar) in 23 nt
overlap (23-1:967-989)
                                       20        10

-                                    ATTTATTCTCCAGGAAGCACTTC

                                     ::::::::::::::::::::::

AT2G24 GGATTTTCTTGATTTAACTCCACAGCCTTCATTTATTCTCCAGGAAGCACTTCCTGATAT

        940       950       960       970       980       990
```

4) The primers for candidate gene of At3g18780

A. Primer information for gene of At3g18780 (*ACTIN2*):

Official gene symbol	ACT2
Sequence length	1,756bp
Product length	122bp
Sense primer	5'GGTGGTTCCATTCTTGCTTCC3'
Anti-sense primer	5'CCTTTGATCTTGAGAGCTTAGAAAC3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5'GGTGGTTCCATTCTTGCTTCC3'

```
>>AT3G18780.2 | Symbols: DER1, LSR2, ENL2, ACT2 (1756 nt)
 initn: 105 init1: 105 opt: 105  Z-score: 156.4  bits: 35.7 E(): 0.023
banded Smith-Waterman score: 105;  100.000% identity (100.000% similar) in 21 nt
overlap (1-21:1280-1300)
                                              10        20

                                     GGTGGTTCCATTCTTGCTTCC

                                     :::::::::::::::::::::

AT3G18 CCACCTGAAAGGAAGTACAGTGTCTGGATCGGTGGTTCCATTCTTGCTTCCCTCAGCACA

    1250      1260      1270      1280      1290      1300
```

b. Anti-sense Primer: 5'CCTTTGATCTTGAGAGCTTAGAAAC3'

```
>>AT3G18780.2 | Symbols: DER1, LSR2, ENL2, ACT2 (1756 nt)
rev-comp initn: 125 init1: 125 opt: 125  Z-score: 174.2  bits: 39.2 E(): 0.0023
banded Smith-Waterman score: 125;  100.000% identity (100.000% similar) in 25 nt
overlap (25-1:1377-1401)
                                         20        10

-                                    GTTTCTAAGCTCTCAAGATCAAAGG

                                     :::::::::::::::::::::::::

AT3G18 AGGCAGGTCCAGGAATCGTTCACAGAAAATGTTTCTAAGCTCTCAAGATCAAAGGCTTAA

       1350      1360      1370      1380      1390      1400
```

5) The primers for candidate gene of At1g19180

A. Primer information for At1g19180 (*JAZ1*):

Official gene symbol	JAZ1
Sequence length	1,329 bp
Product length	102bp
Sense primer	5'CAACATCGCTACTATCGCAAAC 3'
Anti-sense primer	5'GCAATAGGAAGTTCTGTCAATGG 3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5' CAACATCGCTACTATCGCAAAC 3'

```
>>AT1G19180.1 | Symbols: JAZ1, TIFY10A | JAZ1/TIFY10A (1329 nt)

 initn: 110 init1: 110 opt: 110  Z-score: 174.6  bits: 38.7 E(): 0.0029

banded Smith-Waterman score: 110;  100.000% identity (100.000% similar) in 22 nt overlap
(1-22:728-749)
                                            10        20

                                          CAACATCGCTACTATCGCAAAC

                                        ::::::::::::::::::::::

AT1G19 CTTAGCCAAGAATCAAACCGATATCAGAAGCAACATCGCTACTATCGCAAACCAAGTTCC

           700       710       720       730       740       750
```

b. Anti-sense Primer: 5' GCAATAGGAAGTTCTGTCAATGG 3'

```
>>AT1G19180.1 | Symbols: JAZ1, TIFY10A | JAZ1/TIFY10A (1329 nt)

rev-comp initn: 115 init1: 115 opt: 115  Z-score: 179.7  bits: 39.7 E(): 0.0015

banded Smith-Waterman score: 115;  100.000% identity (100.000% similar) in 23 nt overlap
(23-1:807-829)

                                  20       10

-                                       CCATTGACAGAACTTCCTATTGC

                                        :::::::::::::::::::::::

AT1G19 ACACAAGAGCCAATCCAATCCTCCCCAACACCATTGACAGAACTTCCTATTGCTAGAAGA

           780       790       800       810       820       830
```

6) The primers for candidate gene of At5g13220

A. Primer information for At5g13220 (*JAZ10*):

Official gene symbol	JAZ10
Sequence length	1,033 bp
Product length	103bp
Sense primer	5'CTTTATTGTCATTGATAGCTTGATTTA3'
Anti-sense primer	5'GCGTGTTATAATTTTCTTTACCATATA 3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5' CTTTATTGTCATTGATAGCTTGATTTA 3'

```
>>AT5G13220.1 | Symbols: JAZ10, TIFY9, JAS1, AT5G13220 (1019 nt)

 initn: 135 init1: 135 opt: 135  Z-score: 189.8  bits: 41.5 E(): 0.00054

banded Smith-Waterman score: 135;  100.000% identity (100.000% similar) in 27 nt
overlap (1-27:921-947)
                                              10        20

                                       CTTTATTGTCATTGATAGCTTGATTTA

                                       ::::::::::::::::::::::::::

AT5G13 TATTGGTTTGTTTTTAAAAATGTCTATTATCTTTATTGTCATTGATAGCTTGATTTAAGA

              900       910       920       930       940       950
```

b. Anti-sense Primer: 5' GCGTGTTATAATTTTCTTTACCATATA 3'

```
>>AT5G13220.1 | Symbols: JAZ10, TIFY9, JAS1, AT5G13220 (1019 nt)

rev-comp initn: 115 init1: 115 opt: 115  Z-score: 156.8  bits: 35.3 E(): 0.037

banded Smith-Waterman score: 115;  100.000% identity (100.000% similar) in 23 nt
overlap (27-5:997-1019)
                                     20       10

-                                     TATATGGTAAAGAAAATTATAACACGC

                                      :::::::::::::::::::::::::::

AT5G13 GTGACCTTCTACTTTTGTTTTATTTTTTAGTATATGGTAAAGAAAATTATAACACGC

            970       980       990      1000      1010
```

7) The primers for candidate gene of At3g49620

A. Primer information for At3g49620 (*DIN11*):

Official gene symbol	DIN11
Sequence length	1,304 bp
Product length	123bp
Sense primer	5' ACTATGGCTTGTTGACGCTTAT3'
Anti-sense primer	5' CACCGATGTTGCAGATAAATGAT3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5' ACTATGGCTTGTTGACGCTTAT 3'

```
>>AT3G49620.1 | Symbols: DIN11 | DIN11 (DARK INDUCIBLE 11)  (1304 nt)

 initn: 110 init1: 110 opt: 110  Z-score: 179.1  bits: 39.5 E(): 0.0017

banded Smith-Waterman score: 110;  100.000% identity (100.000% similar) in 22 nt
overlap (1-22:774-795)
                                      10       20

                                     ACTATGGCTTGTTGACGCTTAT

                                     ::::::::::::::::::::::

AT3G49 AAAATGTTATTGGATGTGGAGCTCACACTGACTATGGCTTGTTGACGCTTATAAATCAAG

              750       760       770       780       790       800
```

b. Anti-sense Primer: 5' CACCGATGTTGCAGATAAATGAT 3'

```
>>AT3G49620.1 | Symbols: DIN11 | DIN11 (DARK INDUCIBLE 11)  (1304 nt)

rev-comp initn: 115 init1: 115 opt: 115  Z-score: 174.1  bits: 38.7 E(): 0.0031

banded Smith-Waterman score: 115;  100.000% identity (100.000% similar) in 23 nt
overlap (23-1:874-896)
                                  20       10

-                                      ATCATTTATCTGCAACATCGGTG

                                       :::::::::::::::::::::::

AT3G49 TGATTGGATACCAGCTATTCCGATCCCTGGATCATTTATCTGCAACATCGGTGACATGTT

              850       860       870       880       890       900
```

8) The primers for candidate gene of At1g66690

A. Primer information for At1g66690 (S-adenosyl-L-methionine:carboxyl ,ethyltransferase family protein):

Official gene symbol	At1g66690
Sequence length	1,114 bp
Product length	115bp
Sense primer	5' AACCTCGACCTAGACTTGATTTC3'
Anti-sense primer	5' ACTTCTCTTCCACGGCATCT3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5' AACCTCGACCTAGACTTGATTTC 3'

```
>>AT1G66690.1 | Symbols:  | S-adenosyl-L-methionine:carboxyl,ethyltransferase
(1114 nt)

 initn: 115 init1: 115 opt: 115  Z-score: 182.7  bits: 40.0 E(): 0.0012

banded Smith-Waterman score: 115;  100.000% identity (100.000% similar) in 23 nt
overlap (1-23:179-201)
                                        10       20

                                       AACCTCGACCTAGACTTGATTTC

                                       :::::::::::::::::::::::

AT1G66 AAGGACAAGATGACCGAGGCGATCTCCGCCAACCTCGACCTAGACTTGATTTCGAATCGC

          150       160       170       180       190       200
```

b. Anti-sense Primer: 5' ACTTCTCTTCCACGGCATCT 3'

```
>>AT1G66690.1 | Symbols:  | S-adenosyl-L-methionine:carboxyl,ethyltransferase
(1114 nt)

 initn: 140 init1: 140 opt: 140  Z-score: 127.2  bits: 29.6 E():  1.1

Smith-Waterman score: 140;  100.000% identity (100.000% similar) in 20 aa overlap
(1-20:274-293)
                                              10        20

                                       AGATGCCGTGGAAGAGAAGT

                                       ::::::::::::::::::::

AT1G66 TAACACTTTTGTGGCAGTCCAAAACATAATAGATGCCGTGGAAGAGAAGTATCTTAGAGA

                  250       260       270       280       290       300
```

9) The primers for candidate gene of At1g53885

A. Primer information for At1g53885 (senescence-associated protein-related):

Official gene symbol	At1g53885
Sequence length	637 bp
Product length	134bp
Sense primer	5'ACAACTCCATCAAGACAAAGAT3'
Anti-sense primer	5' AAGCCAACATCATCTTCGTC3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5' ACAACTCCATCAAGACAAAGAT 3'

```
>>AT1G53885.1 | Symbols:  | senescence-associated protein (637 nt)

 initn: 110 init1: 110 opt: 110  Z-score: 164.6  bits: 35.8 E(): 0.022

banded Smith-Waterman score: 110;  100.000% identity (100.000% similar) in 22 nt
overlap (1-22:228-249)
                                       10       20

                                      ACAACTCCATCAAGACAAAGAT

                                      ::::::::::::::::::::::

AT1G53 TTTCCTTAAAACATGTCATCTCTGCAATAAACAACTCCATCAAGACAAAGATGTTTACAT

           200       210       220       230       240       250
```

b. Anti-sense Primer: 5' AAGCCAACATCATCTTCGTC 3'

```
>>AT1G53885.1 | Symbols:  | senescence-associated protein (637 nt)

 initn: 137 init1: 137 opt: 137  Z-score: 129.0  bits: 29.1 E(): 0.87

Smith-Waterman score: 137;  100.000% identity (100.000% similar) in 20 aa overlap
(1-20:342-361)
                                       10       20

                                      GACGAAGATGATGTTGGCTT

                                      ::::::::::::::::::::

AT1G53 GATTGATGATAGAAAAGAACTAGAGGCTTCGACGAAGATGATGTTGGCTTCGTACAGACG

                320       330       340       350       360       370
```

10) The primers for candidate gene of At2g26020

Chapter 2: Materials and Methods

A. Primer information for At2g26020 (*PDF1.2b*):

Official gene symbol	PDF1.2b
Sequence length	274 bp
Product length	104bp
Sense primer	5' AGAAGTTGTGCGAGAAGCC3'
Anti-sense primer	5' AGATCCATGTTTTGCTCCTTCA3'

B. Primer blast results based on the FASTA in TAIR database

a. Sense Primer: 5' AGAAGTTGTGCGAGAAGCC 3'

```
>>AT2G26020.1 | Symbols: PDF1.2b (274 nt)

 initn: 140 init1: 140 opt: 140  Z-score: 143.2  bits: 30.4 E(): 0.14

Smith-Waterman score: 140;  100.000% identity (100.000% similar) in 19 aa overlap
(1-19:120-138)
                                      10

                                     AGAAGTTGTGCGAGAAGCC

                                     ::::::::::::::::::::

AT2G26 CTTTTGAAGTACCAACAATGGTGGAAGCACAGAAGTTGTGCGAGAAGCCAAGTGGTACTT

          90       100       110       120       130       140
```

b. Anti-sense Primer: 5' AGATCCATGTTTTGCTCCTTCA 3'

```
>>AT2G26020.1 | Symbols: PDF1.2b (274 nt)

rev-comp initn: 110 init1: 110 opt: 110  Z-score: 173.1  bits: 36.2 E(): 0.017

banded Smith-Waterman score: 110;  100.000% identity (100.000% similar) in 22 nt
overlap (22-1:202-223)
                                  20       10

-                                      TGAAGGAGCAAAACATGGATCT

                                       ::::::::::::::::::::::

AT2G26 CAATGCATGCAAGAATCAGTGCATTAACCTTGAAGGAGCAAAACATGGATCTTGCAACTA

               180       190       200       210       220       230
```

Chapter 3

Investigation of Jasmonate Responsive Transcription Factors

3.1 Introduction

As described in the **section 1.4.3**, MYC2 has been identified as an important transcription regulator in the JA signal pathway. In this chapter, the expression profile of wounding and JA responsive transcription factor coding genes are investigated from publically available databases. T-DNA insertion mutants of selected candidates are verified, and one of these TF was characterised here as a component of the JA signal pathway.

T-DNA originates from the tumour-inducing (Ti) plasmid of *Agrobacterium tumefaciens*, and is stably inserted into the host plant genomic DNA (Krysan et al., 1999), more or less randomly into the promoter and transcriptional regions on the Arabidopsis genome (Kim SJ, 2007). The standard analysis for T-DNA insertion mutants is based on the PCR-based identification of the T-DNA insertion in the target genes (Tax FE, 2001).

In this Chapter, PCR was used to confirm the T-DNA insertions in transcription factor genes. A goal for this is to identify a homozygous line for each T-DNA insertion, which could then be used to examine the effect of MeJA on that line. In this chapter, the root growth inhibition of the T-DNA mutants and wild type was compared.

3.2 Results

3.2.1 Investigation of Wounding and JA Responsive TFs

3.2.1.1 Search for Arabidopsis Transcription Factors via the Plant TF Database.

Arabidopsis contains 135 bHLH (**Appendix Table A.1**), 143 MYB (**Appendix Table A.2**) and 145 AP2-EREBP transcription factors (**Appendix Table A.3**). Lists of these transcription factors were downloaded from a plant TF Database (http://plntfdb.bio.uni-potsdam.de/v2.0/), and then their transcription regulation was analysed by Genevestigator (https://www.genevestigator.com/gv/index.jsp).

3.2.1.2 Investigating Gene Expression Profile from Genevestigator

As described in **section 2.1.1.2**, a subset of the 4069 arrays on Genevestigator were used to analyse gene expression profiles following treatments (wounding and MeJA) and in specific genetic backgrounds (eg. *coi1*). For this analysis, 135 bHLH TF, 143 MYB TF and 146 AP2-EREBP TF were entered into the gene selection window and their expression was analysed. The original microarray data collected from wounding and MeJA treatment were downloaded from ArrayExpress (http://www.ebi.ac.uk/microarray-as/ae/) for an investigation of responsive genes.

According to the expression profiles after wounding and MeJA treatments in comparison with the mock treatment, six transcription factor coding genes were selected as the wounding and MeJA responsive genes. In 135 bHLH TF coding genes, At1g32640 and At2g46510 were selected as the up-regulated genes at 15min, 30min and 1h after wounding treatment (the ratio of $Expression_{treated}/Expression_{control}$ >2). These two genes were also selected as the up-regulated genes at 0.5h after MeJA treatment (the ratio of $Expression_{treated}/Expression_{control}$ >2). In 143 MYB TF coding genes, At3g50060 was selected as the up-regulated gene at 15min after wounding treatment (the ratio of $Expression_{treated}/Expression_{control}$ >2). The MYB TF coding gene

Chapter3: Investigation of Jasmonate Responsive Transcription Factors

At2g23290 was selected as the down-regulated gene at 30min after wounding treatment (the ratio of $Expression_{treated}/Expression_{control} < 0.5$). In 146 AP2/ERF TF coding genes, At5g47220 and At4g17500 were selected as the up-regulated genes at 15min, 30min and 1h after wounding treatment (the ratio of $Expression_{treated}/Expression_{control} < 0.5$).

The plots of gene expression after wounding and MeJA treatments of this six selected transcription factors are illustrated in **Figure 3.1** and **Figure 3.2,** respectively. The expression of At1g32640, At2g46510, At5g47220 and At4g17500 was up-regulated at 15min, 30min, and 1h after wounding on shoots in comparison with the untreated control (**Figure 3.1**). The expression of At3g50060 was up-regulated at 15min after wounding on shoots in comparison with the untreated control (**Figure 3.1**). The expression of At2g23290 was down-regulated at 15min and 30min after wounding on shoots in comparison with the untreated control (**Figure 3.1**). The expression of At1g32640, At2g46510 and At4g17500 was up-regulated at 0.5h, 2h and 6h after MeJA treatment in comparison with the untreated control (**Figure 3.2**). The exprssion of At3g50060 and At2g23290 was down-regulated at 0.5h after MeJA treatment in comparison with the untreated control (**Figure 3.2**).

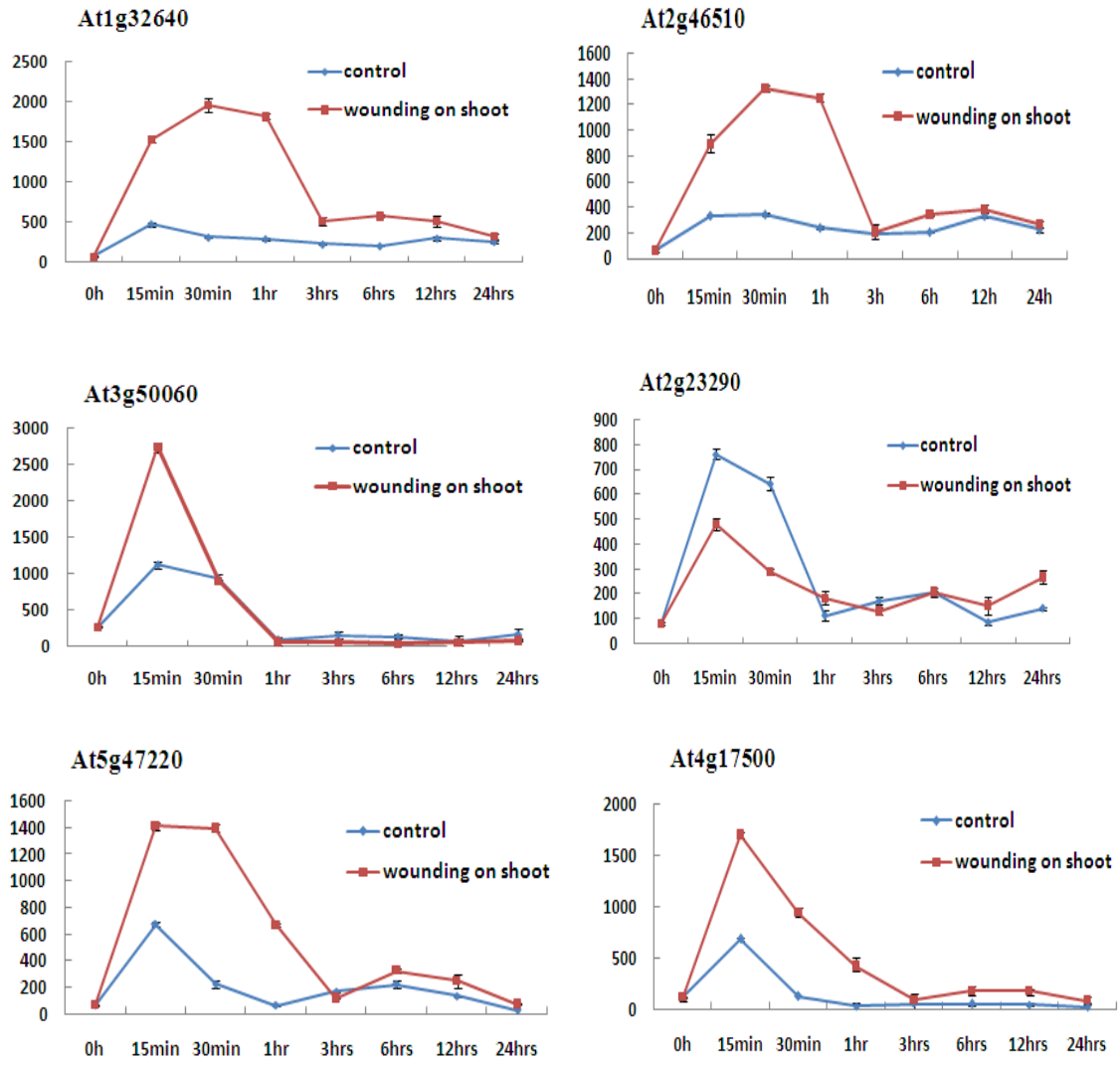


Figure 3.1: Expression of six transcription factors after wounding on shoots.
Sixteen-day-old wild type (Col-0) Arabidopsis seedlings were punctured with pins for wounding reatment. Blue line shows the change in gene expression of control shoot tissue and red line shows the change in gene expression of wounded shoot tissue. Each value at the indicated time point displays the mean value of three biological replicates. Error bars denote the value of standard errors. The units on y axes display the absolute value of gene expression.

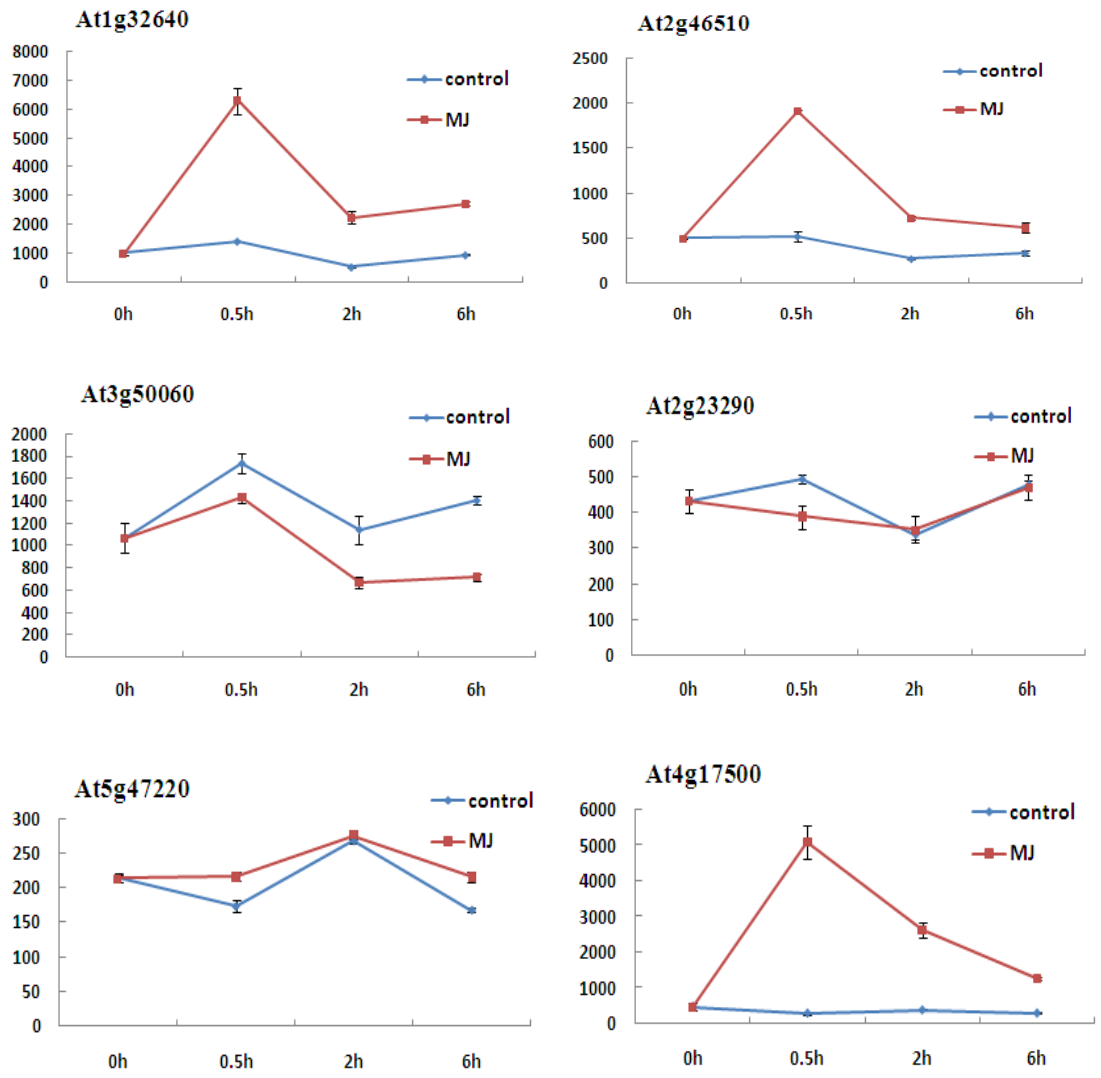


Figure 3.2: Expression of six transcription factors after MeJA treatment.
Blue line shows the change in gene expression of control samples and red line shows the change in gene expression of Arabidopsis thaliana (Col-0) cell suspension treated with 50μM MeJA for 0.5h, 2h and 6h. Each value at the indicated time point displays the mean value of three biological replicates. Error bars denote the value of standard errors. The units on y axes display the absolute value of gene expression.

At1g32640 encodes MYC2, a bHLH TF, and functions as a transcription regulator in the JA signal pathway (as described in the **section 1.4.3**). At2g46510 encodes a bHLH TF and functions as a positive regulator in the ABA signal pathway (as described in the **section 1.6.2.5**). The gene expression profiles of At1g32640 and At2g46510 are similar after wounding. The expression of At1g32640 and At2g46510 were up-regulated compared to controls at 15min, 30min and 1h after wounding on shoots (**Figure 3.1**). Additionally, At1g32640 and At2g46510 have a similar gene expression profile after MeJA treatment. The expression of At1g32640 and At2g46510 was up-regulated at 0.5h after MeJA treatment (**Figure 3.2**). At2g46510 was named here Jasmonate Up-regulated Transcription Factor (***JUT***). The expression profile of At1g32640 and At2g46510 also shares a similar pattern of expression at different stages of Arabidopsis development (**Figure 3.3**).

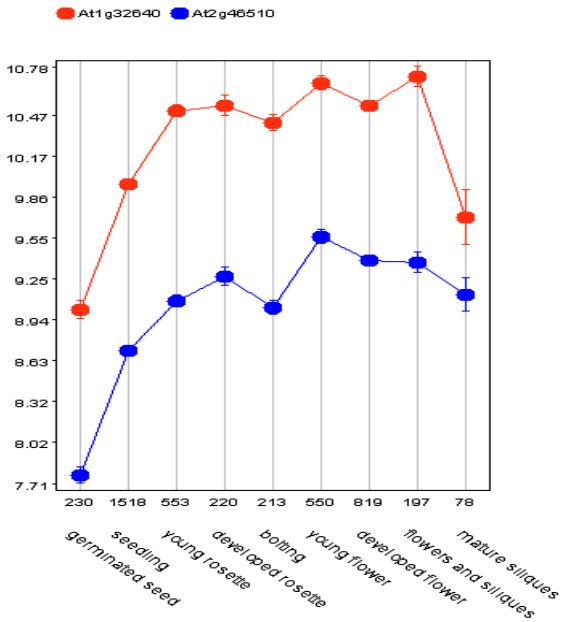


Figure 3.3: Expression of *AtMYC2* (At1g32640) and *JUT* (At2g46510) at different developmental stages.

The Development tool on Genevestigator database was used for investigating the expression of *AtMYC2* and *JUT* at different developmental stages in Arabidopsis thaliana (Col-0). The output image is downloaded here. At1g32640 is shown in red and At2g46510 is shown in blue. The numbers on x axes display the different developmental stages of Arabidopsis. The units on y axes display the log2 value of gene expression.

Chapter3: Investigation of Jasmonate Responsive Transcription Factors

The F-box protein COI1 regulates the expression of a considerable number of genes in the JA and wounding signal pathways (as described in the **section 1.4.1.2**). In order to investigate whether the expression of six TF genes At1g32640, At2g46510, At3g50060, At2g23290, At5g47220 and At4g17500 are dependent on COI1 or not, the gene expression profiles of these six TFs were analysed in *coi1* under non-induced condition by the mutation tool of Genevestigator. **Table 3.1** displays the signal ratio and log2 signal ratio of **Expression** $_{coi1}$ **/ Expression** $_{control}$ for the six TF genes. The expression of At1g32640, At2g46510, At5g47220 and At4g17500 is significantly down-regulated in *coi1* in comparison with the wild type. These results indicate that COI1 is required for the expression of At1g32640, At2g46510, At5g47220 and At4g17500.

Table 3.1: The signal ratio and log2 signal ratio of Expression $_{coi1}$ **/ Expression** $_{Col-0}$.

Gene ID	Signal ratio	log2 Signal Ratio
At1g32640 *	0.13	-2.99
At2g46510 *	0.08	-3.59
At3g50060	1.99	0.99
At2g23290	0.86	-0.22
At5g47220 *	0.12	-3.12
At4g17500 *	0.16	-2.68

Notes: Data in this table are referenced from Genevestigator. The signal ratio for each input gene is calculated by the average signal of the coi1 mutant in a subset of selected arrays divided by those in the wild type Arabidopsis Col-0 (Tomas et al., 2008). The asterisk denotes the **log2 signal ratio of Expression** $_{coi1}$ **/ Expression** $_{Col-0}$ smaller than "-2".

3.2.1.3 Search for T-DNA Insertion Mutants of At3g50060, At2g46510 and At2g23290

From the results of the investigation on gene expression profiles (**section 3.2.1.2**), the expression of At2g46510 (*JUT*) was up-regulated after wounding and MeJA treatment in comparison with the untreated control. Additionally, At2g46510 had the similar expression profile with *AtMYC2* after wounding and MeJA treatment (**Figure 3.1** & **Figure 3.2**), at different developmental stages (**Figure 3.3**) and also depended on COI1 (**Table 3.1**). From above, the gene of At2g46510 has been selected as candidate gene for further investigation. On the other hand, the expression of At3g50060 and At2g23290 was up-regulated at 15min after wounding and down-regulated at 0.5h after MeJA treatment (**Figure 3.1** & **Figure 3.2**). These two genes have been selected as the supplementary candidates for identifying the regulation of JA responses.

By utilizing the Arabidopsis gene mapping tool, T-DNA Express (http://signal.salk.edu/cgi-bin/tdnaexpress) on the SALK (http://signal.salk.edu/) database, MeJA and wounding responsive genes, At2g46510, At3g50060, and At2g23290, identified in section **2.1.1** were searched for suitable T-DNA insertion lines. These T-DNA insertion lines were then ordered from NASC-European Stock Centre (http://arabidopsis.info/). Information for four mutant lines of three target genes was retrieved from the TAIR and SALK Database. **Table 2.1** displays the details of T-DNA insertion lines used in this research.

3.2.2 Confirmation of the Location of the T-DNA Insertion Mutations

3.2.2.1 Plant Material Preparation

Seeds of indicated T-DNA insertion lines and wild type Col-0 were germinated and grown in a LD growth room for four weeks. Next, the genomic DNA of indicated T-DNA insertion lines and wild type Col-0 were isolated from fresh leaves of four-week-old Arabidopsis seedlings. The genomic DNA of indicated T-DNA insertion lines and wild type were used for PCR reactions to identify the homozygous line of T-DNA insertion mutation in the genes At3g50060, At2g46510 and At2g23290, respectively.

3.2.2.2 PCR-based Confirmation of the Presence of T-DNA Insertion in the Target Gene

Forward and reverse primers used for PCR in this research were designed by the T-DNA primer design tool at http://signal.salk.edu/tdnaprimers.2.html. Primer LP was designed on the 5' end of T-DNA insertion and primer RP was designed on the 3'end of T-DNA insertion in the region of the flanking sequence. T-DNA left border primers were downloaded from the SALK database (http://signal.salk.edu/). Primers used for confirming the T-DNA insertion on target genes were shown in **Table 2.2**. Two paired PCR reactions were used in this section: the PCR with LP and RP; the PCR with LB and RP. In wild type (WT), only one product can be amplified by LP and RP, while no product could be amplified by LB and RP. In a homozygous T-DNA insertion line, no product could be amplified by LP and RP, whilst there is a product by LB and RP. In a hemizygous T-DNA insertion line (the T-DNA inserted in one of the pair chromosomes) there is a product in both PCR amplified with LP and RP, and PCR amplified with LB and RP.

Primer LP(1) and RP(1) of gene At3g50060 amplified one product (about 1035bp) in wild type Col-0, but not in N662814 (**Figure 3.4.A**). The T-DNA left border primer

LBa1 and RP(1) amplified one product in N662814, but not in wild type Col-0 (**Figure 3.4.A**). There is no product amplified by primer LBa1 and LP(1) in both wild type Col-0 and N662814. These results indicate that the N662814 seedling used in this PCR experiment is a homozygous T-DNA insertion in At3g50060. My interpretation of the orientation of T-DNA insertion and the locations of primers are shown in **Figure 3.4.B**.

Primer LP(2) and RP(2) of gene At3g50060 amplified one product (about 1156bp) in wild type Col-0, but not in N662523 (**Figure 3.4.C**). The T-DNA left border primer LBb1 and RP(2) amplified one product in N662523, but not in wild type Col-0 (**Figure 3.4.C**). These results indicate that this N662523 seedling used in this PCR experiment is a homozygous T-DNA insertion in At3g50060. My interpretation of the orientation of T-DNA insertion and the locations of primers are shown in **Figure 3.4.D**.

Primer LP(3) and RP(3) of gene At2g46510 amplified one product (about 795bp) in wild type Col-0, but none from the N874647 (**Figure 3.4.E**). The T-DNA left border primer LB1 and RP(3) amplified one product in N874647, but not in wild type Col-0 (**Figure 3.4.G**). These results indicate that the N874647 seedling used in this PCR experiment is a homozygous T-DNA insertion in At2g46510. My interpretation of the orientation of the T-DNA insertion and the locations of primers are shown in **Figure 3.4.F**.

Primer LP(4) and RP(4) of gene At2g23290 amplified one product (about 792bp) in wild type Col-0, but not in N876044 (**Figure 3.4.E**). The T-DNA left border primer LB1 and RP(4) amplified one product in N876044, but not in wild type Col-0 (**Figure 3.4.G**). These results indicate that this N876044 seedling used in this PCR experiment is a homozygous T-DNA insertion in At2g23290. My interpretation of the orientation of T-DNA insertion and the locations of primers are shown in **Figure 3.4.H**.

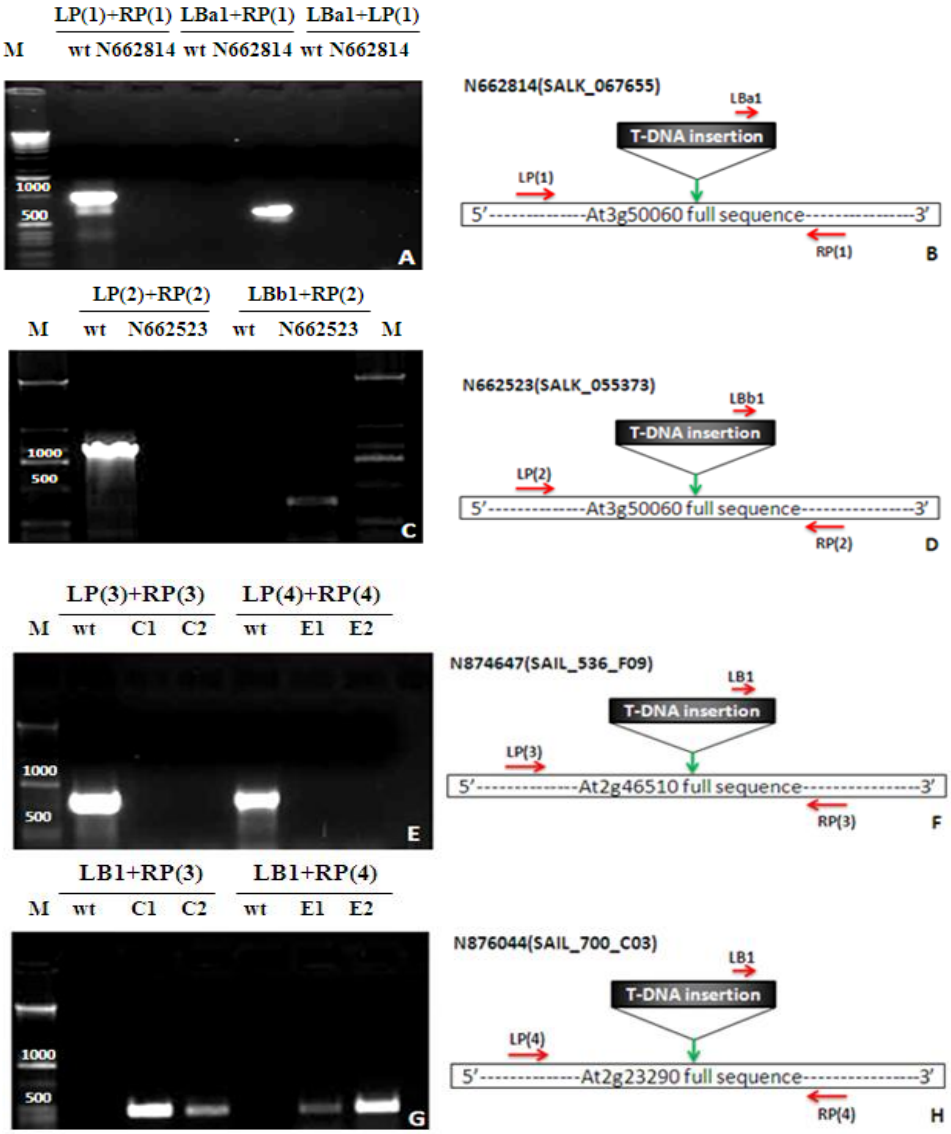


Figure 3.4: Identification of homozygous lines of T-DNA insertion mutants in the target genes.

A. LP(1) and RP(1) amplified one product in wild type Col-0. LBa1 and RP(1) amplified one product in N662814. **Figure B, D, F and H** displays my interpretation of the T-DNA insertion (black bar) inserts into the target gene (white bar) in individual T-DNA insertion line. The location of each primer is shown as red arrow. The predicted T-DNA insertion site is shown as green arrow. **C.** LP(2) and RP(2) amplified one product in wild type Col-0. LBb1 and RP(2) amplified one product in N662523. **E.** LP(3) and RP(3), LP(4) and RP(4) amplified one product in wild type, but not in N874647 and N876044. **G.** LB1 and RP(3) amplified one product in N874647, but not in wild type. LB1 and RP(4) amplified one product in N876044, but not in wild type. C1 and C2 stand for N874647. E1 and E2 stand for N876044.

3.2.2.3 Identifying the T-DNA Insertion Sites in the Target Genes

The left border primer of T-DNA insertion was used for sequencing the T-DNA/genomic DNA junction. The DNA sequencing reaction followed the protocol mentioned in the **section 2.3.8**. Then the sequence of T-DNA/genomic DNA junction was aligned with Arabidopsis genome DNA sequence on the FASTA tool (http://www.arabidopsis.org/cgi-bin/fasta/nph-TAIRfasta.pl). **Figure 3.5** to **Figure 3.8** display the sequencing and blast results of the T-DNA/genomic DNA junction of T-DNA insertion lines. The blast results were used to identify the starting site of the matched genomic DNA sequence, which corresponds to the insertion site of the T-DNA. **Table 3.2** shows the summary of T-DNA insertion sites in the homozygous T-DNA insertion lines. **Figure 3.9** illustrates the schematic diagrams of the T-DNA insertion in the target gene.

```
N662814:
GCGGTCAGCATCAGCTGTTGCCCGTCTCACTGGTGAAAGAAAAACCACCC 50
CAGTACATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTCTCTATCTC 100
TCTCTCTGTTATAAATACCTTTTTTCTCCTCTCTTCAGTTTCCTCCACCT 150
TCTTCTTCTTCTTCTTCTTCTCTCCTCTACATTAATCTTCATCTGACTCT 200
CAAATCTCGGAAACATCAACGCGTTGTTGTTATTGTTA              238

>>AT3G50060.1 | Symbols: MYB77 | myb domain protein 77 |  (1137 nt)
rev-comp initn: 350 init1: 350 opt: 386  Z-score: 198.8  bits: 46.4 E(): 0.00024
banded Smith-Waterman score: 386;  80.992% identity (80.992% similar) in 121 nt overlap (238-122:712-832)

                                      230       220       210
-                                   TAACAATAACAACAACGCGTTGATGTTTCC
                                    ::::::::::::::::::::::::::::::
AT3G50 ACCTGGAGCTGAGAACACGAGTTCGAGCCATAACAATAACAACAACGCGTTGATGTTTCC
             690       700       710       720       730       740

             200       190       180       170       160       150
-      GAGATTTGAGAGTCAGATGAAGATTAATGTAGAGGAGAGAAGAAGAAGAAGAAGAAGAAG
       :::::::::::::::::::::::::::::::::::::::: ::  : :: : ::: :
AT3G50 GAGATTTGAGAGTCAGATGAAGATTAATGTAGAGGAGAGAGGAGAAGGACGTAGAGGTGA
             750       760       770       780       790       800

             140           130       120       110       100
-      GTGGAGGAAACTG----AAGAGAGGAGAAAAAAGGTATTTATAACAGAGAGAGAGATAGA
       ::  : ::   ::    : :::: :: ::::
AT3G50 GTTTATGACGGTGGTGCAGGAGATGATAAAAGCTGAAGTGAGGAGTTACATGGCGGAAAT
             810       820       830       840       850       860

        90        80        70        60        50        40
-      GAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGGGGTGGTTTTTCTTTCAC

AT3G50 GCAGAAAACAAGTGGTGGATTCGTCGTCGGAGGTTTATACGAATCCGGCGGCAATGGTGG
             870       880       890       900       910       920
```

Figure 3.5: Sequence of the T-DNA/genomic DNA junction in T-DNA insertion line N662814 and its best match, determined by Blast. The Blast analysis on dataset of TAIR10 Genes (+introns, +UTRs) (DNA). The genomic DNA sequence of At3g50060 gene is shown in red. The T-DNA border sequence is shown in black.

```
N6622523:
CAGCATCAGCTGTTGCCCGTCTCACTGGTGAAAGAAAAACCACCCCAGTA  50
CATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTCTCTATCTCTCTCT  100
CTGTTAACAAAAGATCTGTTTCAATGGCGGATCGTGTTAAAGGTCCATGG  150
AGTCAAGAAGAAGATGAGCAGCTACGAAGGATGGTTGAGAAATACGGACC  200
GAGGAATTGGTCTGCGATTAGCAAATCG                        229

>>AT3G50060.1 | Symbols: MYB77 | myb domain protein 77 |  (1137 nt)
 initn: 615 init1: 615 opt: 615  Z-score: 480.8  bits: 98.5 E(): 4.7e-20
banded Smith-Waterman score: 615;  100.000% identity (100.000% similar) in 123 nt overlap (106-228:38-160)

          80        90       100       110       120       130
       TTAAGTTGTCTCTCTATCTCTCTCTCTGTTAACAAAAGATCTGTTTCAATGGCGGATCGT
                                       ::::::::::::::::::::::::::::::
AT3G50 TCTTCTTCTTCTTCTTCTCTACAACAAACAAACAAAAGATCTGTTTCAATGGCGGATCGT
        10        20        30        40        50        60

         140       150       160       170       180       190
       GTTAAAGGTCCATGGAGTCAAGAAGAAGATGAGCAGCTACGAAGGATGGTTGAGAAATAC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
AT3G50 GTTAAAGGTCCATGGAGTCAAGAAGAAGATGAGCAGCTACGAAGGATGGTTGAGAAATAC
        70        80        90       100       110       120

         200       210       220
       GGACCGAGGAATTGGTCTGCGATTAGCAAATCG
       :::::::::::::::::::::::::::::::::
AT3G50 GGACCGAGGAATTGGTCTGCGATTAGCAAATCGATTCCAGGTCGATCTGGTAAATCGTGT
       130       140       150       160       170       180

AT3G50 AGATTACGTTGGTGTAATCAGTTATCTCCGGAGGTTGAGCATCGTCCTTTCTCGCCGGAG
       190       200       210       220       230       240
```

Figure 3.6: Sequence of the T-DNA/genomic DNA junction in T-DNA insertion line N662523 and its best match, determined by Blast. The Blast analysis on dataset of TAIR10 Genes (+introns, +UTRs) (DNA). The genomic DNA sequence of At3g50060 gene is shown in red. The T-DNA border sequence is shown in black.

```
N874647:
CGCAATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATA 50
TATCCTGCCACCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAA 100
AATCACCACTCGATACAGGCAGCCCATCAGTCCGGGACGGCGTCAGCGGG 150
AGAGCCGTTGTAAGGCGGCAGACTTTGCTCATGTTACCGATGCTATTCGG 200
AAGAACGGCAACTAAGCTGCCGGGTTTGAAACACGGATGATCTCGCGGAG 250
GGTAGCATGTTGATTGTAACGATGACAGAGCGTTGCTGCCTGTGATCAGG 300
ATACAGATATGAGGAAGAGAGTGTTGCAGAAGCTTCATAGGTTGTTTGGT 350
GGATCTGATGAAGACAATTATGCTTTGAGCTTAGAGAAAGTTACTGCTAC 400
TGAGATTTTCTTCTTAG                                   417
>>AT2G46510.1 | Symbols: ATAIB, AIB | ABA-inducible BHLH  (2363 nt)
 initn: 565 init1: 565 opt: 565  Z-score: 490.1  bits: 102.2 E(): 6.9e-21
banded Smith-Waterman score: 565;  100.000% identity (100.000% similar) in 113 nt overlap (305-417:785-897)

          280       290       300       310       320       330
       ACAGAGCGTTGCTGCCTGTGATCAGGATACAGATATGAGGAAGAGAGTGTTGCAGAAGCT
                                       ::::::::::::::::::::::::::::::
AT2G46 CAACATGGGGGCAGAGGAAGAGACATGGCAAGATATGAGGAAGAGAGTGTTGCAGAAGCT
          760       770       780       790       800       810

          340       350       360       370       380       390
       TCATAGGTTGTTTGGTGGATCTGATGAAGACAATTATGCTTTGAGCTTAGAGAAAGTTAC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
AT2G46 TCATAGGTTGTTTGGTGGATCTGATGAAGACAATTATGCTTTGAGCTTAGAGAAAGTTAC
          820       830       840       850       860       870

          400       410
       TGCTACTGAGATTTTCTTCTTAG
       :::::::::::::::::::::::
AT2G46 TGCTACTGAGATTTTCTTCTTAGCTTCCATGTATTTCTTCTTCAATCACGGTGAAGGCGG
          880       890       900       910       920       930
```

Figure 3.7: Sequence of the T-DNA/genomic DNA junction in T-DNA insertion line N874647 and its best match, determined by Blast. The Blast analysis on dataset of TAIR10 Genes (+introns, +UTRs) (DNA). The genomic DNA sequence of At2g46510 gene is shown in red. The T-DNA border sequence is shown in black.

Figure 3.8: Sequence of the T-DNA/genomic DNA junction in T-DNA insertion line N876044 and its best match, determined by Blast. The Blast analysis on dataset of TAIR10 Loci Downstream Sequences - 1000 bp (DNA). The genomic DNA sequence of At2g23290 gene is shown in red. The T-DNA border sequence is shown in black.

Table 3.2: T-DNA insertion sites in the homozygous T-DNA insertion lines.

Line	Gene	Gene Product	Insertion site	No. of insertion
N662814	At3g50060	MYB77	exon	1
N662523	At3g50060	MYB77	5'UTR	1
N874647	At2g46510	bHLH TF	exon	1
N876044	At2g23290	MYB70	3'UTR	1

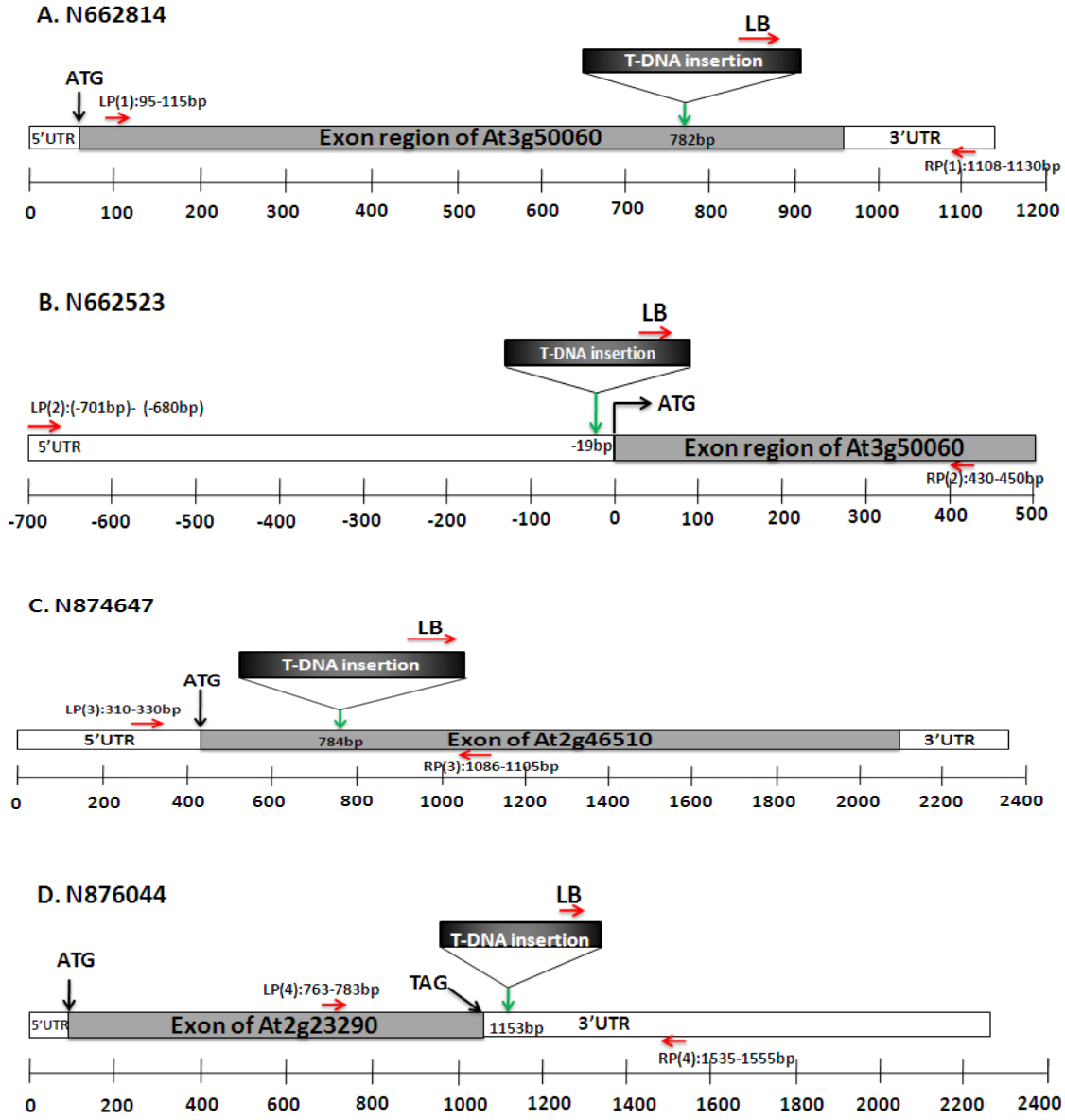


Figure 3.9: Schematic diagrams of the T-DNA insertions in the target genes.
The white bars denote the 5'UTR and 3'UTR; gray bars denote exon; black bars denote the T-DNA insertion. Start code of the gene is shown as ATG. Red arrows show primer locations and the green arrows show the T-DNA insertion site in target gene. The numbers of nucleotides are shown below each diagram. A. In N662814, the T-DNA insertion site locates on the exon of At3g50060. B. In N662523, the T-DNA insertion site locates on the 5'UTR of At3g50060. C. In N874647, the T-DNA insertion site locates on the exon of At2g46510. D. In N876044, the T-DNA insertion site locates on the 3'UTR of At2g23290.

3.2.3 Identify the Phenotype of T-DNA Mutants

3.2.3.1 The Effect of MeJA on Root Growth Inhibition of the T-DNA Mutants

To test the effect of MeJA on Arabidopsis root growth, fifteen Arabidopsis seeds of Col-0, *jin1*-1, T-DNA insertion mutant lines N874647, N876044, N662523 and N662814 were germinated and grown on 1/2 MS media in a LD growth chamber. After one week, fifteen seedlings of each line were carefully transferred with sterilised forceps onto 1/2 MS media containing 50μM MeJA for 48h MeJA treatment. An equal number of Arabidopsis seedlings of each line were transferred onto 1/2MS media for 48h control treatment. The root lengths of individual seedlings were then analysed with the ImageJ software. **Figure 3.10.A shows** root length of Col-0, *jin1*-1, T-DNA insertion mutants N874647, N876044, N662523 and N662814 in untreated mock and 48h MeJA-treated samples. Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups.

A two-way ANOVA ($p < 0.05$) was used to determine whether Arabidopsis root length after MeJA treatment was affected by a) genotypic variation b) the application of MeJA treatment or c) by the two factors (genotype x treatment) combined. To test our hypothesis, six different genotypes (Col-0, *jin1*-1, N8746477, N876044, N662523 and N662814) and two treatments (mock and 50uM MeJA 48h treatment) were used in this experiment. Statistical analyses were conducted using SPSS18 program (SPSS Inc., Chicago IL). Data of Arabidopsis root length was normally distributed and met the assumptions of two-way ANOVA. Results of the analysis (**Figure 3.10.C**) indicate that the factor of Genotype had significant effect on the Arabidopsis root length ($F_{\text{Genotype}}(5, 178) = 6.798$, $p < 0.001$), as well as the factor Treatment ($F_{\text{Treatment}}(1,178) = 57.292$, $p < 0.001$). There is also a significant effect from Genotype x Treatment on Arabidopsis root length ($F_{\text{Genotype} \times \text{Treatment}}(5, 178) = 1.858$, $p < 0.05$).

Pair-wise comparisons (**Figure 3.10.D**) revealed that there is a statistically significant difference between group of Col-0 and N874647 ($p < 0.05$) and Col-0 and N662814 ($p< 0.001$). These results confirmed the observation that N874647 displays a significant difference after MeJA treatment compared to the wild type Col-0. In N874647, the percentage of root length after MeJA treatment to its untreated mock group is 83.88%, while in wild type Col-0 that percentage is 74.90% (**Figure 3.10.A**). This result indicates that N874647 has a reduced responsiveness to MeJA-induced root growth inhibition than wild type Col-0. In addition, in N662814, the percentage of root length after MeJA treatment to its untreated mock group is 85.23%, while in wild type Col-0 that percentage is 74.90% (Figure 3.10.A). This result suggests that N662814 has a reduced responsiveness to MeJA-induced root growth inhibition than wild type Col-0.

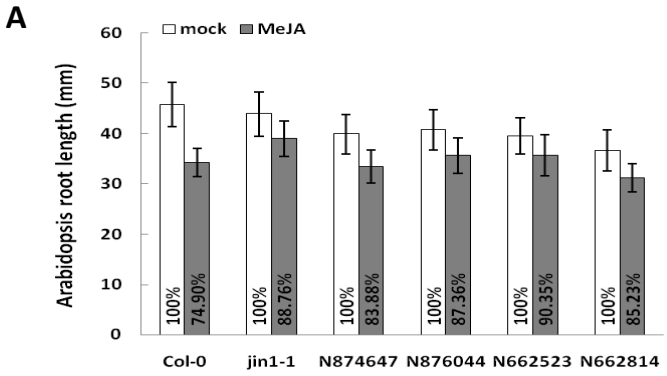


Table B: Effect of MeJA treatment on Arabidopsis root length (mean ± SD).

Genotype	mock	MeJA treated
col-0	45.81 ± 4.37	34.31 ± 2.82
***jin1*-1**	43.94 ± 4.4	39 ± 3.47
N874647	39.94 ± 3.92	33.5 ± 3.29
N876044	40.75 ± 4.06	35.6 ± 3.54
N662523	39.5 ± 3.63	35.69 ± 4.12
N662814	36.67 ± 4.10	31.25 ± 2.77

Table C: Summary of Genotype and treatment on Arabidopsis root length by two-way ANOVA.

Source	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Genotype	5	2.171	6.798	0.000	0.16	0.998
Treatment	1	18.302	57.292	0.000	0.243	1
Genotype × Treatment	5	0.594	1.858	0.041	0.05	0.624
Error	178	0.319				
Total	190					
Corrected Total	189					

Table D: Pair-wise comparisons (p< 0.05) between different groups by Tukey's LSD. Significantly different groups are highlighted by "$p < 0.05$: *", "$p < 0.01$: **", $p < 0.001$: *** ".

comparisons	Mean difference	Std. Error	Sig.	95% Confidence Interval Lower Bound, Upper Bound	
Col-0 vs *jin1*-1	-.141	.141	.321	-.419	.138
Col-0 vs N874647	.334	.141	**.019***	.056	.613
Col-0 vs N876044	.189	.142	.187	-.092	.470
Col-0 vs N662523	.247	.141	.082	-.032	.526
Col-0 vs N662814	.610	.142	**.000*****	.329	.892
***jin1*-1 vs Col-0**	.141	.141	.321	-.138	.419
***jin1*-1 vs N874647**	.475	.141	**.001***	.196	.754
***jin1*-1 vs N876044**	.329	.142	**.022***	.048	.611
***jin1*-1 vs N662523**	.388	.141	**.007****	.109	.666
***jin1*-1 vs N662814**	.751	.142	**.000*****	.470	1.032
N874647 vs Col-0	-.334	.141	**.019***	-.613	-.056
N874647 vs *jin1*-1	-.475	.141	**.001***	-.754	-.196
N874647 vs N876044	-.146	.142	.308	-.427	.136
N874647 vs N662523	-.087	.141	.537	-.366	.191

Table D: Pair-wise comparisons ($p< 0.05$) between different groups by Tukey's LSD. Significantly different groups are highlighted by "$p < 0.05$: *", "$p < 0.01$: **", $p < 0.001$: *** " (continued)

comparisons	Mean difference	Std. Error	Sig.	95% Confidence Interval Lower Bound, Upper Bound	
N874647 vs N662814	.276	.142	.054	-.005	.557
N876044 vs Col-0	-.189	.142	.187	-.470	.092
N876044 vs *jin1*-1	-.329	.142	**.022***	-.611	-.048
N876044 vs N874647	.146	.142	.308	-.136	.427
N876044 vs N662523	.058	.142	.684	-.223	.339
N876044 vs N662814	.422	.144	**.004****	.138	.705
N662523 vs Col-0	-.247	.141	.082	-.526	.032
N662523 vs *jin1*-1	-.388	.141	**.007****	-.666	-.109
N662523 vs N874647	.087	.141	.537	-.191	.366
N662523 vs N876044	-.058	.142	.684	-.339	.223
N662523 vs N662814	.364	.142	**.012***	.082	.645
N662814 vs Col-0	-.610	.142	**.000*****	-.892	-.329
N662814 vs *jin1*-1	-.751	.142	**.000*****	-1.032	-.470
N662814 vs N874647	-.276	.142	.054	-.557	.005
N662814 vs N876044	-.422	.144	**.004****	-.705	-.138
N662814 vs N662814	-.364	.142	**.012***	-.645	-.082

Figure 3.10: Effect of MeJA treatment on Arabidopsis root length of Col-0, *jin1*-1, T-DNA insertion mutants N874647, N876044, N662523 and N662814.

A. Root length of Col-0, *jin1*-1, T-DNA insertion mutants N874647, N876044, N662523 and N662814 in untreated mock and 48h MeJA-treated samples. Bar charts display the value of mean ± SD of fifteen seedlings. Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups. **Table B** shows the effect of MeJA treatment on Arabidopsis root length (mean ± SD). **Table C** displays the summary of effects analyzed by two-way ANOVA. **Table D** illustrates the pair-wise comparisons between different groups by Tukey's LSD ($p< 0.05$).

3.2.3.2 The Effect of Wounding on Shoot Growth of *jut*

To test the effect of wounding on the shoot growth of the T-DNA mutant *jut*, sixteen Arabidopsis seeds of Col-0, *jin1*-1 and *jut* (N874647) were germinated and grown on soil in two square pots (eight seeds per pot) in the short day (8h light/16h dark) growth room. Eight 21-day-old seedlings for each indicated line were selected for wounding treatment. An equal number of same age unwounded seedlings of each indicated line were selected as the control group. For wounding treatment, one leaf of each seedling was lightly squeezed by forceps once per day, and this was then repeated for ten consecutive days. Pictures of wounded and unwounded seedlings are taken on day 31 (**Figure 3.11**). Arabidopsis Leaf Measurement software was then used to measure the Arabidopsis leaf area in the wounded group and control group. **Figure 3.12.A** shows the Arabidopsis leaf area of 31-day-old control and wounded seedlings. Percentages inside the charts represent the root length of wounding-treated different lines to their respective untreated (mock) groups.

A two-way ANOVA ($p < 0.05$) was used to determine whether Arabidopsis leaf area after wounding was affected by a) genotypic variation b) the application of wounding treatment or c) by the two factors (genotype × treatment) combined. To test our hypothesis, three different genotypes (Col-0, *jin1*-1, *jut*) and two treatments (with or without wounding treatment) were used in this experiment. Statistical analyses were conducted using SPSS18 program (SPSS Inc., Chicago IL). Data of Arabidopsis leaf area was normally distributed and met the assumptions of two-way ANOVA. Results of the analysis (**Figure 3.12.C**) indicate that the factor of Genotype had significant effect on the Arabidopsis leaf area ($F_{\text{Genotype}}(2, 42) = 6.244$, $p < 0.01$), as well as the factor Treatment ($F_{\text{Treatment}}(1, 42) = 223.433$, $p < 0.001$). There is also a significant effect from Genotype x Treatment on Arabidopsis leaf area ($F_{\text{Genotype} \times \text{Treatment}}(2, 42) = 2.408$, $p < 0.05$).

Pair-wise comparisons (**Figure 3.12.D**) revealed that there is a statistically significant difference between group of Col-0 and *jut* ($p < 0.01$). This result confirmed the observation that *jut* mutant displays a significant difference after wounding treatment compared to the wild type Col-0. In *jut* mutant, the percentage of leaf area after wounding to its untreated mock group is 83.03%, while in wild type Col-0 that percentage is 66.27% (**Figure 3.12.A**). These results indicate that *jut* has a reduced responsiveness to wound-induced shoot growth inhibition than wild type Col-0.

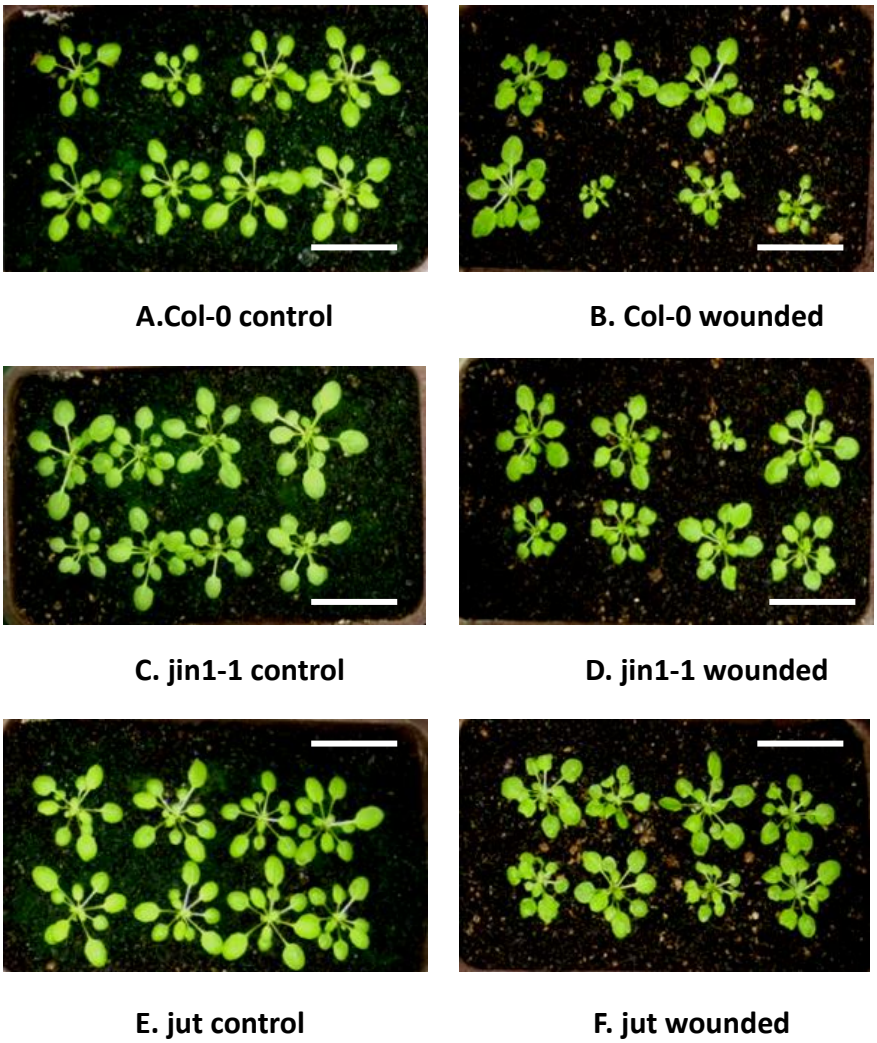


Figure 3.11: The unwounded control and wounded seedlings of Col-0, *jin1*-1 and *jut*. Eight 21-day-old seedlings of each indicated line were selected for wounding treatment. One leaf of each seedling was lightly squeezed by forceps once per day, and then was repeated for ten consecutive days. Pictures of wounded and unwounded seedlings are taken on the 31st day after seed germination. Scale equals 5cm.

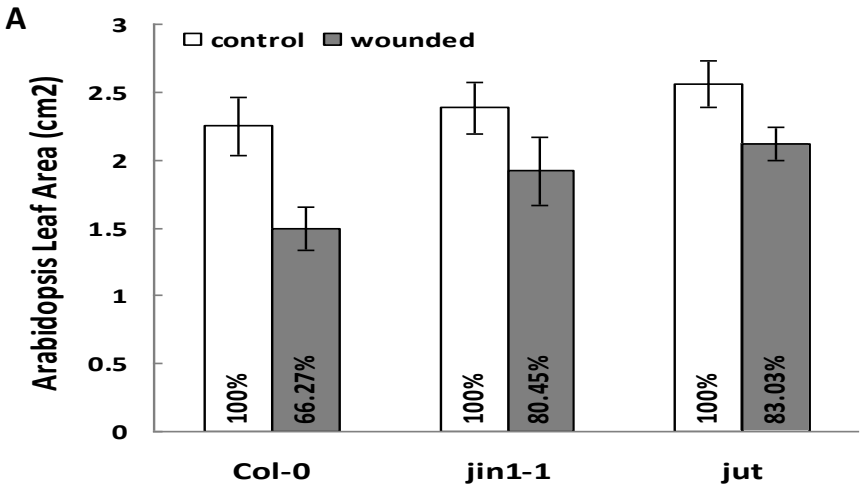


Table B: Effect of wounding treatment on Arabidopsis leaf area (mean ± SD).

Genotype	mock	MeJA treated
col-0	2.25 ± 0.21	1.49 ± 0.16
***jin1*-1**	2.38 ± 0.19	1.92 ± 0.25
jut	2.55 ± 0.17	2.12 ± 0.12

Table C: Summary of Genotype and treatment on Arabidopsis leaf are by two-way ANOVA.

Source	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Genotype	2	4.689	6.244	.004	.229	.872
Treatment	1	167.792	223.43 3	.000	.842	1.000
Genotype × Treatment	2	1.809	2.408	.020	.103	.459
Error	42	.751				
Total	48					
Corrected Total	47					

Table D: Pair-wise comparisons between different groups.
Significantly different groups are highlighted by "$p < 0.05$: *", "$p < 0.01$: **".

Multiple comparisons	Mean difference	Std. Error	Sig.	99% Confidence Interval Lower Bound, Upper Bound
Col-0 vs *jin1*-1	-.1648	.30639	.853	-1.1082, .7787
Col-0 vs *jut*	-1.0091	.30639	**.006****	**-1.9526, -.0657**
***jin1*-1 vs Col-0**	.1648	.30639	.853	-.7787, 1.1082
jin*1-1 vs *jut	-.8444	.30639	**.023***	-1.7878, .0991
***jut* vs Col-0**	1.0091	.30639	**.006****	.0657, 1.9526
***jut* vs *jin1*-1**	.8444	.30639	**.023***	-.0991, 1.7878

Figure 3.12: Effect of wounding treatment on Arabidopsis leaf area of Col-0, *jin1*-1and *jut*. A. Arabidopsis leaf area of Col-0, *jin1*-1and *jut* after 10 days continuous wounding treatment. Three biological experiments were repeated for ten days continuous wounding on the plants of Col-0, *jin1*-1and *jut*. Bar charts display the value of mean ± SD (n=8). Percentages inside the charts represent the leaf area of wounding-treated different lines to their respective untreated (mock) groups. **Table B** shows the effect of wounding treatment on Arabidopsis leaf area (mean ± SD). **Table C** displays the summary of wounding effect on Arabidopsis leaf area by two-way ANOVA. **Table D** illustrates the pair-wise comparisons between different groups by Tukey's LSD.

3.2.4 Predicted Amino Acid Sequences and Protein Structural Models of MYC2 and JUT

3.2.4.1 Fourteen Amino Acid Sequences from Arabidopsis Closely Related to JUT

In order to identify the proteins which share the high-similarity with JUT, the DNA sequence of *JUT* (At2g46510) was entered into the BLASTx program on the Plant Transcription Factor Database (http://planttfdb.cbi.pku.edu.cn/blast.php). The amino acid identity greater than 20% was clarified as the selection criteria for JUT closely related amino acid sequences. **Figure 3.13** shows the BLASTx results of fourteen amino acid sequences which share over 20% amino acid identity. These results indicate that MYC2 (At1g32640) is one of fourteen genes identified as having amino acid sequence closely related to JUT. For instance, bHLH013 (At1g01260) has very high similarity to bHLH017 (At2g046510). This result suggests that bHLH013 shares the putative biological function with bHLH17.

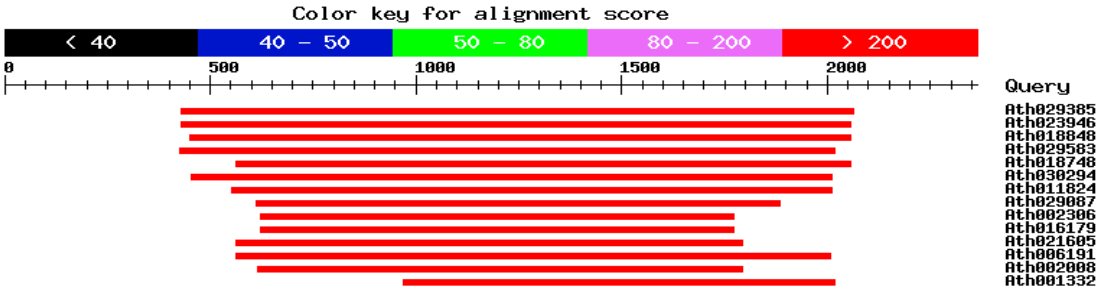


Table A: Fourteen JUT closely related amino acid sequences from Arabidopsis.

Hit ID	AtHLH number	Gene ID	amino acid identity/similarity
Ath029385	bHLH 017	At2g46510	100%
Ath023946	bHLH 013	At1g01260	47%
Ath018848	bHLH 003	At4g16430	34%
Ath029583	bHLH 004	At4g17880	32%
Ath018748	bHLH 006	At1g32640	30%
Ath030294	bHLH 005	At5g46760	31%
Ath011824	bHLH028	At5g46830	29%
Ath029087	bHLH042	At4g09820	26%
Ath002306	bHLH 012	At4g00480	22%
Ath016179	bHLH 012	At4g00480	22%
Ath021605	bHLH 002	At1g63650	33%
Ath006191	bHLH 014	At4g00870	31%
Ath002008	bHLH 001	At5g41315	29%
Ath001332	bHLH 021	At2g16910	24%

Figure 3.13: BLASTx results of fourteen JUT closely related amino acid sequences from Arabidopsis. Hit IDs, AtHLH numbers and Gene IDs are shown in the table A.

3.2.4.2 Phylogenetic Analysis of JUT-related bHLH TFs from *Arabidopsis thaliana*

From a previous phylogenetic analysis of bHLH TFs in Arabidopsis (Toledo-Ortiz et al., 2003), nine JUT-related bHLH TFs were selected for analysis in this section. They are AtMYC1, AtMYC2, AtMYC3, AtMYC4, AtAIB (JUT), bHLH003, bHLH013, bHLH014 and bHLH028. The phylogenetic tree was generated based on amino acid sequences of these nine candidate transcription factors using Phylogeny.fr (http://www.phylogeny.fr/version2_cgi/index.cgi). **Figure 3.14** illustrates a phylogenetic tree of JUT and related bHLH TFs. For instance, bHLH013 (At1g01260) has very high similarity to JUT (bHLH017). The sub-cluster of AtMYC2 (bHLH006), bHLH028, bHLH014, AtMYC4 (bHLH004) and AtMYC3 (bHLH005) shares similarity with the sub-cluster of JUT (bHLH017), bHLH013 and bHLH003.

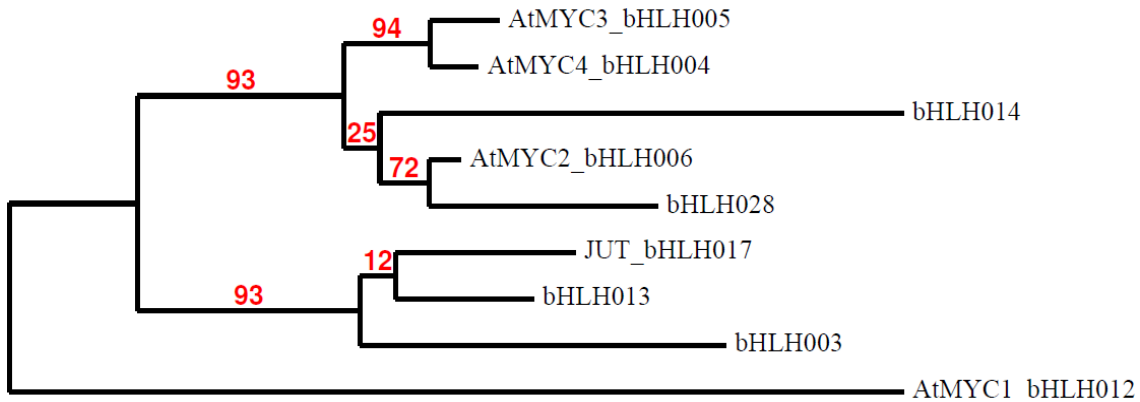
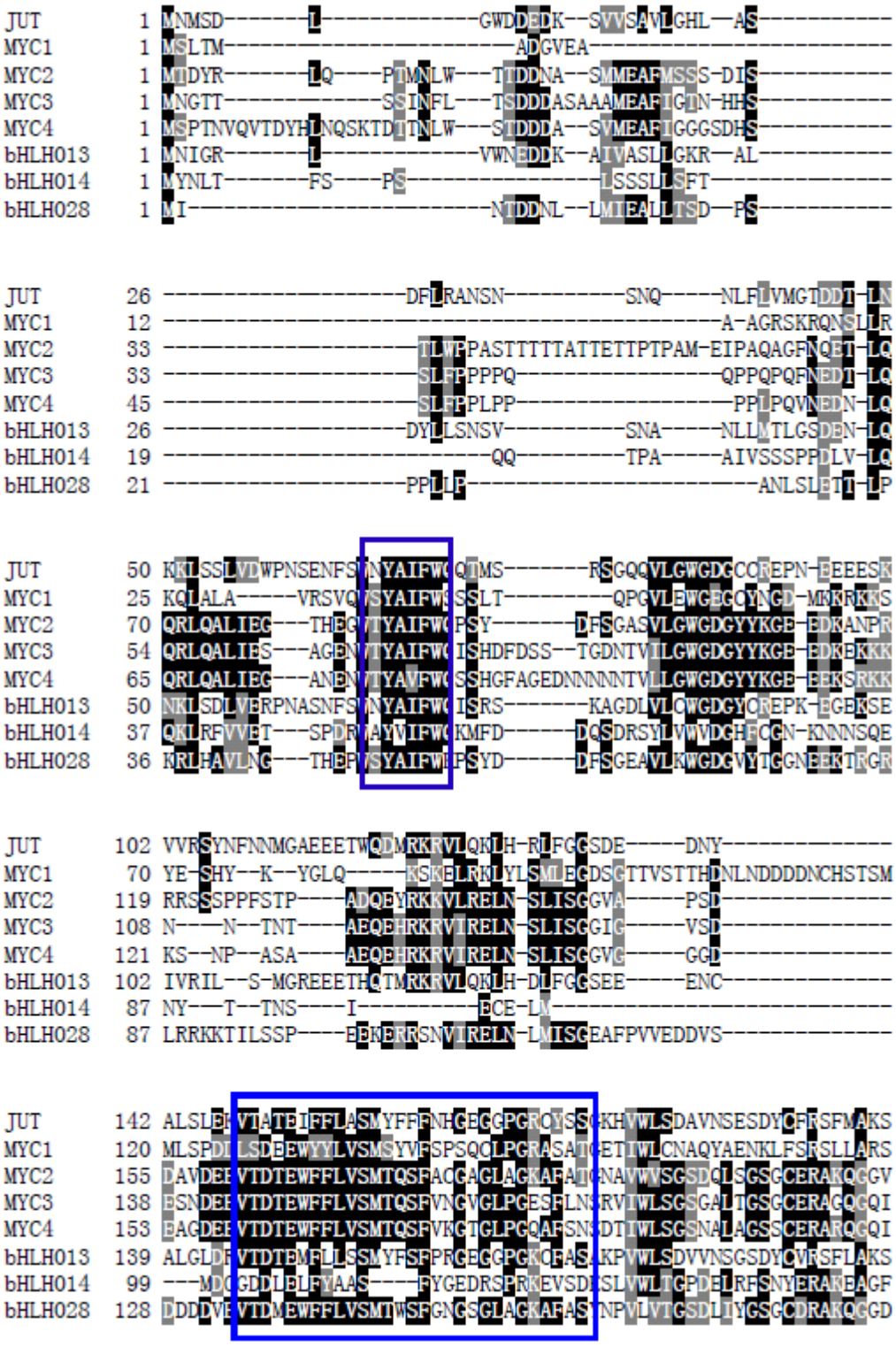
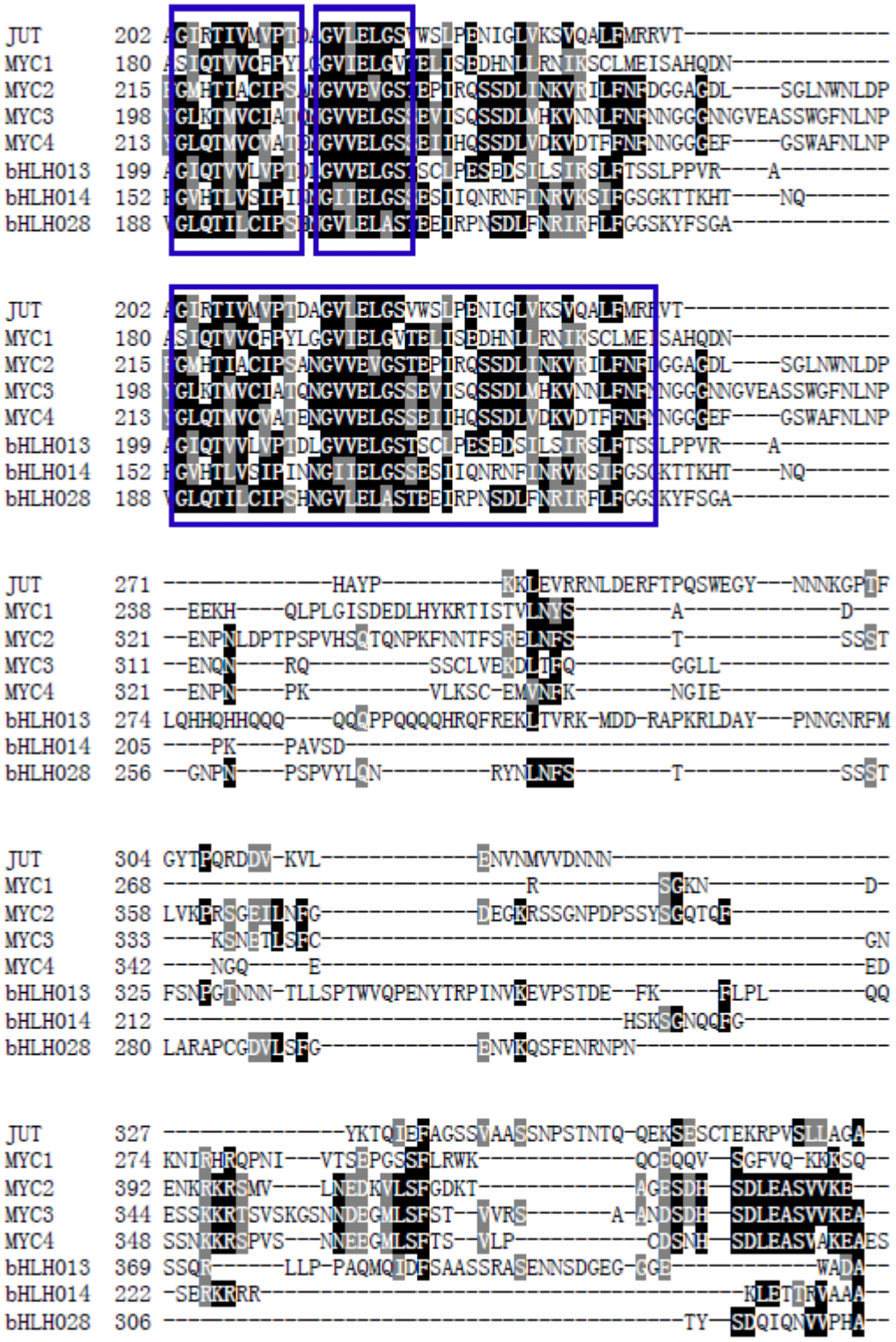


Figure 3.14: Phylogenetic tree of JUT-related bHLH TFs.
Phylogenetic tree is generated based on the amino acid sequences of AtMYC1, AtMYC2, AtMYC3, AtMYC4, JUT, bHLH003, bHLH013, bHLH014 and bHLH028. Branch support percentage values are shown in red for each node.

3.2.4.3 Amino Acid Sequences of JUT and JUT-related bHLH TFs

The amino acid sequences of JUT and JUT-related bHLH TFs used in **Figure 3.14** were aligned with the ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and transformed by BoxShade (http://www.ch.embnet.org/software/BOX_form.html). **Figure 3.15** displays the amino acid alignments of JUT related TFs.


JUT
MYC1
MYC2
MYC3
MYC4
bHLH013
bHLH014
bHLH028


JUT 202
MYC1 180
MYC2 215
MYC3 198
MYC4 213
bHLH013 199
bHLH014 152
bHLH028 188
JUT 271
MYC1 238
MYC2 321
MYC3 311
MYC4 321
bHLH013 274
bHLH014 205
bHLH028 256
JUT 304
MYC1 268
MYC2 358
MYC3 333
MYC4 342
bHLH013 325
bHLH014 212
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bHLH028 306

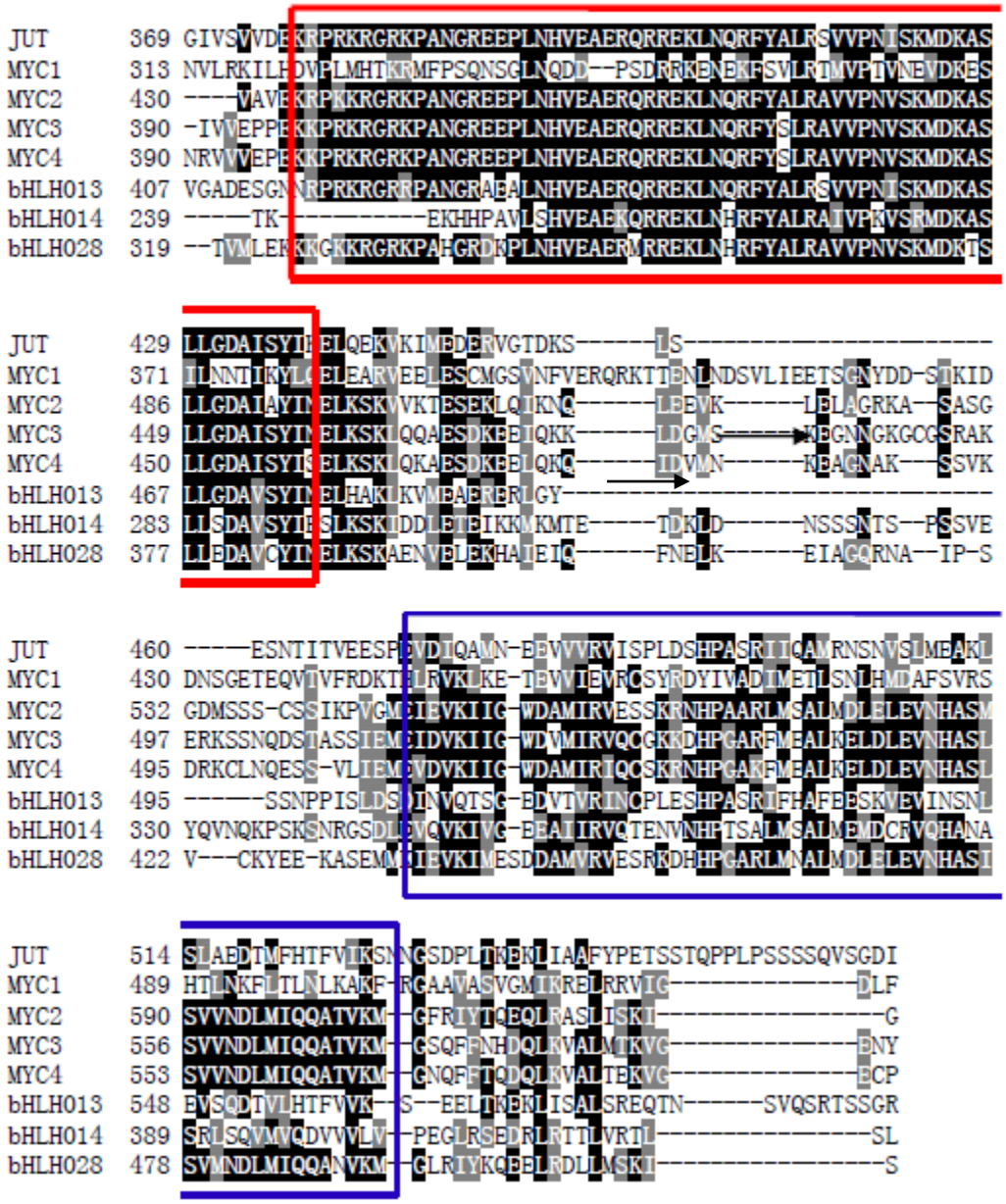


Figure 3.15: The alignments on amino acid sequences of JUT and JUT-related bHLH TFs. The high identical amino acids are shaded in black. The bHLH domain is shown in the red box. Besides the bHLH domain, there are several highly similarity amino acid sequences in JUT and JUT-related bHLH TFs.

3.2.5 Gene Co-expression Analyses of *AtMYC2* and *JUT*

In this section, I present an investigation on genes that are co-expressed with *AtMYC2* and *JUT*. The platform of GeneCAT (http://genecat.mpg.de/), which combines gene co-expression analyses and gene cluster tools (Mutwil et al., 2008), was used to identify genes that are co-expressed with *AtMYC2* and *JUT*. GeneCAT for Arabidopsis thaliana uses the expression profiles of Affymetrix 351 ATH1 microarrays containing 22810 probesets. The gene IDs of At1g32640 and At2g46510 were entered into the search blank, and 0.7 was selected as the cut-off of Pearson Correlation Coefficient (r-value) for the gene co-expression analysis. There are eight genes that met this criterion and co-expressed with At1g32640 (*AtMYC2*) (**Table 3.3**), for instance, At2g06050 (*OPR3*), At1g17380 (*JAZ5*) and At1g19180 (*JAZ1*). There are six genes that met this criterion and co-expressed with At2g46510 (*JUT*) (**Table 3.4**), for instance, At1g17380 (*JAZ5*), At1g74950 (*JAZ2*), At1g70700 (*JAZ9*) and At1g72450 (*JAZ6*). Of the co-expressed genes that meet the threshold criteria, only two genes (At1g17380, At1g53885) co-expressed with *AtMYC2* and also co-expressed with *JUT*. This indicates these two genes could be co-regulated by MYC2 and JUT. Presumably the non-overlapping genes could be differentially regulated by MYC2 and JUT.

Table 3.3: Genes co-expressed with At1g32640 (*AtMYC2*) (r-value > 0.7).

r-value	Probe ID	Gene ID	Description of gene product
0.7816	265530_at	at2g06050	12-oxophytodienoate reductase (OPR3)
0.76685	263972_at	at2g42760	expressed protein
0.75885	261033_at	at1g17380	expressed protein (JAZ5)
0.74306	262226_at	at1g53885	senescence-associated protein-related
0.73966	259518_at	at1g20510	4-coumarate--CoA ligase family protein
0.71946	252679_at	at3g44260	CCR4-NOT transcription complex protein
0.70257	256017_at	at1g19180	expressed protein (JAZ1)
0.70118	261405_at	at1g18740	expressed protein

Table 3.4: Genes co-expressed with At2g46510 (*JUT*) (r-value > 0.7).

r-value	Probe ID	Gene ID	Description of gene product
0.76674	261033_at	at1g17380	expressed protein (JAZ5)
0.75811	262171_at	at1g74950	expressed protein (JAZ2)
0.75268	261037_at	at1g17420	lipoxygenase
0.74274	260205_at	at1g70700	expressed protein (JAZ9)
0.71489	262226_at	at1g53885	senescence-associated protein-related
0.71145	260429_at	at1g72450	expressed protein (JAZ6)

The tool of Gene Expresssion Tree calculates a Pearson Correlation Coefficient (r-value) of all input genes, and then generates the matrix data of r-values. This matrix data was then transformed to one distance matrix (Mutwil et al., 2008). The distance between two genes on the tree is transformed by "d (a, b) =1- r(a, b)". For instance, the r-value of two highly co-expressed genes is close to 1, so the distance of these two highly co-expressed genes is close to 0. The input genes, At1g32640 (*AtMYC2*), At2g46510 (*JUT*), and a subgroup of JA related, closely co-expressed genes (At1g19180 (*JAZ1*), At1g74950 (*JAZ2*), At1g17380 (*JAZ5*), At1g72450 (*JAZ6*), At1g70700 (*JAZ9*), At2g06050 (*OPR3*) and At1g53885), were then submitted to generate a Gene Expression Tree using the Gene Expression Tree tool on GeneCAT. **Figure 3.16** displays the output of Gene Expression Tree for these input genes. These results verify that expression of MYC2 and JUT are co-expressed in distinct sub-clusters, which are in turn expressed similarly.

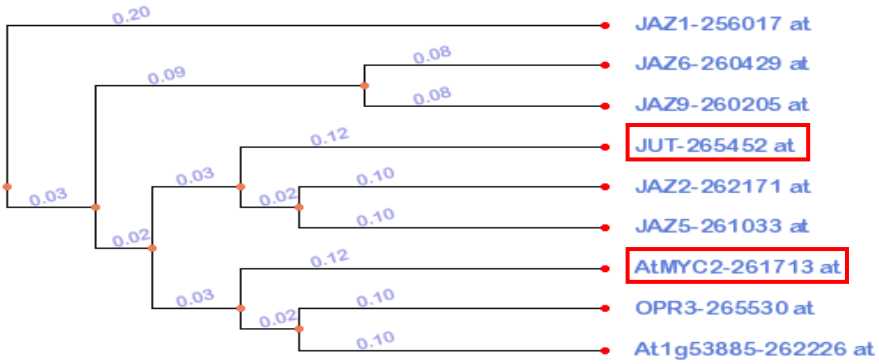


Figure 3.16: Gene Expression Tree based on the Distance of co-expressed genes. Image is downloaded from Gene Expresssion Tree Tool on GeneCAT dadabase. The distance between two genes on the tree is transformed by "d (a, b) =1- r(a, b)". *AtMYC2* and *JUT* are highlighted with red box.

Chapter3: Investigation of Jasmonate Responsive Transcription Factors

In order to further investigate the gene expression profile of *JUT* and its co-expressed genes, the Cluster cutting Tool was used to explore the hierarchical clustering data of candidate genes in *AtGenExpress* database (Goda et al., 2008). The gene ID At2g46510 was entered into the search blank and 20 was set as the cut-off number of genes to be selected as co-expressed with the input gene. **Figure 3.17** displays the clustering of At2g46510 and twenty co-expressed genes based on the gene expression profile in *AtGenExpress* database. These results indicate that the genes At5g13220 (*JAZ10*), At1g74950 (*JAZ2*), At1g70700 (*JAZ9*), At1g17380 (*JAZ5*), At1g32640 (*AtMYC2*) and At1g72450 (*JAZ6*) share high-similarity of gene expression profile at 30min, 1h and 3h MeJA (MJ) treatment. These results reveal a gene cluster (**Figure 3.17** highlighted with red box), which includes genes identified as co-expressed in **Table 3.5**. Significantly their co-expression shown in **Figure 3.17** occurs in part because of their strong up-regulation by MeJA (MJ) treatment. One gene in this cluster, AT1G44350, however, was not revealed as strongly co-expressed with *JUT* in **Table 3.5**. AT1G44350 is a predicted IAA-amino acid conjugate hydrotase 6 (ILL6). This raises a possibility that MeJA treatment will promote auxin accumulation from stores of inactive auxin conjugates.

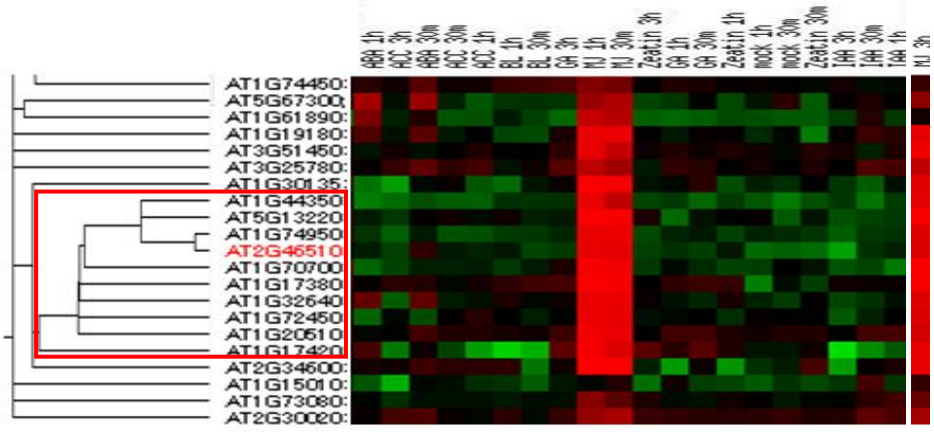


Figure 3.17: *JUT* and its co-expressed genes.

The Gene IDs are listed on the left side of figure. The treatments are shown above the figure. Up-regulated expression and down-regulated expression are indicated in red and green, respectively. *JUT* gene is shown in red. The gene sub-cluster is highlighted with red box.

3.3 Discussion

In this chapter, JA-inducible transcription factors were selected from publically available databases. First of all, the gene expression profile indicated that At2g46510 and At1g32640 was strongly up-regulated by wounding and JA (**Figure 3.1** & **Figure 3.2**), and also dependent on COI1 (**Table 3.1**). The gene At2g46510 was then named as Jasmonate Up-regulated Transcription Factor (***JUT***). The effect of MeJA on the root growth inhibition revealed that the homozygous T-DNA insertion mutant *jut* (N874647) displayed a reduced responsiveness to MeJA-induced root growth inhibition compared to wild type (**Figure 3.10**). Wounding treatment did not reduce shoot growth of *jin1*-1 and *jut* as much as wild type (**Figure 3.12**). Apparently, both MYC2 and JUT are required for MeJA- and wound-induced growth inhibition.

In addition, JUT and MYC2 share high-similarity in both amino acid sequence, beside the bHLH domain, there are several highly similarity amino acid sequences in JUT and JUT-related bHLH TFs (**Figure 3.15**). It has been reported that the N-terminal of MYC2 interacts with the C-terminal domain of JAI3/JAZ3 (Chini et al., 2007). The N-terminal shares sequence similarity in both JUT and MYC2. It is possible therefore that JUT also interacts with JAZ proteins.

Co-expressed genes share a simultaneous expression profile and therefore may regulate multiple aspects of a response to a common stimulus. *JUT* and *AtMYC2* are co-expressed, and with other JA responsive genes, such as *JAZ2*, *JAZ5*, *JAZ6*, *JAZ9* and *JAZ10* (**Figure 3.16** & **Figure 3.17**). Taken together, these results establish that the JA up-regulated transcription factor JUT is a component in the JA signal pathway. This raises the importance of establishing experimentally the expression of *JUT*.

Chapter 4

Genetic Interaction between *AtMYC2* and *JUT*

4.1 Introduction

In Chapter 3, the expression of *JUT* was analysed in the publicly available microarray databases using the program Genevestigator, and co-expression of *JUT* with other genes was analysed using the program GeneCAT and AtGenExpressJapan. These analyses indicated that *JUT* was regulated similarly to, and co-expressed with, *AtMYC2*. This raises the questions whether MYC2 regulates JUT, or *vice versa*, or are they regulated independently of each other? To address these questions, Northern blotting analysis was used to investigate the expression of *AtMYC2* and *JUT* in wild type plants, and in mutants of the JA signal pathway.

The wild type Col-0, Col-gl and 2M and the JA signal mutants *coi1*-16, *jai3*-1, *jin1*-1 and *jut* were used in the experiments of this chapter. Additionally, the lines JUTOE and MYC2OE (**Table 2.1**) were included. As described in the **section 1.4.1.1**, the mutant *coi1* is insensitive to growth inhibition by the bacterial toxin coronatine, which is a structural and functional homologue of JA (Feys et al., 1994). The vegetative storage protein gene (*VSP*) is not expressed in *coi1* (Ellis and Turner, 2002). The mutant *coi1*-16 was first isolated and identified after screening mutagenised Col-gl seeds carrying the JA-responsive reporter *VSP1*::luciferase (2M line) (Ellis and Turner, 2002). In the mutant *jai3*-1, the *JAZ3* gene has a guanine (G) to adenine (A) transition in the splicing acceptor of its fifth intron, and therefore produces a JAZ3 C-terminal truncated protein (Lorenzo et al., 2004). The mutant *jin1*-1 and *jut* are the MYC2 C-terminal deletion mutant and the *JUT* gene T-DNA insertion mutant, respectively (**Table 2.1**).

4.2 Results

4.2.1 The Expression of *AtMYC2* and *JUT* was Up-regulated by MeJA and Wounding

Arabidopsis Col-0 seeds were surface-sterilised and sown on 1/2 MS medium, stratified, and grown in a LD growth chamber as described in the **section 2.2.5**.

For gene expression analyses after MeJA treatment, six groups of fourteen twelve-day-old Col-0 seedlings were carefully transferred with sterilised fine forceps onto 1/2 MS medium containing 50μM MeJA. These groups of MeJA-treated seedlings were harvested by snap freezing in liquid nitrogen at 15mins, 30mins, 45mins, 1h, 3h and 6h after transfer. Fourteen untreated seedlings were harvested (as above) as the control sample.

For gene expression analyses following wounding, six groups of fourteen twelve-day-old Col-0 seedlings were selected for wounding treatment. For wounding treatment, one leaf of each seedling was lightly squeezed with sterilised fine forceps. These groups of fourteen wounded-seedlings were harvested by snap freezing in liquid nitrogen at 15mins, 30mins, 45mins, 1h, 3h and 6h after wounding. Fourteen unwounded seedlings were harvested (as above) as the control sample.

Total RNA of individual samples was extracted (**section 2.3.2**), fractionated by RNA agarose gel electrophoresis, transferred to a Hybond^{TM}-XL membrane, and hybridised to ^{32}P-dCTPs-labelled probes for *AtMYC2* and *JUT*, and then analysed by phosphor-imaging, as described in the **section 2.3.9**.

Northern blotting results revealed that the expression of *AtMYC2* (At1g32640) and *JUT* (At2g46510) was induced in Col-0 within 15mins after transfer to MeJA and 15mins after wounding (**Figure 4.1**). The expression of *AtMYC2* continuously increased up till

3h after MeJA treatment and then reduced at 6h after MeJA treatment (**Figure 4.1**). The expression of *AtMYC2* continuously increased up till 1h after wounding and then reduced at 3h and 6h after wounding treatment (**Figure 4.1**). MeJA-induced expression of *JUT* was maintained at high level from 15mins till 6h after MeJA treatment. However, wound-induced expression of *JUT* continuously increased up till 45min after wounding and then reduced at 1h, 3h and 6h after wounding (**Figure 4.1**). These results verify the gene expression analyses in **Figure 3.1** and **Figure 3.2**.

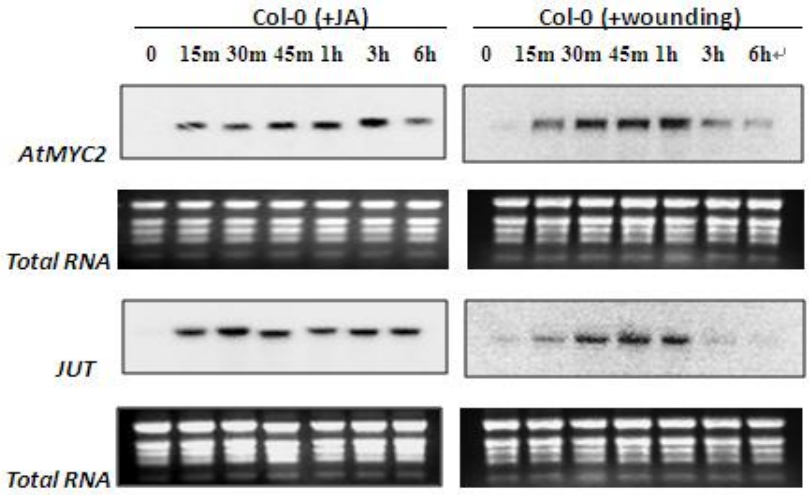


Figure 4.1: *AtMYC2* and *JUT* are rapidly induced by MeJA and wounding.

Twelve-day-old Col-0 Arabidopsis seedlings were treated with 50μM MeJA for 15min, 30min, 45min, 1h, 3h and 6h. 0 stands for non-treated control sample. For the wounding experiment, one leaf was wounded by fine forceps on each seedling. Plant tissues were collected at the indicated times. Blots were hybridised with *AtMYC2* and *JUT* probe, respectively. The primers for probe amplification are shown in **Table 2.4** (**section 2.3.12**). Eight micrograms of total RNA were loaded per lane, and the total RNA for the blots is shown to confirm equal loading.

4.2.2 Requirement of COI1 for MeJA-induced expression of *JUT*

Analysis of the microarray database at the Genvestigator website indicated that the expression of *AtMYC2* and *JUT* was down-regulated in *coi1* in comparison with wild type Col-0 (**Table 3.1**). In order to confirm that the expression of *AtMYC2* and *JUT* are dependent on COI1, I investigated the expression of *AtMYC2* and *JUT* in *coi1*-16.

Arabidopsis seeds of Col-0, *jin1*-1, *jai3*-1, *jut*, Col-gl, *coi1*-16, 2M and JUTOE (**Table 2.1**) were surface-sterilised and sown on 1/2 MS medium, stratified, and grown in a LD growth chamber as described in the **section 2.2.5**. Fourteen twelve-day-old seedlings of each indicated line were carefully transferred with sterilised fine forceps onto 1/2 MS medium containing 50μM MeJA. These MeJA-treated seedlings of each indicated line were harvested at 15mins after transfer, and snap froze in liquid nitrogen. Fourteen untreated seedlings were harvested as the control sample.

Total RNA of individual samples was extracted (**section 2.3.2**), fractionated by RNA agarose gel electrophoresis, transferred to a Hybond^{TM}-XL membrane, and hybridised to ^{32}P-dCTPs-labelled probes for *AtMYC2* and *JUT*, and then analysed by phosphor-imaging, as described in the **section 2.3.9**.

The expression of *AtMYC2* and *JUT* increased at 15mins after MeJA treatment in the wild type of Col-0, Col-gl and 2M (**Figure 4.2**). However, in *coi1*-16, the expression of *AtMYC2* was only slightly increased after JA treatment in comparison with its wild type parent Col-gl and Col-0 (**Figure 4.2**). *JUT* was not increased in expression after MeJA treatment in *coi1*-16 (**Figure 4.2**). These results confirm that the expression of *AtMYC2* is partially dependent on COI1 and the expression of *JUT* is totally dependent on COI1.

In order to verify the incomplete transcription of AtMYC2, the primers for the *AtMYC2* probe were designed on the C-terminal deletion sequence of *AtMYC2* (**Figure 2.2.A**). There is therefore no hybridisation of the probe in the *jin1*-1 background (**Figure 4.2**). In order to verify the incomplete transcription of *JUT*, the primers for the *JUT* probe were designed on the 3' end of the gene (**Figure 2.2.B**). There is no hybridisation of the probe in the *jut* background (**Figure 4.2**). Unexpectedly, there was *AtMYC2* expression in the *jai3*-1 mutant after MeJA treatment. Also the *JUT* expression increased at 15mins after MeJA treatment in the *jai3*-1 mutant (**Figure 4.2**). In JUTOE, *JUT* expression was expressed constitutively, and was increased at 15mins after MeJA treatment compared to Col-0. However, *AtMYC2* expression was reduced at 15mins after MeJA treatment compared to Col-0 (**Figure 4.2**). This result indicates that over-expression of *JUT* represses MeJA-induced expression of *AtMYC2*.

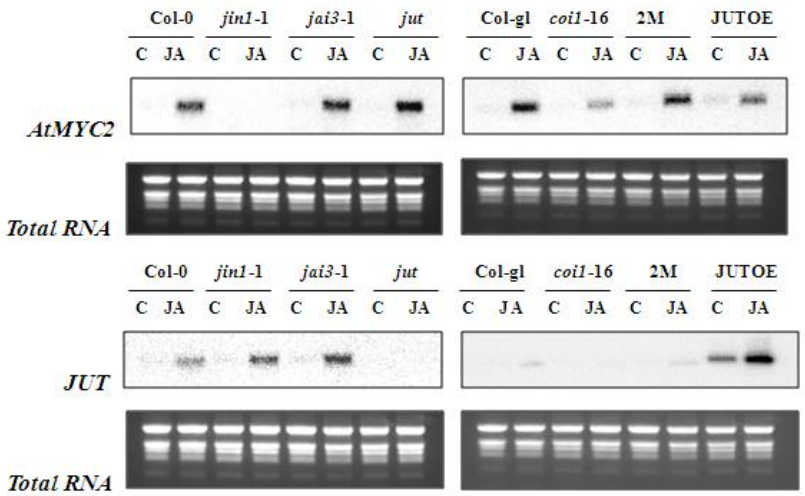


Figure 4.2: Expression of *AtMYC2* and *JUT* are dependent on COI1.

Twelve-day-old Col-0, *jin1*-1, *jai3*, *jut*, col-gl, *coi1*-16, 2M and JUTOE Arabidopsis seedlings were treated with 50μM MeJA for 15min. C stands for non-treated control and JA for MeJA-treated. Plant tissues were collected at the indicated times. Blots were hybridised with *AtMYC2* and *JUT* probe, respectively. The primers for probe amplification are shown in **Table 2.4** (**section 2.3.12**). Eight micrograms of total RNA were loaded per lane.

4.2.3 MYC2 and JUT Are Not Required for the Initial MeJA-induced Expression of Each Other

In order to identify whether the expression of *AtMYC2* and *JUT* are dependent on each other or not, the expression of *AtMYC2* was analysed in the T-DNA insertion mutant *jut*, and the expression of *JUT* was analysed in the MYC2 C-terminal deletion mutant *jin1*-1.

Arabidopsis seeds of Col-0, *jin1*-1 and *jut* were surface-sterilised and sown on 1/2 MS medium, stratified, and grown under LD in a growth chamber as described in **section 2.2.5** in **Chapter 2**. Six groups of fourteen twelve-day-old Col-0 seedlings were carefully transferred with sterilised fine forceps onto 1/2 MS medium containing 50μM MeJA. These MeJA-treated seedlings of each indicated line were harvested by snap freezing in liquid nitrogen at 15mins, 30mins, 45mins, 1h, 3h and 6h after transfer. Fourteen untreated seedlings were harvested (as above) as the control sample.

Total RNA of individual samples was extracted (**section 2.3.2**), fractionated by RNA agarose gel electrophoresis, transferred to a Hybond XL membrane, and hybridised to ^{32}P-dCTPs-labelled probes for *AtMYC2* and *JUT*, and then analysed by phosphor-imaging, as described in the **section 2.3.9**.

In the wild type Col-0, the expression of *AtMYC2* was induced at 15mins up till 3h after MeJA treatment and then reduced at 6h after MeJA treatment. In the T-DNA insertion mutant line *jut*, *AtMYC2* continued to be expressed in samples taken at 15min, 30min, 45min, 1h, 3h and 6h after MeJA treatments (**Figure 4.3**). These results indicate that JUT is not required for the initial MeJA-induced the expression of *AtMYC2*.

Chapter 4: Genetic Interaction between *AtMYC2* and *JUT*

In the wild type Col-0, the expression of *JUT* was maintained at high level at 15mins up till 6h after MeJA treatment. The expression of *JUT* was slightly increased at 15min and 30min after MeJA treatment in *jin1*-1, but gradually decreased in samples taken at 45mins, 1h, 3h and 6h after MeJA treatments (**Figure 4.3**). These results indicate that MYC2 is partially required for the initial MeJA-induced the expression of *JUT*, and also required for maintaining the high level expression of *JUT* at 45mins after MeJA treatment. These results therefore imply that MYC2 partially regulates MeJA-induced expression of *JUT*.

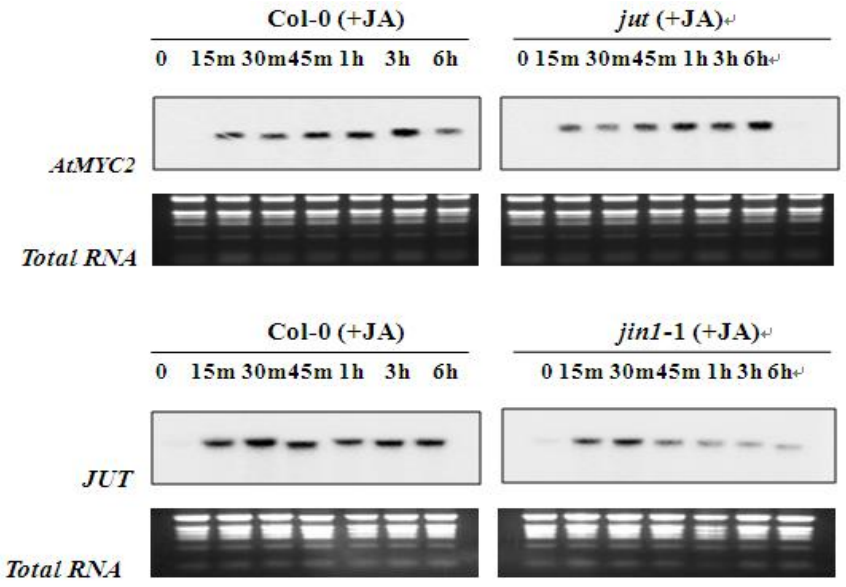


Figure 4.3: MYC2 and JUT are not required for the initial MeJA-induced expression of each other.

Twelve-day-old Col-0, *jut* and *jin1*-1 Arabidopsis seedlings were treated with 50μM MeJA for 15min, 30min, 45min, 1h, 3h and 6h. 0 stands for non-treated. Plant tissues were collected at the indicated times. Blots were hybridized with *AtMYC2* and *JUT* probe, respectively. The primers for probe amplification are shown in **Table 2.4** (section **2.3.12**). Eight micrograms of total RNA were loaded per lane as a control.

4.2.4 Identifying the JA Response of the Double Mutant *jin1*-1/*jut*

4.2.4.1 Generation of the Double Mutant *jin1*-1/*jut*

In order to identify the interaction of JUT and MYC2 in the JA pathway, the double mutant *jin1*-1/ *jut* was generated by crossing Arabidopsis lines of *jin1*-1 and *jut*. The method used for Arabidopsis crossing is grown in **section 2.2.6**.

Plants of the *JUT* T-DNA insertion line (SAIL_536_F09) were crossed into a background lacking a functional *AtMYC2* gene (*jin1*-1). The styles elongated after successful crossing. The F1 seeds were sown and, after the siliques become mature, the F2 seeds were harvested. As the results show in **Figure 3.10**, the single mutants *jin1-1* and *jut* are both less sensitive to root growth inhibition induced by MeJA in comparison with the wild type Col-0. Sixty F2 seeds were grown on 1/2 MS medium containing 50μM MeJA. Only ten of these seedlings displayed MeJA insensitivity, and these were numbered from 1 to 10 (**Figure 4.4.A**). These results indicate that these ten MeJA less sensitive plants should be homozygous at least for the *jin1*-1 or *jut* mutation.

These ten seedlings were then transferred into soil, and F3 seeds were harvested from the mature siliques. Seedlings 1, 2 and 5 did not reach maturity. Ten F3 seeds of individuals 3, 4, 6, 7, 8, 9, 10 were collected and sown on 1/2 MS media in a LD growth chamber. After two-days, five F3 seedlings from these lines were transferred onto 1/2 MS media containing 50μM MeJA, and five F3 seedlings from each line were transferred onto 1/2 MS as a control (**Figure 4.4.B**). After one week, growth of the seedlings was compared. Seedlings from line 3 were less sensitive to MeJA than *jin1*-1 and *jut* (**Figure 4.4.C**).

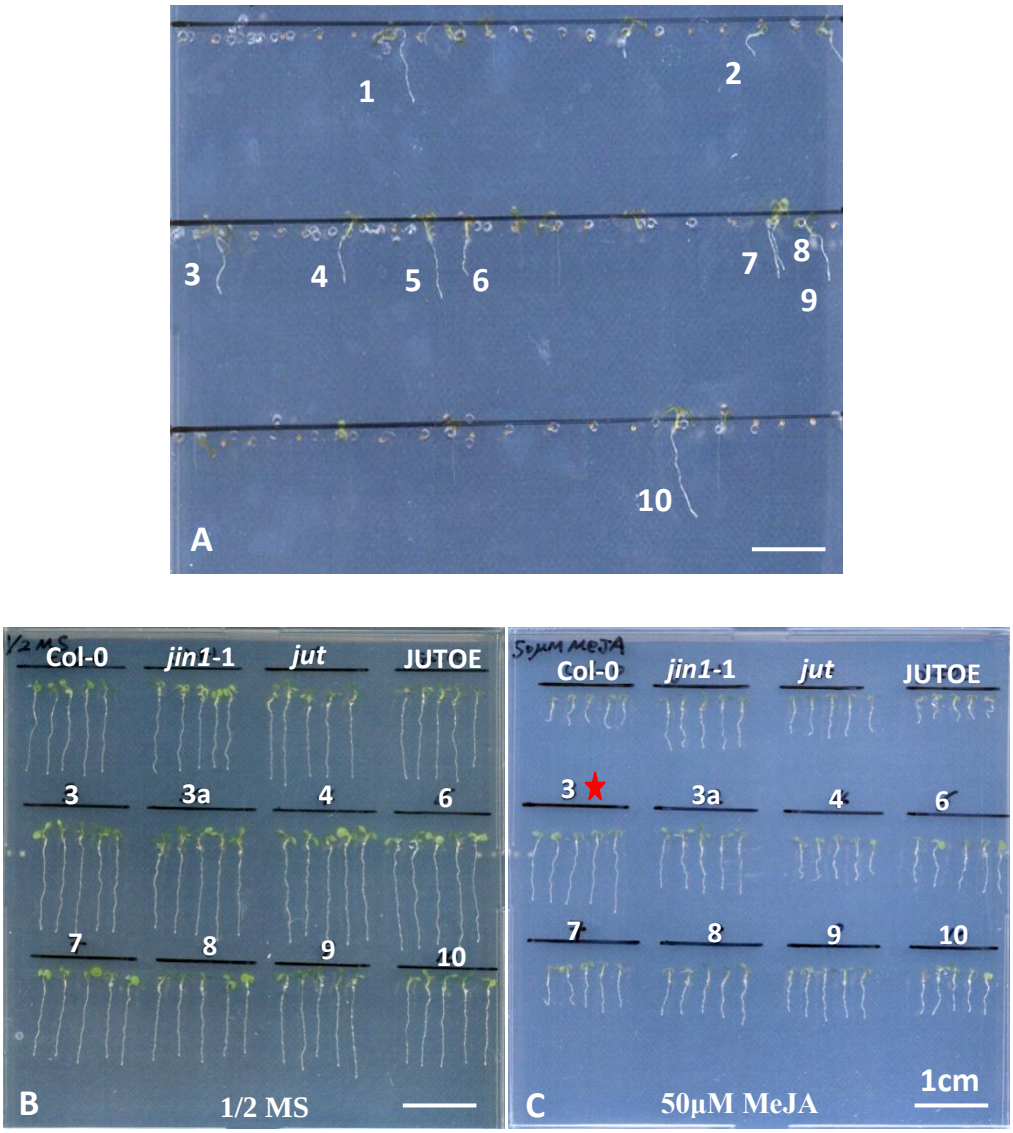


Figure 4.4: Root growth inhibition assay on the double mutant *jin1*-1/*jut*.

Seedlings of Col-0, *jin1*-1, *jut*, JUTOE and lines No.3, No.3a, No.4, No.6, No.7, No.8, No.9 and No.10 were grown on 50μM MeJA for one week treatment. **A.** Ten of F2 seedlings (numbered from 1 to 10) are less sensitive to MeJA treatment; **B.** Five F3 seedlings of *jin1*-1, *jut*, No.3, No.3a, No.4, No.6, No.8, No.9 and No.10 were grown on 1/2 MS media for control experiment; **C.** Seedlings of *jin1*-1, *jut*, No.3, No.3a, No.4, No.6, No.8, No.9 and No.10 are less sensitive to MeJA-induced root growth inhibition. The No. 3 seedlings (highlighted with a red star) are less sensitive to MeJA than *jin1*-1 and *jut*. Line 3a is the F4 progeny of line 3.

4.2.4.2 PCR Identification of the Double Mutant *jin1*-1/*jut*

The Arabidopsis seedlings grown on 1/2 MS media containing 50μM MeJA (**Figure 4.4.D**) were transferred to soil and grown at 22°C LD for another three weeks. Genomic DNA of each indicated line was extracted following the protocol described in **section 2.3.1**.

PCR-based methods were used to identify lines with the double mutation, *jin1*-1/*jut*. Primers used for PCR identification of the double mutant *jin1*-1/*jut* are displayed in **Table 2.5** in **section 2.3.12**. Primers in group A (MYC2F and MYC2R in **Figure 2.2.A**) were designed to amplify a *AtMYC2* product from wild type, but not from *jin1*-1 mutant. Primers in group B (LP(3) and RP(3), F(1) and R(1) in **Figure 2.3**) were designed to amplify two *JUT* specific products from wild type, but not from the *jut* mutant, where the presence of the T-DNA would make the fragment too large to be amplified. Primers in group C (LBb1 and R(1) in **Figure 2.3**) were designed to amplify the T-DNA insertion specific product from *jut*, but not from wild type. Primers in group D (CF1 and R1 in **Figure 2.1**) were designed to amplify a 35S::*JUT* product. PCR reaction programs are described in **section 2.3.6.4**. PCR products were analysed by agarose gel electrophoresis following the protocol described in **section 2.3.3**.

Genomic DNA from wild type (Col-0) gave a product with primers A, indicating that presence of C-terminal of *AtMYC2*. In Col-0 background, two products were amplified separately by each pair of primers in Group B, indicating the presence of *JUT*. No product was amplified by primers pair C, indicating the absence of T-DNA insertion in *JUT*. No product was amplified by primers pair D, indicating the absence of 35S::JUT in wild type background (**Figure 4.5.A**).

Genomic DNA from *jin1*-1 did not amplify any product with primers A, indicating that absence of C-terminal of *AtMYC2*. In *jin1*-1 background, two products were amplified separately by each pair of primers in Group B, indicating the presence of *JUT*. No product was amplified by primers pair C, indicating the absence of T-DNA insertion in

JUT. No product was amplified by primers pair D, indicating the absence of 35S::JUT in *jin1*-1 background (**Figure 4.5.A**).

Genomic DNA from *jut* gave a product with primers A, indicating that presence of C-terminal of *AtMYC2*, however no product with both primer pairs B, indicating the absence of *JUT*. One product was amplified by primers pair C, indicating the presence of T-DNA insertion in *JUT*. No product was amplified by primers pair D, indicating the absence of 35S::JUT in *jut* background (**Figure 4.5.A**).

Genomic DNA from JUTOE gave a product with primers A, indicating that presence of C-terminal of *AtMYC2*, and with both primer pairs B, indicating the presence of *JUT*. No product was amplified by primers pair C, indicating the absence of T-DNA insertion in *JUT*. One product was amplified by primers pair D, indicating the presence of 35S::JUT in JUTOE background (**Figure 4.5.B**).

Genomic DNA from No. 3 seedling did not amplify any product with primers A, indicating that absence of C-terminal of *AtMYC2*, and no product with both primer pairs B, indicating the absence of *JUT*. One product was amplified by primers pair C, indicating the presence of T-DNA insertion in *JUT* (pointed with red arrow in **Figure 4.5.B**). No product was amplified by primers pair D, indicating the absence of 35S::JUT in this background (**Figure 4.5.B**).

Genomic DNA from No. 4 and No.6 seedlings gave one product with primers A, indicating that presence of C-terminal of *AtMYC2*, and with both primer pairs B, indicating the presence of *JUT*. One product was amplified by primers pair C, indicating the presence of T-DNA insertion in *JUT*. No product was amplified by primers pair D, indicating the absence of 35S::JUT in this background (**Figure 4.5.C**).

Genomic DNA from No. 7 seedling did not amplify any product with primers A, indicating that absence of C-terminal of *AtMYC2*, and with both primer pairs B, indicating the presence of *JUT*. One product was amplified by primers pair C,

indicating the presence of T-DNA insertion in *JUT*. No product was amplified by primers pair D, indicating the absence of 35S::JUT in this background (**Figure 4.5.C**).

Genomic DNA from No. 8 seedling did not amplify any product with primers A, indicating that absence of C-terminal of *AtMYC2*, and with one primer pairs B, indicating the presence of *JUT*. One product was amplified by primer pairs C, indicating the presence of T-DNA insertion in *JUT*. No product was amplified by primer pairs D, indicating the absence of 35S::JUT in this background (**Figure 4.5.D**).

Genomic DNA from No. 9 seedling did not amplify any product with primers A, indicating that absence of C-terminal of *AtMYC2*, and with both primer pairs B, indicating the presence of *JUT*. One product was amplified by primer pairs C, indicating the presence of T-DNA insertion in *JUT*. No product was amplified by primer pairs D, indicating the absence of 35S::JUT in this background (**Figure 4.5.D**).

Genomic DNA from No. 10 seedling gave one product with primers A, indicating that presence of C-terminal of *AtMYC2*, however no product with both primer pairs B, indicating the absence of *JUT*. No product was amplified by primer pairs C, indicating the absence of T-DNA insertion in *JUT*. No product was amplified by primer pairs D, indicating the absence of 35S::JUT in this background (**Figure 4.5.D**).

Genomic DNA from 3a-2, 3a-3, 3a-4 did not amplify any product with primers A, indicating that absence of C-terminal of *AtMYC2*, and no product with both primer pairs B, indicating the absence of *JUT*. One product was amplified by primer pairs C, indicating the presence of T-DNA insertion in *JUT* (**Figure 4.5.E**). There was one failure PCR amplification by primer pair C in the 3a-5 background (**Figure 4.5.E**). These results suggest that No. 3a-2, 3a-3, 3a-4 are candidates of homozygous double mutant *jin1*-1/*jut*.

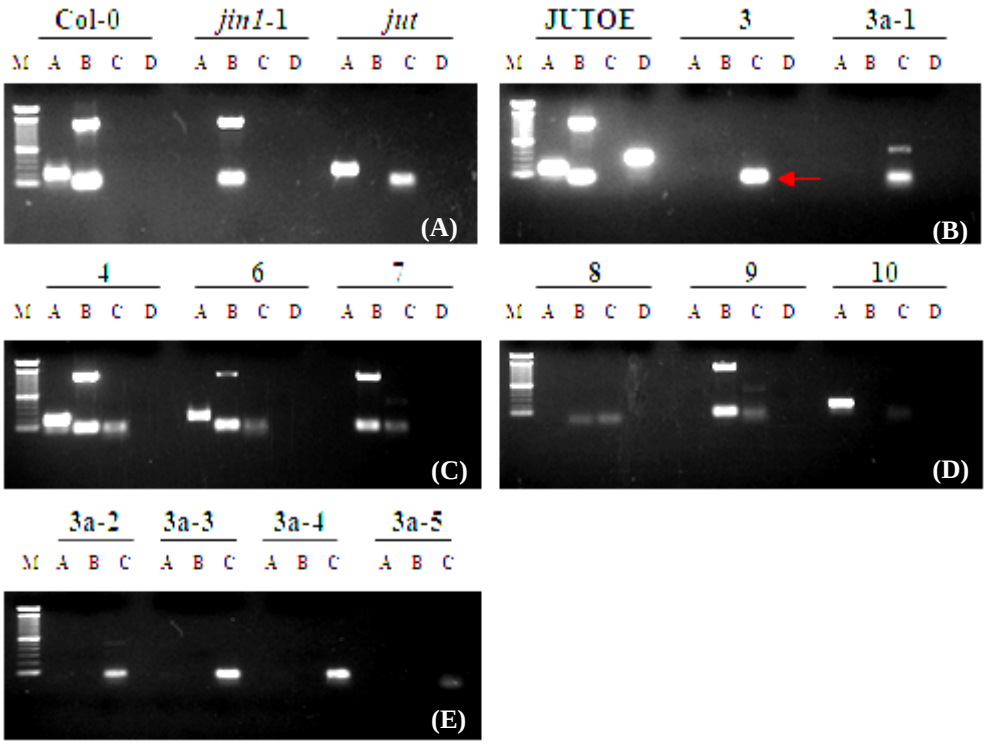


Figure 4.5: PCR identification of the double mutant *jin1*-1/*jut*.

Genomic DNA was extracted from Col-0, *jin1*-1, *jut*, JUTOE, F2 seedlings from a cross of *jin1*-1×*jut* (**Figure 4.4.B**), numbered 3, 4, 6, 7, 8, 9 and 10, and F3 progeny of seedling 3, numbered 3a-1, 3a-2, 3a-3, 3a-4 and 3a-5. These genomic DNA samples were amplified with primer groups A, B, C and D. Primer pair A (**Figure 2.2.A**) amplifies a product from wild type, but not *jin1*-1. Primer group B (**Figure 2.3**) has two pair of Primers which both amplifying a product from wild type, but not *jut*. Those amplifications were performed separately, but the products were run together as B. Primer pair C amplifies a product from the T-DNA insertion in *jut*, but not wild type. Primer pair D amplifies a product from JUTOE but not wild type, *jin1*-1 or *jut*.

4.2.4.3 Effect of MeJA on Root Growth and Shoot Growth of *jin1*-1, *jut* and *jin1*-1/*jut*

In order to identify the effect of MeJA on root growth of the double mutant *jin1*-1/*jut*, nine lines of Arabidopsis seeds were directly germinated and grown on 1/2 MS media (with or without 20μM MeJA) for seven days (**section 2.2.7** in **Chapter 2**). The root length of Col-0 is shorter than other lines (**Figure 4.6** & **Figure 4.7.A**). It has been discovered that wound-induced endogenous jasmonates negatively regulate mitosis and therefore repress plant growth in wild type background (Zhang and Turner, 2008). This might be the reasonable reason why mutants in JA signal pathway displayed the longer root length than wild type. This The mutants of *jin1*-1, *jin1*-2, *jut*, *jin1*-1/*jut* and *coi1*-16 were less sensitive to MeJA compared to wild type Col-0, however, the JUTOE line, *jai3*-1 and two wild type lines of Col-0 and Col-gl were sensitive to MeJA (**Figure 4.6**).

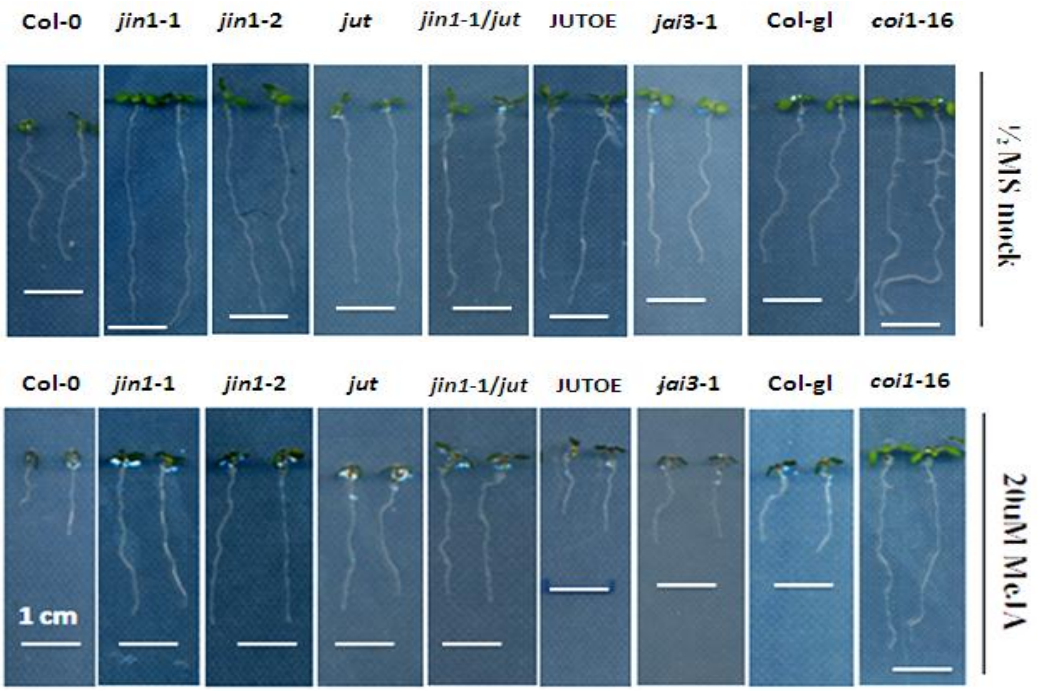


Figure 4.6: Effect of MeJA on root growth of nine Arabidopsis lines.

Arabidopsis seedlings of Col-0, *jin1*-1, *jin1*-2, *jut*, *jin1*-1/*jut*, JUTOE, *jai3*-1, Col-gl and *coi1*-16 display different root growth phenotypes after seven days 20μM MeJA treatment. The white scale equals 1cm.

Chapter 4: Genetic Interaction between *AtMYC2* and *JUT*

In order to identify the effect of MeJA on root growth of the double mutant *jin1*-1/*jut*, nine lines of Arabidopsis seeds were directly germinated and grown on 1/2 MS media (with or without 20μM MeJA) for seven days (**section 2.2.7** in **Chapter 2**). The root lengths of individual seedlings were then analysed with the ImageJ software. **Figure 4.7.A shows** root length of Col-0 and other eight different lines of untreated mock and MeJA-treated samples. Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups.

A two-way ANOVA ($p < 0.05$) was used to determine whether Arabidopsis root length was affected by a) genotypic variation b) the application of MeJA treatment or c) by the two factors (genotype x treatment) combined. To test our hypothesis, we used nine different genotypes (Col-0, *jin1*-1, *jin1*-2, *jut*, JUTOE, *jin1*-1/*jut*, *jai3*-1, col-gl and *coi1*-16) and two treatments (mock and 20μM MeJA treatment). Statistical analyses were conducted using SPSS18 program (SPSS Inc., Chicago IL). Data of Arabidopsis root length was normally distributed and met the assumptions of two-way ANOVA.

Results of the analysis (**Figure 4.7.C**) indicate that the factor of Genotype had significant effect on the Arabidopsis root length ($F_{\text{Genotype}}(8, 192) = 99.753$, $p < 0.001$), as well as the factor Treatment ($F_{\text{Treatment}}(1,192) = 1205.807$, $p < 0.001$). There is also a significant effect from Genotype x Treatment on Arabidopsis root length ($F_{\text{Genotype} \times \text{Treatment}}(8, 192) = 13.442$, $p < 0.001$). Pair-wise comparisons (**Figure 4.7.D**) revealed that *jin1*-1, *jin1*-2, *jut*, JUTOE, *jin1*-1/*jut*, *jai3*-1, col-gl and *coi1*-16 has statistically significant difference to wild type Col-0 ($p < 0.001$). These results confirmed the observation that *jin1*-1, *jin1*-2, *jut*, JUTOE, *jin1*-1/*jut*, *jai3*-1 display significant difference after MeJA treatment compared to the wild type Col-0, and *coi1*-16 shows a significant difference after MeJA treatment compared to its background col-gl. In *jin1*-1, *jin1*-2, *jut*, *jin1*-1/*jut*, *jai3*-1, the percentage of root length after MeJA treatment to its untreated mock group are 51.75%, 52.72%, 68.03%, 66.56% and 42.78% respectively, while in wild type Col-0 that percentage is 28.7% **(Figure 4.7.A)**. These results indicate

that *jin1*-1, *jin1*-2, *jut*, *jin1*-1/*jut* and *jai3*-1 display a reduced responsiveness to MeJA-induced root growth inhibition than wild type Col-0. In *coi1*-16, the percentage of root length after MeJA treatment to its untreated mock group is 72.04%, while in its background col-gl that percentage is 34.96% **(Figure 4.7.A)**. This result indicates *coi1*-16 has a reduced responsiveness to MeJA-induced root growth inhibition than col-gl. In JUTOE, the percentage of root length after MeJA treatment to its untreated mock group is 26.05% **(Figure 4.7.A)** indicating JUTOE shows an increased responsiveness to MeJA-induced root growth inhibition than wild type Col-0.

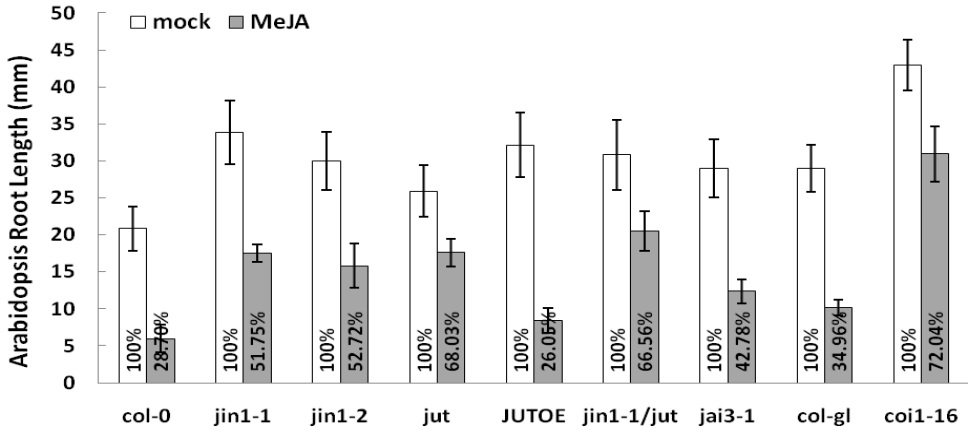


Table B: Effect of MeJA treatment on Arabidopsis root length (mean ± SD).

Genotype	mock	MeJA treated
Col-0	20.86 ± 3.02	5.99 ± 4.25
***jin1*-1**	33.86 ± 1.84	17.52 ± 1.17
jin1-2	30.03 ± 3.94	15.83 ± 2.95
jut	25.91 ± 3.49	17.63 ± 1.86
JUTOE	32.15 ± 4.35	8.37 ± 1.67
***jin1*-1**/jut	30.81 ± 4.67	20.51 ± 2.70
***jai3*-1**	28.98 ± 3.88	12.40 ± 1.61
col-gl	29 ± 3.21	10.14 ± 1.09
***coi1*-16**	42.94 ± 3.46	30.93 ± 3.75

Table C: Summary of Genotype and treatment on Arabidopsis root length by two-way ANOVA.

Source	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Genotype	8	962.020	99.753	.000	.806	798.025
Treatment	1	11628.818	1205.807	.000	.863	1205.807
Genotype × Treatment	8	129.631	13.442	.000	.359	107.533
Error	192	9.644				
Total	210					
Corrected Total	209					

Figure 4.7: Effect of MeJA treatment on Arabidopsis root length of nine different lines. A. Root length of nine different lines in untreated mock and 48h MeJA-treated samples. Bar charts display the value of mean ± SD (n=15). Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups. **Table B** displays effect of MeJA treatment on Arabidopsis root length (mean ± SD). **Table C** displays the summary of effects analyzed by two-way ANOVA. **Table D** (on next page) illustrates the pair-wise comparisons between different groups by Tukey's LSD.

Table D: Multiple comparisons between different groups by Tukey's LSD.

Significantly different groups are highlighted by "$p < 0.05$: *", "$p < 0.01$: **", $p < 0.001$: *** ".

comparisons	Mean difference	Std. Error	Sig.	95% Confidence Interval Lower Bound, Upper Bound	
Col-0 vs *jin1*-1	-12.268	.935	.000***	-14.112	-10.425
Col-0 vs *jin1*-2	-9.504	.923	.000***	-11.324	-7.684
Col-0 vs *jut*	-8.343	.923	.000***	-10.163	-6.523
Col-0 vs jutOE	-6.838	.874	.000***	-8.563	-5.113
Col-0 vs *jin1*-1/*jut*	-12.238	.913	.000***	-14.038	-10.437
Col-0 vs *jai3*-1	-7.268	.911	.000***	-9.064	-5.471
Col-0 vs col-gl	-6.147	.845	.000***	-7.814	-4.480
Col-0 vs *coi1*-16	-23.511	.901	.000***	-25.288	-21.734
jin1-1 vs col-0	12.268	.935	.000***	10.425	14.112
jin1-1 vs *jin1*-2	2.764	.971	.005**	.850	4.679
jin1-1 vs *jut*	3.926	.971	.000***	2.011	5.840
jin1-1 vs jutOE	5.431	.925	.000***	3.606	7.255
jin1-1 vs *jin1*-1/*jut*	.031	.961	.974	-1.865	1.927
jin1-1 vs *jai3*-1	5.001	.959	.000***	3.108	6.893
jin1-1 vs col-gl	6.121	.897	.000***	4.351	7.891
jin1-1 vs coil-16	-11.243	.950	.000***	-13.116	-9.369
jin1-2 vs col-0	9.504	.923	.000***	7.684	11.324
jin1-2 vs *jin1*-1	-2.764	.971	.005**	-4.679	-.850
jin1-2 vs *jut*	1.161	.959	.228	-.731	3.054
jin1-2 vs jutOE	2.666	.913	.004**	.866	4.467
jin1-2 vs *jin1*-1/*jut*	-2.733	.950	.004**	-4.607	-.860
jin1-2 vs *jai3*-1	2.236	.948	.019*	.367	4.106
jin1-2 vs col-gl	3.357	.885	.000***	1.611	5.102
jin1-2 vs coil-16	-14.007	.938	.000***	-15.858	-12.156
jut vs Col-0	8.343	.923	.000***	6.523	10.163
jut vs *jin1*-1	-3.926	.971	.000***	-5.840	-2.011
jut vs *jin1*-2	-1.161	.959	.228	-3.054	.731
jut vs jutOE	1.505	.913	.101	-.296	3.306
jut vs *jin1*-1/*jut*	-3.895	.950	.000***	-5.768	-2.021
jut vs *jai3*-1	1.075	.948	.258	-.795	2.945
jut vs col-gl	2.195	.885	.014*	.450	3.941
jut vs coi1-16	-15.168	.938	.000***	-17.019	-13.317
jutOE vs Col-0	6.838	.874	.000***	5.113	8.563
jutOE vs *jin1*-1	-5.431	.925	.000***	-7.255	-3.606
jutOE vs *jin1*-2	-2.666	.913	.004*	-4.467	-.866

jutOE vs *jut*	-1.505	.913	.101	-3.306	.296
jutOE vs *jin1-1/jut*	-5.400	.903	.000***	-7.180	-3.619
jutOE vs *jai3-1*	2.195	.885	.014*	.450	3.941
jutOE vs col-gl	-15.168	.938	.000***	-17.019	-13.317
jutOE vs coi1-16	6.838	.874	.000***	5.113	8.563
jin1-1/jut vs Col-0	12.238	.913	.000***	10.437	14.038
jin1-1/jut vs *jin1-1*	-.031	.961	.974	-1.927	1.865
jin1-1/jut vs *jin1-2*	2.733	.950	.004**	.860	4.607
jin1-1/jut vs *jut*	3.895	.950	.000***	2.021	5.768
jin1-1/jut vs jutOE	5.400	.903	.000***	3.619	7.180
jin1-1/jut vs *jai3-1*	4.970	.938	.000***	3.119	6.821
jin1-1/jut vs col-gl	6.090	.875	.000***	4.365	7.815
jin1-1/jut vs coi1-16	-11.273	.929	.000***	-13.105	-9.442
jai3-1 vs Col-0	7.268	.911	.000***	5.471	9.064
jai3-1 vs *jin1-1*	-5.001	.959	.000***	-6.893	-3.108
jai3-1 vs *jin1-2*	-2.236	.948	.019*	-4.106	-.367
jai3-1 vs *jut*	-1.075	.948	.258	-2.945	.795
jai3-1 vs jutOE	.430	.901	.634	-1.347	2.207
jai3-1 vs *jin1-1/jut*	-4.970	.938	.000***	-6.821	-3.119
jai3-1 vs col-gl	1.120	.873	.201	-.601	2.841
jai3-1 vs coi1-16	-16.243	.927	.000***	-18.071	-14.416
Col-gl vs Col-0	6.147	.845	.000***	4.480	7.814
Col-gl vs *jin1-1*	-6.121	.897	.000***	-7.891	-4.351
Col-gl vs *jin1-2*	-3.357	.885	.000***	-5.102	-1.611
Col-gl vs *jut*	-2.195	.885	.014*	-3.941	-.450
Col-gl vs jutOE	-.691	.834	.409	-2.336	.955
Col-gl vs *jin1-1/jut*	-6.090	.875	.000***	-7.815	-4.365
Col-gl vs *jai3-1*	-1.120	.873	.201	-2.841	.601
Col-gl vs coi1-16	-17.364	.862	.000***	-19.064	-15.663
coi1-16 vs Col-0	23.511	.901	.000***	21.734	25.288
coi1-16 vs *jin1-1*	11.243	.950	.000***	9.369	13.116
coi1-16 vs *jin1-2*	14.007	.938	.000***	12.156	15.858
coi1-16 vs *jut*	15.168	.938	.000***	13.317	17.019
coi1-16l vs jutOE	16.673	.891	.000***	14.916	18.430
coi1-16 vs *jin1-1/jut*	11.273	.929	.000***	9.442	13.105
coi1-16 vs *jai3-1*	16.243	.927	.000***	14.416	18.071
coi1-16 vs col-gl	17.364	.862	.000***	15.663	19.064

The fresh weights of individual seedlings were then analysed. **Figure 4.8.A shows** fresh weight of Col-0 and other eight different lines of untreated mock and MeJA-treated samples. Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups.

A two-way ANOVA ($p < 0.05$) was used to determine whether Arabidopsis fresh weight after MeJA treatment was affected by a) genotypic variation b) the application of MeJA treatment or c) by the two factors (genotype x treatment) combined. To test our hypothesis, nine different genotypes (Col-0, *jin1*-1, *jin1*-2, *jut*, JUTOE, *jin1*-1/*jut*, *jai3*-1, col-gl and *coi1*-16) and two treatments (mock and 20μM MeJA treatment) were used in this experiment. Statistical analyses were conducted using SPSS18 program (SPSS Inc., Chicago IL). Data of Arabidopsis root length were was normally distributed and met the assumptions of two-way ANOVA.

Results of the analysis (**Figure 4.8.C**) indicate that the factor of Genotype had significant effect on the Arabidopsis fresh weight (F_{Genotype} (8, 182) = 222.027, $p < 0.001$), as well as the factor Treatment ($F_{\text{Treatment}}$ (1,182) = 470.633, $p < 0.001$). There is also a significant effect from Genotype x Treatment on Arabidopsis fresh weight ($F_{\text{Genotype} \times \text{Treatment}}$ (8, 182) = 6.737, $p < 0.001$). Pair-wise comparisons (**Figure 4.8.D**) revealed that *jin1*-1, *jin1*-2, *jut*, JUTOE, *jin1*-1/*jut*, *jai3*-1, col-gl and *coi1*-16 has statistically significant difference to wild type Col-0 ($p < 0.05$). These results confirmed the observation that *jin1*-1, *jin1*-2, *jut*, JUTOE, *jin1*-1/*jut*, *jai3*-1 display significant difference after MeJA treatment compared to the wild type Col-0, and *coi1*-16 shows a significant difference after MeJA treatment compared to its background col-gl. In *jin1*-1, *jin1*-2, *jut*, *jin1*-1/*jut*, *jai3*-1, the percentage of fresh weight after MeJA treatment to its untreated mock group are 73.93%, 78.14%, 84.62%, 79.27% and 65.16% respectively, while in wild type Col-0 that percentage is 63.29% **(Figure 4.8.A)**. These results indicate that *jin1*-1, *jin1*-2, *jut*, *jin1*-1/*jut* and *jai3*-1 display a reduced responsiveness to MeJA-induced growth inhibition than wild type Col-0. In *coi1*-16, the percentage of

fresh weight after MeJA treatment to its untreated mock group is 78.43%, while in its background col-gl that percentage is 69.23% **(Figure 4.8.A)**. This result indicates *coi1*-16 has a reduced responsiveness to MeJA-induced growth inhibition than col-gl. In JUTOE, the percentage of fresh weight after MeJA treatment to its untreated mock group is 54.94% **(Figure 4.8.A)** indicating JUTOE shows an increased responsiveness to MeJA-induced growth inhibition than wild type Col-0.

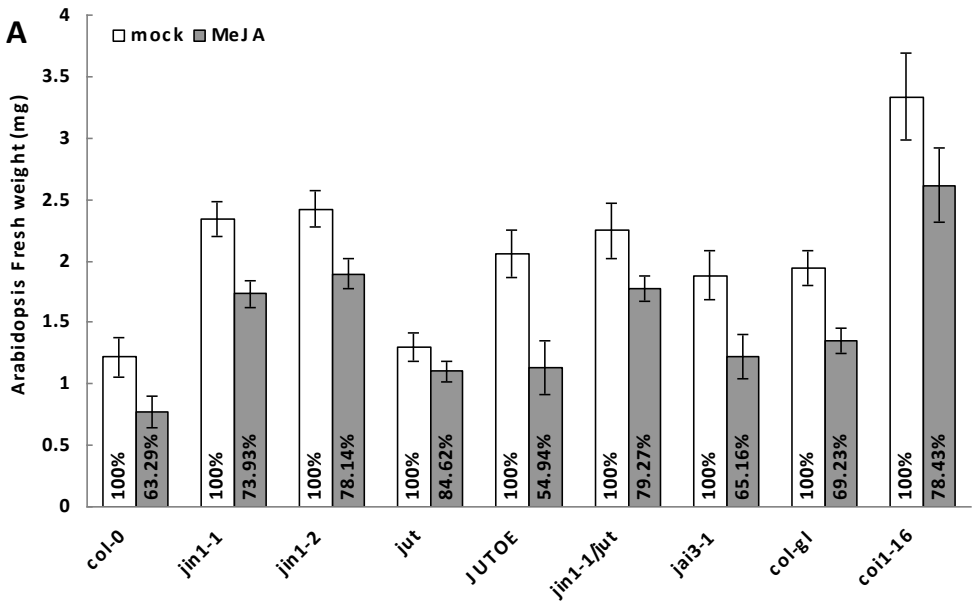


Table B: Effect of MeJA treatment on Arabidopsis root length (mean ± SD).

Genotype	mock	MeJA treated
Col-0	1.22 ± 0.16	0.77 ± 0.13
jin1-1	2.34 ± 0.14	1.73 ± 0.11
jin1-2	2.42 ± 0.15	1.89 ± 0.12
jut	1.3 ± 0.12	1.1 ± 0.09
JUTOE	2.06 ± 0.20	1.13 ± 0.22
*Jin*1-1/*jut*	2.25 ± 0.23	1.78 ± 0.10
jai3-1	1.88 ± 0.2	1.23 ± 0.18
col-gl	1.94 ± 0.14	1.35 ± 0.10

Table C: Summary of Genotype and treatment on Arabidopsis root length by two-way ANOVA.

Source	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Genotype	8	7.683	222.027	.000	.907	1776.213
Treatment	1	16.285	470.633	.000	.721	470.633
Genotype × Treatment	8	.233	6.737	.000	.228	53.895
Error	182	.035				
Total	200					
Corrected Total	199					

Table D: Multiple comparisons between different groups by Tukey's LSD (Alpha=0.05). Significantly different groups are highlighted by "$p < 0.05$: *", "$p < 0.01$: **", $p < 0.001$: *** ".

comparisons	Mean difference	Std. Error	Sig.	95% Confidence Interval Lower Bound, Upper Bound	
Col-0 vs *jin1*-1	-1.0214	.05747	.000***	-1.2018	-.8409
Col-0 vs *jin1*-2	-1.1292	.05675	.000***	-1.3074	-.9511
Col-0 vs *jut*	-.1773	.05609	.047*	-.3534	-.0012
Col-0 vs jutOE	-.5624	.05438	.000***	-.7331	-.3916
Col-0 vs *jin1*-1/*jut*	-1.0102	.05675	.000***	-1.1883	-.8320
Col-0 vs *jai3*-1	-.5818	.05609	.000***	-.7579	-.4057
Col-0 vs col-gl	-.6155	.05490	.000***	-.7879	-.4432
Col-0 vs *coi1*-16	-1.9472	.05547	.000***	-2.1214	-1.7731
jin1-1 vs col-0	1.0214	.05747	.000***	.8409	1.2018
jin1-1 vs *jin1*-2	-.1079	.05812	.645	-.2903	.0746
jin1-1 vs *jut*	.8441	.05747	.000***	.6637	1.0245
jin1-1 vs jutOE	.4590	.05580	.000***	.2838	.6342
jin1-1 vs *jin1*-1/*jut*	.0112	.05812	1.000	-.1713	.1937
jin1-1 vs *jai3*-1	.4395	.05747	.000***	.2591	.6200
jin1-1 vs col-gl	.4058	.05632	.000***	.2290	.5826
jin1-1 vs coi1-16	-.9259	.05687	.000***	-1.1044	-.7473
jin1-2 vs col-0	1.1292	.05675	.000***	.9511	1.3074
jin1-2 vs *jin1*-1	.1079	.05812	.645	-.0746	.2903
jin1-2 vs *jut*	.9519	.05675	.000***	.7738	1.1301
jin1-2 vs jutOE	.5669	.05506	.000***	.3940	.7397
jin1-2 vs *jin1*-1/*jut*	.1190	.05741	.494	-.0612	.2993
jin1-2 vs *jai3*-1	.5474	.05675	.000***	.3692	.7256

jin1-2 vs col-gl	.5137	.05558	.000***	.3392	.6882
jin1-2 vs coi1-16	-.8180	.05614	.000***	-.9943	-.6417
*jut*vs Col-0	.1773	.05609	.047*	.0012	.3534
jut vs *jin1*-1	-.8441	.05747	.000***	-1.0245	-.6637
jut vs *jin1*-2	-.9519	.05675	.000***	-1.1301	-.7738
jut vs jutOE	-.3851	.05438	.000***	-.5558	-.2144
jut vs *jin1*-1/*jut*	-.8329	.05675	.000***	-1.0111	-.6547
jut vs *jai3*-1	-.4045	.05609	.000***	-.5806	-.2285
jut vs col-gl	-.4383	.05490	.000***	-.6106	-.2659
*jut*vs coi1-16	-1.7700	.05547	.000***	-1.9441	-1.5958
jutOE vs Col-0	.5624	.05438	.000***	.3916	.7331
jutOE vs *jin1*-1	-.4590	.05580	.000***	-.6342	-.2838
jutOE vs *jin1*-2	-.5669	.05506	.000***	-.7397	-.3940
jutOE vs *jut*	.3851	.05438	.000***	.2144	.5558
jutOE vs *jin1*-1/*jut*	-.4478	.05506	.000***	-.6207	-.2749
jutOE vs *jai3*-1	-.0195	.05438	1.000	-.1902	.1513
jutOE vs col-gl	-.0532	.05316	.985	-.2201	.1137
jutOE vs coi1-16	-1.3849	.05374	.000***	-1.5536	-1.2161
jin1-1/*jut*vs Col-0	1.0102	.05675	.000***	.8320	1.1883
jin1-1/*jut* vs *jin1*-1	-.0112	.05812	1.000	-.1937	.1713
jin1-1/*jut* vs *jin1*-2	-.1190	.05741	.494	-.2993	.0612
jin1-1/*jut* vs *jut*	.8329	.05675	.000***	.6547	1.0111
jin1-1/*jut*vs jutOE	.4478	.05506	.000***	.2749	.6207
jin1-1/*jut*vs *jai3*-1	.4284	.05675	.000***	.2502	.6065
jin1-1/*jut* vs col-gl	.3946	.05558	.000***	.2201	.5691
jin1-1/*jut* vs coi1-16	-.9371	.05614	.000***	-1.1133	-.7608
jai3-1 vs Col-0	.5818	.05609	.000***	.4057	.7579
jai3-1 vs *jin1*-1	-.4395	.05747	.000***	-.6200	-.2591
jai3-1 vs *jin1*-2	-.5474	.05675	.000***	-.7256	-.3692
jai3-1 vs *jut*	.4045	.05609	.000***	.2285	.5806
jai3-1 vs jutOE	.0195	.05438	1.000	-.1513	.1902
jai3-1 vs *jai3*-1	-.4284	.05675	.000***	-.6065	-.2502
jai3-1 vs col-gl	-.0337	.05490	1.000	-.2061	.1387
jai3-1 vs coi1-16	-1.3654	.05547	.000***	-1.5396	-1.1913
Col-gl vs Col-0	.6155	.05490	.000***	.4432	.7879
Col-gl vs *jin1*-1	-.4058	.05632	.000***	-.5826	-.2290
Col-gl vs *jin1*-2	-.5137	.05558	.000***	-.6882	-.3392
Col-gl vs *jut*	.4383	.05490	.000***	.2659	.6106
Col-gl vs jutOE	.0532	.05316	.985	-.1137	.2201
Col-gl vs *jin1*-1/*jut*	-.3946	.05558	.000***	-.5691	-.2201

Col-gl vs *jai3*-1	.0337	.05490	1.000	-.1387	.2061
Col-gl vs coi1-16	-1.3317	.05428	.000***	-1.5021	-1.1613
coi1-16 vs Col-0	1.9472	.05547	.000***	1.7731	2.1214
coi1-16 vs *jin1*-1	.9259	.05687	.000***	.7473	1.1044
coi1-16 vs *jin1*-2	.8180	.05614	.000***	.6417	.9943
coi1-16 vs *jut*	1.7700	.05547	.000***	1.5958	1.9441
coi1-16l vs jutOE	1.3849	.05374	.000***	1.2161	1.5536
coi1-16 vs *jin1*-1/*jut*	.9371	.05614	.000***	.7608	1.1133
coi1-16 vs *jai3*-1	1.3654	.05547	.000***	1.1913	1.5396
coi1-16 vs col-gl	1.3317	.05428	.000***	1.1613	1.5021

Figure 4.8: Effect of MeJA treatment on Arabidopsis fresh weight of nine different lines. **A.** Arabidopsis fresh weight of nine different lines in untreated mock and 48h MeJA-treated samples. Bar charts display the value of mean ± SD (n=15). Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups. **Table B** displays effect of MeJA treatment on Arabidopsis root length (mean ± SD). **Table C** displays the summary of effects analyzed by two-way ANOVA. **Table D** (on next page) illustrates the pair-wise comparisons between different groups by Tukey's LSD.

Chapter 4: Genetic Interaction between *AtMYC2* and *JUT*

To test the role of JUT on MeJA-induced root growth inhibition, Arabidopsis seeds of each Col-0, *jin1*-1, *jut*, JUTOE, *jin1*-1/*jut*, Col-gl and *coi1*-16 were germinated and grown on 1/2 MS media in a LD growth chamber. After one week, more than fourteen seedlings of each line were carefully transferred with sterilised forceps onto 50μM MeJA MS plate for 48h treatment. An equal number of Arabidopsis seedlings of each line were transferred onto 1/2MS media for 48h control treatment. The root lengths of individual seedlings were then mearsured with ImageJ software. **Figure 4.9.A shows** root length of Col-0, *jin1*-1, *jut*, JUTOE, *jin1*-1/*jut*, col-gl and *coi1*-16 in untreated mock and 48h MeJA-treated samples. Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups.

A two-way ANOVA ($p < 0.05$) was used to determine whether Arabidopsis root length after MeJA treatment was affected by a) genotypic variation b) the application of MeJA treatment or c) by the two factors (genotype x treatment) combined. To test our hypothesis, seven different genotypes (Col-0, *jin1*-1, *jut*, JUTOE, *jin1*-1/*jut*, col-gl and *coi1*-16) and two treatments (mock and 50uM MeJA for 48h treatment) were used in this experiment. Statistical analyses were conducted using SPSS18 program (SPSS Inc., Chicago IL). Data of Arabidopsis root length was normally distributed and met the assumptions of two-way ANOVA.

Results of the analysis (**Figure 4.9.C**) indicate that the factor of Genotype had a significant effect on the Arabidopsis root length (F_{Genotype} (6, 182) = 65.576, $p < 0.001$), as well as the factor Treatment ($F_{\text{Treatment}}$ (1,182) = 211.152, $p < 0.001$). There is also a significant effect from Genotype x Treatment on Arabidopsis root length ($F_{\text{Genotype} \times \text{Treatment}}$ (6, 182) = 2.868, $p < 0.05$). Pair-wise comparisons (**Figure 4.9.D**) revealed that there is a statistically significant difference between groups of Col-0 and *jin1*-1 ($p < 0.001$), Col-0 and *jut* ($p < 0.01$), Col-0 and *jin1*-1/*jut* ($p < 0.01$), Col-0 and *coi1*-16 ($p < 0.001$). These results confirmed the observation that *jin1*-1, *jut* and *jin1*-1/*jut* display significant difference after MeJA treatment compared to the wild type Col-0. In *jin1*-1

mutant, the percentage of root length after MeJA treatment to its untreated mock group is 78.63%, while in wild type Col-0 that percentage is 43.75% (**Figure 4.9.A**). This result indicates that *jin1*-1 has a reduced responsiveness to MeJA-induced root growth inhibition than wild type Col-0. In *jut* mutant, the percentage of root length after MeJA treatment to its untreated mock group is 63.57%, and in *jin1*-1/*jut* that percentage is 68.95% (**Figure 4.9.A**). These results indicate that *jin1*-1/*jut* has a reduced responsiveness to MeJA-induced root growth inhibition than *jut*, but an increased responsiveness than *jin1*-1.

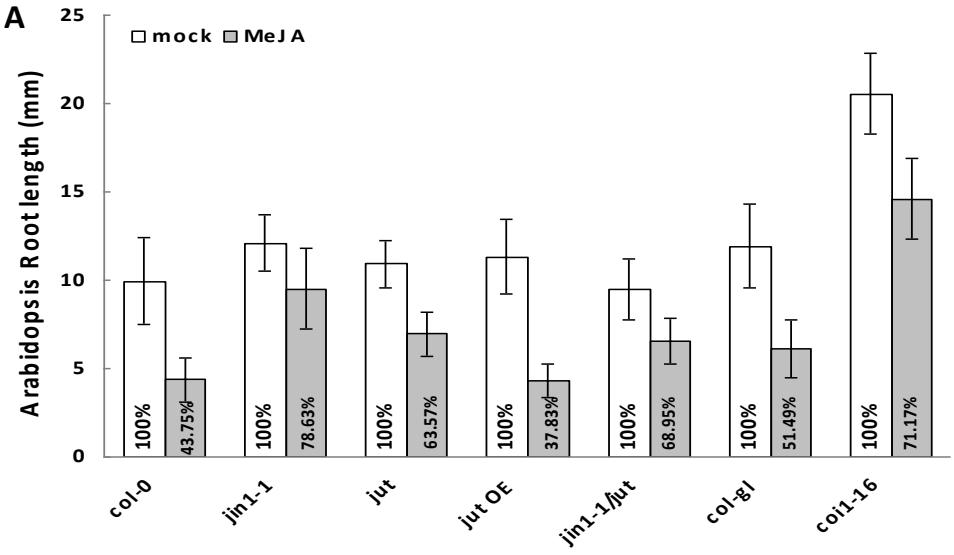


Table B: Effect of MeJA treatment on Arabidopsis root length (mean ± SD).

Genotype	mock	MeJA treated
col-0	9.95 ± 2.4	4.35 ± 1.2
jin1-1	12.1 ± 1.58	9.52 ± 2.25
jut	10.29 ± 1.35	6.94 ± 1.23
JUTOE	11.33 ± 2.14	4.28 ± 0.95
Jin1-1/jut	9.47 ± 1.74	6.53 ± 1.28
col-gl	11.92 ± 2.37	6.14 ± 1.62
coi1-16	20.53 ± 2.28	14.61 ± 2.29

Table C: Summary of Genotype and treatment on Arabidopsis root length by two-way ANOVA.

Source	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Genotype	6	3.669	65.576	.000	.684	1.000
Treatment	1	11.814	211.152	.000	.537	1.000
Genotype × Treatment	6	.160	2.868	.011	.086	.884
Error	182	.056				
Total	196					
Corrected Total	6	3.669	65.576	.000		

Table D: Pair-wise comparisons ($p < 0.05$) between different groups by Tukey's LSD. Significantly different groups are highlighted by "$p < 0.05$: *", "$p < 0.01$: **", $p < 0.001$: *** ".

comparisons	Mean difference	Std. Error	Sig.	95% Confidence Interval Lower Bound, Upper Bound	
Col-0 vs *jin1*-1	-.4051	.06322	**.000*****	-.5936	-.2167
Col-0 vs *jut*	-.2296	.06322	**.007****	-.4181	-.0412
Col-0 vs jutOE	-.1225	.06322	.459	-.3110	.0660
Col-0 vs *jin1*-1/*jut*	-.2374	.06322	**.004****	-.4258	-.0489
Col-0 vs col-gl	-.1790	.06322	.075	-.3675	.0095
Col-0 vs coi1-16	-1.0964	.06322	**.000*****	-1.2849	-.9079
jin1-1 vs col-0	.4051	.06322	**.000*****	.2167	.5936
jin1-1 vs *jut*	.1755	.06322	.086	-.0130	.3640
jin1-1 vs jutOE	.2827	.06322	**.000*****	.0942	.4712
jin1-1 vs *jin1*-1/*jut*	.1678	.06322	.116	-.0207	.3563
jin1-1 vs col-gl	.2262	.06322	**.008****	.0377	.4147
jin1-1 vs coi1-16	-.6913	.06322	**.000*****	-.8797	-.5028
jut vs Col-0	.2296	.06322	**.007****	.0412	.4181
jut vs *jin1*-1	-.1755	.06322	.086	-.3640	.0130
jut vs jutOE	.1072	.06322	.620	-.0813	.2957
jut vs *jin1*-1/*jut*	-.0077	.06322	1.000	-.1962	.1808
jut vs col-gl	.0507	.06322	.985	-.1378	.2392
jut vs coi1-16	-.8668	.06322	**.000*****	-1.0552	-.6783
jutOE vs Col-0	.1225	.06322	.459	-.0660	.3110
jutOE vs *jin1*-1	-.2827	.06322	**.000*****	-.4712	-.0942
jutOE vs *jut*	-.1072	.06322	.620	-.2957	.0813
jutOE vs *jin1*-1/*jut*	-.1149	.06322	.538	-.3034	.0736
jutOE vs col-gl	-.0565	.06322	.973	-.2450	.1320
jutOE vs coi1-16	-.9739	.06322	**.000*****	-1.1624	-.7854
jin1-1/*jut* vs Col-0	.2374	.06322	**.004****	.0489	.4258

***jin1*-1/*jut* vs *jin1*-1**	-.1678	.06322	.116	-.3563	.0207
jin1*-1/*jut* vs *jut	.0077	.06322	1.000	-.1808	.1962
***jin1*-1/*jut* vs jutOE**	.1149	.06322	.538	-.0736	.3034
***jin1*-1/*jut* vs col-gl**	.0584	.06322	.968	-.1301	.2469
***jin1*-1/*jut* vs coi1-16**	-.8590	.06322	**.000***	-1.0475	-.6705
Col-gl vs Col-0	.1790	.06322	.075	-.0095	.3675
Col-gl vs *jin1*-1	-.2262	.06322	**.008****	-.4147	-.0377
Col-gl vs *jut*	-.0507	.06322	.985	-.2392	.1378
Col-gl vs jutOE	.0565	.06322	.973	-.1320	.2450
Col-gl vs *jin1*-1/*jut*	-.0584	.06322	.968	-.2469	.1301
Col-gl vs coi1-16	-.9174	.06322	**.000***	-1.1059	-.7289
coi1-16 vs Col-0	1.0964	.06322	**.000***	.9079	1.2849
coi1-16 vs *jin1*-1	.6913	.06322	**.000***	.5028	.8797
coi1-16 vs *jut*	.8668	.06322	**.000***	.6783	1.0552
coi1-16l vs jutOE	.9739	.06322	**.000***	.7854	1.1624
coi1-16 vs *jin1*-1/*jut*	.8590	.06322	**.000***	.6705	1.0475
coi1-16 vs col-gl	.9174	.06322	**.000***	.7289	1.1059

Figure 4.9: Effect of MeJA treatment on Arabidopsis root length of Col-0, *jin1*-1, *jut*, JUTOE, *jin1*-1/*jut*, col-gl and *coi1*-16. A. Root length of Col-0, *jin1*-1, *jut*, JUTOE, *jin1*-1/*jut*, col-gl and *coi1*-16 in untreated mock and 48h MeJA-treated samples. Bar charts display the value of mean ± SD (n=14). Percentages inside the charts represent the root length of MeJA-treated different lines to their respective untreated (mock) groups. **Table B:** Effect of MeJA treatment on Arabidopsis root length (mean ± SD). **Table C** displays the summary of effects analyzed by two-way ANOVA. **Table D** (on next page) illustrates the multiple comparisons between different groups by Tukey's LSD.

The Arabidopsis seeds of wild type Col-0 and Col-gl, MYC2OE and JUTOE, the single mutants *coi1*-16, *jin1*-2, *jin1*-1, *jut*, *jai3*-1 and the double mutant *jin1*-1/*jut* were directly germinated and grown on both 1/2 MS media and 1/2 MS media containing 20μM MeJA in a LD growth room for twelve days (**section 2.2.7** in **Chapter 2**). The double mutant *jin1*-1/*jut* was less sensitive to MeJA-indued growth inhibition than either of the single mutants (**Figure 4.10**). This result indicates that both MYC2 and JUT are required for MeJA-induced shoot growth inhibition.

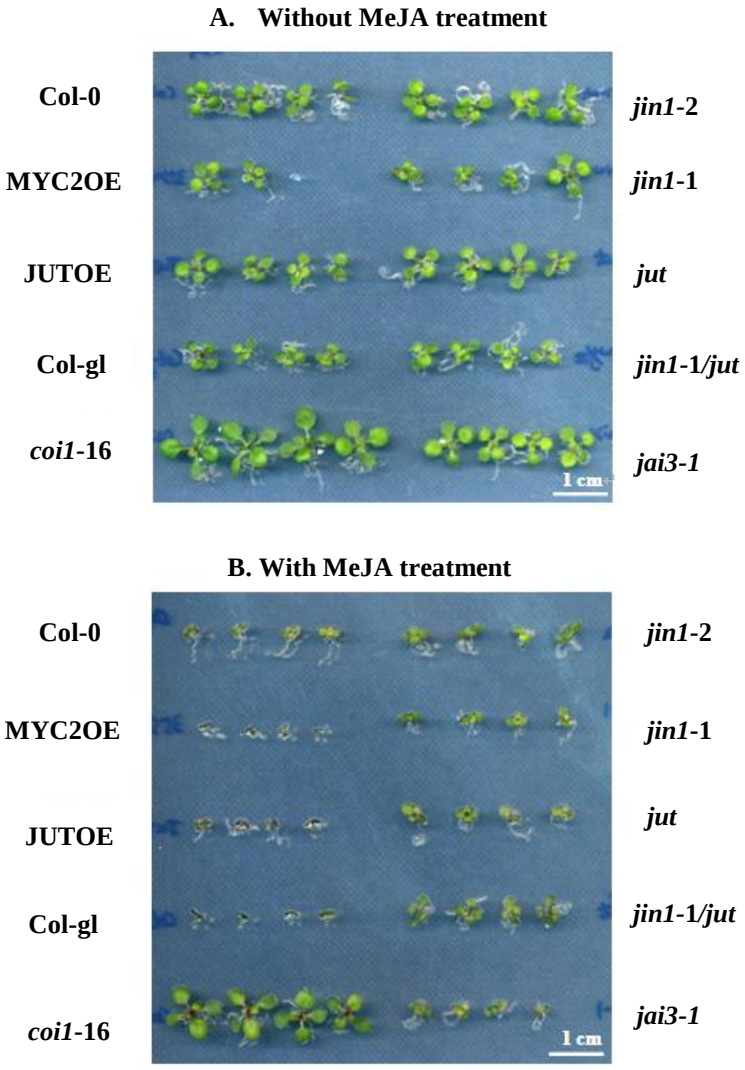


Figure 4.10: Arabidopsis shoot growth without and with MeJA treatment. Pictures of Arabidopsis seedlings on 1/2 MS (without MeJA treatment) and 1/2 MS media containing 20μM MeJA (after MeJA treatment) are taken on the 12th day after seed germination.

4.2.4.4 Flowering time of *jin1*-1 and *jut*

Arabidopsis plants of Col-0, *jin1*-1, MYC2OE, *jut*, JUTOE, *jin1*-1/jut, *jai3*-1 and *coi1*-16 were grown on soil in a LD growth room for four weeks. The double mutant *jin1*-1/*jut* displays an earlier flowering phenotype than the single mutant *jin1*-1 and *jut*.

A one-way ANOVA was used to test the preference differences among all genotypes. The output of "F (7, 88) = 535.7, $p<0.0001$" indicates the preferences for stem length differed significantly across the different genotypes (**A** in **Figure 4.11**). A one-way ANOVA was used to test for preference differences among all genotypes. The output of "F (7, 88) = 91.07, $p<0.0001$." indicates the preferences for flowering time differed significantly across the different genotypes (**B** in **Figure 4.11**). A one-way ANOVA was used to test the preference differences between *jut* and *coi1*-16. The output of "F (1, 22) = 24.27, $p<0.0001$." indicates the preferences for flowering time differed significantly in *jut* and *coi1*-16. The mutant of *coi1*-16 displays early flowering phenotype compared with its wild type background (Robson et al., 2010). The flowering time of *jut* is early than wild type col-0 and later than *coi1*-16 (**B** in **Figure 4.11)**. The over-expression line MYC2OE and JUTOE show delayed flowering phenotype in comparison with wild type Col-0 (**B** in **Figure 4.11**). These results indicate that MYC2 and JUT negative regulate flowering time.

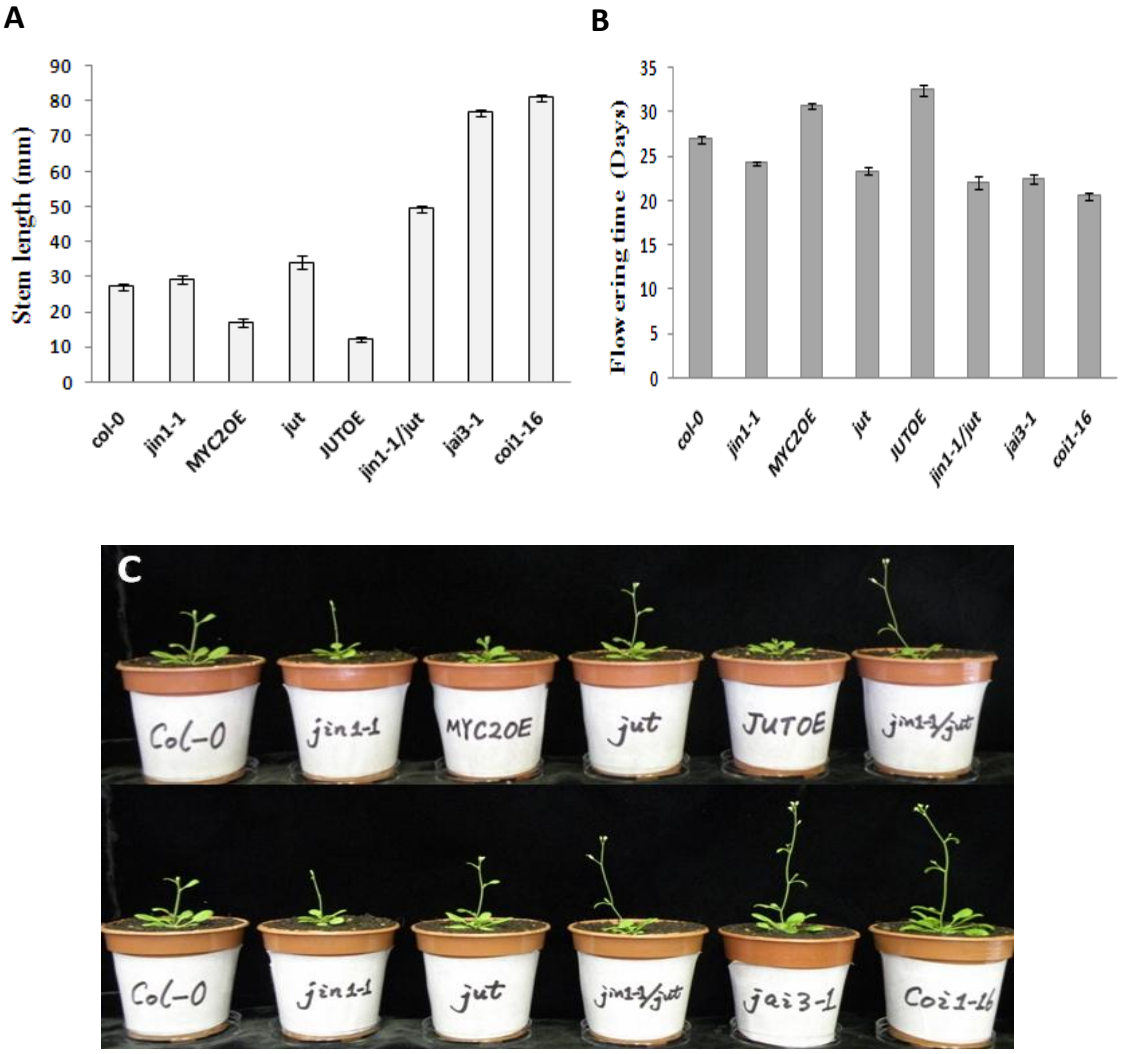


Figure 4.11: Double mutant *jin1*-1/*jut* flowers earlier than *jin1*-1 and *jut*.

A. Stem length of 28-day-old Arabidopsis seedlings. Arabidopsis seeds were directly germinated and grown in LD growth room for 28 days. Bar chart displays the value of mean ± SE of 12 seedlings for each line. **B.** Flowering time in LD condition. The number of days records the first flower appearance on the seedling of individual line. Bar chart displays the value of mean ± SE of 12 seedlings for each line. **C.** The 28-day-old Arabidopsis seedlings. Arabidopsis seeds were directly germinated and grown in LD growth room. The pictures are taken on the 28th day after seed germination.

4.3 Discussion

4.3.1 Expression of *JUT* and *AtMYC2* are COI1-dependent

Previous research identified that the up-regulated expression of *AtMYC2* was dependent on COI1 (Lorenzo at al., 2004). The expression of *AtMYC2* was induced by JA or ABA treatment in wild type plants. On the contrary, there was no inducible expression of *AtMYC2* by JA or ABA treatment in *coi1*-1 (**Figure 4.12**). This study indicates that expression of *AtMYC2* was enhanced by treatment with JA or ABA, and was COI1-dependent.

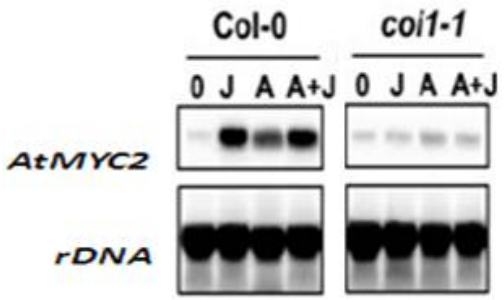


Figure 4.12: JA- and ABA-induced *AtMYC2* expression depends on COI1. (This Figure was edited from **Figure 7B** in Lorenzo at al., 2004). Four-week-old wild type (Col-0) and *coi1*-1 were treated with 50 µM of jasmonic acid (J), 100 µM of ABA (A) and both JA and ABA (A+J) for 30min. 0 stands for non-treated control. Twenty micrograms of total RNA were loaded per lane. *AtMYC2* probe was hybridised with all blots and rDNA was used for loading control.

In this chapter, I have examined whether the expression of *JUT* induced by JA, and whether COI1 is required for this inducible expression of *JUT*. As expected, the expression of *JUT* was induced by MeJA in wild type plants, but not in *coi1*-16 (**Figure 4.2**). This result supports the hypothesis that COI1 is required for the MeJA-induced expression of *JUT*. The expression of AtMYC2 is slightly induced by MeJA treatment in coi1-16 compared to wild type background (**Figure 4.2**). This result indicates that MeJA-induced expression of *AtMYC2* is partially dependent on COI1.

4.3.2 Transcriptional regulation between JUT and MYC2

JUT was not required for the initial MeJA-induced expression of *AtMYC2* (**Figure 4.3**). These results indicate that MYC2 is partially required for the initial MeJA-induced the expression of *JUT*, and also required for maintaining the high level expression of *JUT* at 45mins after MeJA treatment (**Figure 4.3**). These results therefore imply that MYC2 partially regulates MeJA-induced expression of *JUT*. Additionally, in MeJA-treated JUTOE, *AtMYC2* expression was less than wild type after MeJA treatment (**Figure 4.2**). This indicates that constitutively expressed *JUT* represses MeJA-induced expression of *AtMYC2*.

4.3.3 Phenotype of *jin1*-1 and *jut*

JUTOE was more sensitive to MeJA-induced growth inhibition compared to wild type and mutants. However, *jut* was less sensitive to MeJA-induced growth inhibition compared to wild type and mutants (**Figure 4.7** and **Figure 4.8**). These results demonstrate that JUT functions as a novel component in the JA signal pathway. The double mutant *jin1*-1/*jut* was less sensitive to MeJA than *jut*, but more sensitive than *jin1*-1. This indicates that both MYC2 and JUT are required for MeJA-induced root growth inhibition. Likewise, both MYC2 and JUT are required for MeJA-induced growth inhibition as determined by fresh weight. Constitutive expression of MYC2 demonstrated an exaggerated response to MeJA-induced growth inhibition (Lorenzo et al., 2004). In agreement, lines with constitutive expression of MYC2 and JUT were more sensitive to MeJA-induced growth inhibition (**Figure 4.10**). The cotyledons of MYC2OE and JUTOE seedlings after MeJA treatment had distinct purple margins, due to the anthocyanin accumulation (**Figure 4.10**). These results indicating that MYC2 and JUT are components of the jasmonate signal pathway that stimulate the anthocyanin accumulation.

4.3.4 Double Mutant *jin1*-1/*jut* Flowers Earlier than *jin1*-1 and *jut*

The mutant *coi1*-16 flowers earlier than wild type (Robson et al., 2010). Therefore the flowering time of *jin1*-1, *jut* has been investigated in this Chapter. Both mutants flowered earlier than wild type. However, the double mutant *jin1*-1/*jut* flowered earlier than either of the single mutant, at a similar time to *coi1*-16. Apparently, therefore JUT and MYC2 regulate flowering time at least partially independently. In agreement, MYCOE and JUTOE flowered later than wild type. All of these results indicate that MYC2 and JUT function as negative regulators of flowering time. The reasonable model of flowering time regulation by JA signal pathway is that COI1 positively regulates transcription factor MYC2 and JUT, therefore to repress the flowering time in wild type background (**Figure 4.13**).

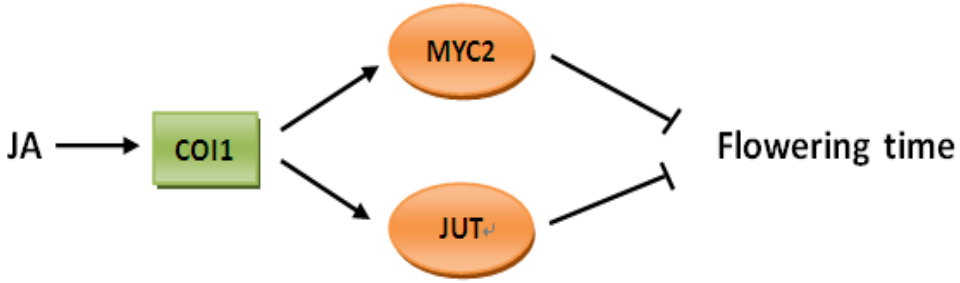


Figure 4.13: A regulatory model of JA signal pathway on flowering time.

Chapter 5

Global Transcriptional Profiling Analyses

5.1 Introduction

Affymetrix technology has been widely used for investigating global transcriptional profiling in Arabidopsis. The principle of Affymetrix technology is described in **section 1.7.2**. According to a previous global transcriptional profiling analysis of wild type Col-gl and the mutant *coi1*-16 after JA and wounding, about 84% of 212 JA-induced genes and 44% of 153 wounding-induced genes have COI1-dependent gene expression. On the other hand, about 53% of 104 JA-suppressed genes and 46% of 83 wounding-suppressed genes are repressed by COI1 (Devoto et al., 2005). Another example, genomic microarray analyses revealed that the expression of tyraminotransferase (*TAT3*), dehydroascorbate reductase (*DHAR*) and mono-dehydroascorbate reductase (*MDHAR*) was reduced in a MYC2 mutant *jin1*-9 in comparison with the wild type (Col-0) (Dombrecht et al., 2007). This result indicates that MYC2 positively regulates JA-induced expression of these oxidative stress tolerance genes.

As described in Chapter 4, the expression of transcription factors MYC2 and JUT are induced within 15min of treatment with MeJA or wounding (**Figure 4.1** in **Chapter 4**). In this chapter, Arabidopsis ATH1 Genome Arrays were used to investigate the genes regulated by MeJA, MYC2 and JUT. This should allow us to identify the role of MYC2 and JUT in transcriptional regulation of the JA signal pathway. Experimentally, the microarray analyses were applied to the following genotypes: wild type (Col-0), *jin1*-1, *jut*, and the double mutant *jin1*-1/*jut*, either untreated as control or treated with MeJA, and the differences in the patterns of gene transcription between genotypes and treatments were compared.

5.2 Methods

5.2.1 Plant Material Preparation

As previously described (**section 2.2.7**), two groups of fourteen Arabidopsis seeds of Col-0, *jin1*-1, *jut* and *jin1*-1/jut were germinated and grown on 1/2 MS media in a LD growth chamber. After two-weeks, fourteen seedlings of each line were carefully transferred with sterilised fine forceps onto 1/2 MS medium containing 20μM MeJA for 30mins, and then snap frozen in liquid nitrogen. Fourteen untreated Arabidopsis seedlings were transferred onto 1/2 MS media and harvested at 30mins after transfer (as above) as the control samples.

5.2.2 Arabidopsis RNA Extraction

Fourteen two-weeks old seedlings of Col-0 mock, Col-0 MeJA-treated, *jin1*-1 mock, *jin1*-1 MeJA-treated, *jut* mock, *jut* MeJA-treated, *jin1*-1/*jut* mock and also *jin1*-1/*jut* MeJA-treated were harvested for total RNA extractions using RNeasy Plant Mini Kit QIAGEN ® (**section 2.3.2** in **Chapter 2**). The eight RNA samples were quantified by Nanodrop ND-1000 Spectrophotometer (**section 2.3.5** in **Chapter 2**).

5.2.3 RNA Sample Submission for Microarray Assay at NASC

Ten micrograms dried total RNA of each sample was submitted to NASC's International Affymetrix Service Centre (http://affymetrix.arabidopsis.info/) for processing. A total of eight microarray slides were used.

5.2.4 Raw Microarray Data Normalisation and Transformation

First of all, raw microarray data in CEL files generated from the Affymetrix machine were downloaded from NASCArrays (http://affymetrix.arabidopsis.info/). All of the raw data was normalised and transformed by Dr. Anyela V. Camargo-Rodriguez and Dr. Jan Kim with Bioconductor software which based on the R statistical programming language. After data normalisation and transformation, all microarray date was then extracted and managed in Microsoft Excel datasheets.

5.3 Results

5.3.1 JA Responsive Genes

To identify genes up-regulated by JA, the expression level of each gene in the treatment (wt + MJ) and the treatment (wt + mock) was examined to determine if it met the criterion: "(wt+MJ)/(wt + mock) >1.5". There are 1158 genes meeting this criterion, for instance, At2g34600 (*JAZ7*), At1g17380 (*JAZ5*), At1g30135 (*JAZ8*), At5g13220 (*JAZ10*) and At1g19180 (*JAZ1*). **Appendix Table A.4** just shows the genes which met the criterion "(wt+MJ)/(wt+mock) >10" . To identify genes down-regulated by JA, the expression level of each gene with the treatment (wt +MJ) and the treatment (wt + mock) was examined to determine if it met the criterion "(wt+MJ)/(wt + mock) <0.5". Two hundred and seven genes met this criterion, for instance, At5g04150 (*bHLH*), At1g73830 (*BEE3*) and At2g32100 (*ATOFP16*) (**Appendix Table A.5**).

5.3.2 MYC2-dependent JA Responsive Genes

5.3.2.1 MYC2-dependent and JA Up-regulated Genes

To identify MYC2-dependent and JA up-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jin1*-1+MJ)/(wt+MJ) <0.5" and "(wt+MJ)/(wtmock) >1.5". Sixty-two genes met these two criteria, for instance, At1g76640 (encodes a calmodulin-related protein), At3g49620 (dark inducible 11, *DIN11*), At1g66690 (encodes a S-adenosyl-L-methionine:carboxyl methyltransferase protein) and At4g37850 (encodes a bHLH protein) (**Appendix Table A.6**).

5.3.2.2 MYC2-dependent and JA Down-regulated Genes

To identify MYC2-dependent and JA down-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jin1*-1+MJ)/(wt+MJ) <0.5" and "(wt+MJ)/(wtmock) <0.5". One glutathione transferase coding gene At5g17220 met these two criteria (**Appendix Table A.7**).

5.3.2.3 MYC2-repressed and JA Up-regulated Genes

To identify MYC2-repressed and JA up-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jin1*-1+MJ)/(wt+MJ) >1.5" and "(wt+MJ)/(wtmock) >1.5". Sixty-nine genes met these two criteria, for instance, At4g24570 (encodes a mitochondrial substrate carrier family protein), At1g66090 (encodes a disease resistance protein), At2g46400 (*WRKY46*), At3g23230 (encodes an ethylene response factor, ERF), At1g33760 (*DREB TF*) (**Appendix Table A.8**).

5.3.2.4 MYC2-repressed and JA Down-regulated Genes

To identify MYC2-repressed and JA down-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jin1*-1+MJ)/(wt+MJ) >1.5" and "(wt+MJ)/(wtmock) <0.5". Fifteen met these two criteria, for instance, At3g56400 (*WRKY70*) (**Appendix Table A.9**).

5.3.3 JUT-dependent JA Responsive Genes

5.3.3.1 JUT-dependent and JA Up-regulated Genes

To identify JUT-dependent and JA up-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jut*+MJ)/(wt+MJ) <0.5" and "(wt+MJ)/(wtmock) >1.5". Twenty-five genes met these two criteria, for instance, At3g49620 (Dark Inducible 11, *DIN11*), At4g37710 (encodes a VQ motif-containing protein) and At1g66690 (encodes one S-adenosyl-L-methionine:carboxyl methyltransferase protein) (**Appendix Table A.10**).

5.3.3.2 JUT-dependent and JA Down-regulated Genes

To identify JUT-dependent and JA down-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jut*+MJ)/(wt+MJ) <0.5" and "(wt+MJ)/(wtmock) <0.5". One late-embryogenesis-abundant gene At2g41620 met these two criteria (**Appendix Table A.11**).

5.3.3.3 JUT-repressed and JA Up-regulated Genes

To identify JUT-repressed and JA up-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jut*+MJ)/(wt+MJ) >1.5" and "(wt+MJ)/(wtmock) >1.5". Thirty genes met these two criteria, for instance, At2g46400 (*WRKY46*) and At2g24850 (tyrosine aminotransferase, *TAT3*) (**Appendix Table A.12**).

5.3.3.4 JUT-repressed and JA Down-regulated Genes

To identify JUT-repressed and JA down-regulated genes, the expression level of each gene was examined to determine if it met the criteria: "(*jut*+MJ)/(wt+MJ) >1.5" and "(wt+MJ)/(wtmock) <0.5". Twenty-three genes met these two criteria, for instance, At5g04150 (encodes a bHLH TF), At3g22231 (Pathogen and Circadian Controlled 1, *PCC1*) and At3g56400 (WRKY70) (**Appendix Table A.13**).

5.3.4 JA responsive genes regulated by both MYC2 and JUT

5.3.4.1 JA up-regulated genes dependent by both MYC2 and JUT

To identify JA up-regulated genes that are dependent by both MYC2 and JUT, the expression level of each gene was examined to determine if it met the criteria: "(DM+MJ)/(wt+MJ) <0.5" and "(DM+MJ)/(wtmock) >1.5". DM refers to the double mutant *jin1*-1/*jut*. Twenty-five genes met these two criteria, for instance, At1g76640 (encodes a calmodulin-related protein), At3g49620 (Dark Inducible 11, *DIN11*) and At4g37710 (encodes a VQ motif-containing protein) (**Appendix Table A.14**).

5.3.4.2 JA down-regulated genes dependent by both MYC2 and JUT

To identify JA down-regulated genes that are dependent by both MYC2 and JUT, the expression level of each gene was examined to determine if it met the criteria: "(DM+MJ)/(wt+MJ) <0.5" and "(DM+MJ)/(wtmock) <0.5". Two genes met these two criteria, for instance, At5g02760 (encodes a protein phosphatise 2C family protein) (**Appendix Table A.15**).

5.3.4.3 JA up-regulated genes repressed by both MYC2 and JUT

To identify JA up-regulated genes that are repressed by both MYC2 and JUT, the expression level of each gene was examined to determine if it met the criteria: "(DM+MJ)/(wt+MJ) >1.5" and "(DM+MJ)/(wtmock) >1.5". Thirty-seven genes met these two criteria at the same time, for instance, At1g18710 (*MYB47*), At2g42540 (Cold-Regulated 15A, *COR15A*) and At1g52690 (late-embryogenesis-abundant gene) (**Appendix Table A.16**).

5.3.4.4 JA down-regulated genes repressed by both MYC2 and JUT

To identify JA down-regulated genes that are repressed by both MYC2 and JUT, the expression level of each gene was examined to determine if it met the criteria: "(DM+MJ)/(wt+MJ) >1.5" and "(DM+MJ)/(wtmock) <0.5". Twelve genes met these two criteria, for instance, At3g22231 (Pathogen and Circadian Controlled 1, *PCC1*), At3g56400 (*WRKY70*) (**Appendix Table A.17**).

5.3.5 Overview of MYC2- and JUT-regulated JA responsive genes

The pie charts in **Figure 5.1** illustrate that the genes dependent by MYC2, JUT or both, largely belong to the JA up-regulated genes. In agreement, the genes repressed by MYC2, JUT or both, are mainly distinguished as JA up-regulated genes.

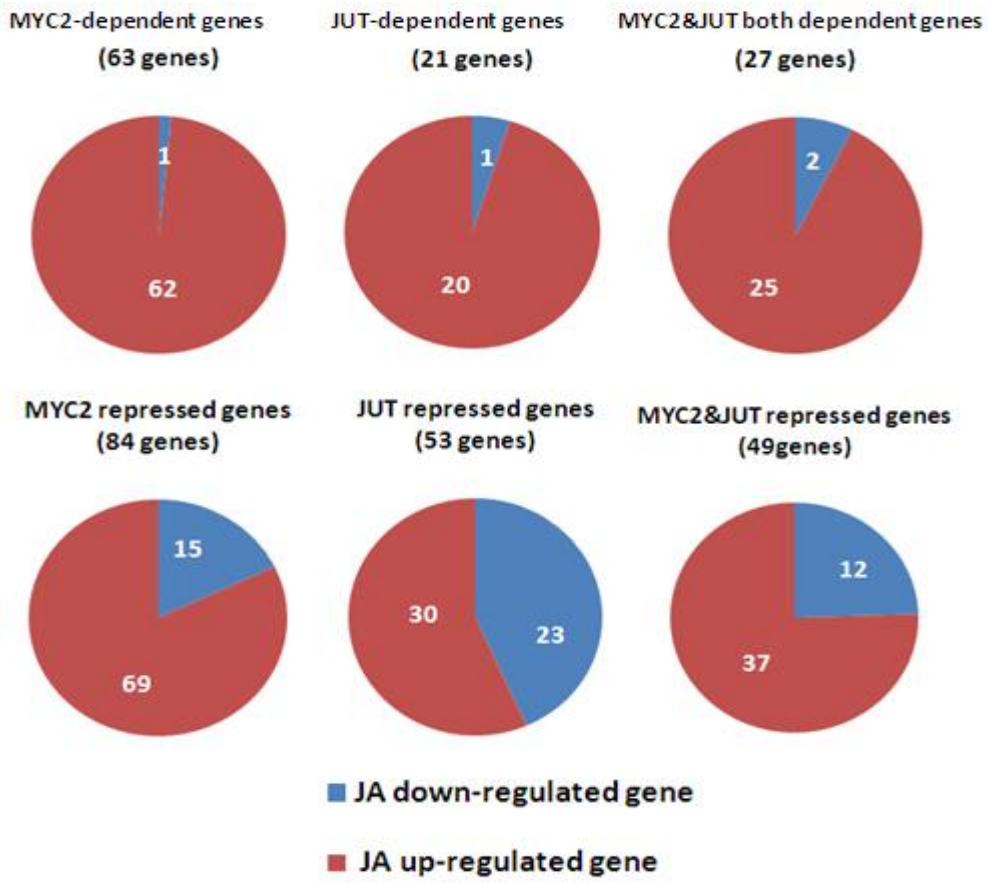


Figure 5.1: Regulation of JA responsive genes which require MYC2 and JUT.
According to the microarray gene transcription profile analyses, there are 62 MYC2-dependent genes and 69 MYC2 repressed genes displaying JA up-regulated pattern, however, only one MYC2-dependent gene and 15 MYC2-repressed genes share the JA down-regulated pattern. Moreover, there are 20 JUT-dependent genes and 30 JUT-repressed genes that have the JA up-regulated pattern, while, only one JUT-dependent gene and 23 JUT-repressed genes share the JA down-regulated pattern. Furthermore, 25 MYC2 & JUT co-dependent genes and 37 MYC2 & JUT co-repressed genes have the JA up-regulated pattern, however, only two MYC2 & JUT co-dependent genes and 12 MYC2 co-repressed genes share the JA down-regulated pattern.

The overlap between MYC2 and JUT regulated genes is shown in **Figure 5.2**. Seven JA up-regulated genes are dependent by MYC2, JUT and MYC2+JUT (**Table 5.1**). Two JA up-regulated genes are repressed by MYC2, JUT and MYC2+JUT (**Table 5.2**). Seven JA down-regulated genes are repressed by MYC2, JUT and MYC2+JUT (**Table 5.3**). For example, At3g56400 (WRKY70) is down-regulated by JA, and repressed by MYC2, JUT and MYC2+JUT (**Table 5.3**). This adds to a previous report that over-expression of *WRKY70* suppresses JA responsive genes, and causes insensitive to root growth inhibition by JA and activates SA pathway (Ren et al., 2008). WRKY70, therefore, suppresses JA responses, and it is shown here that JA suppresses the expression of *WRKY70*, and may thereby suppress SA responses.

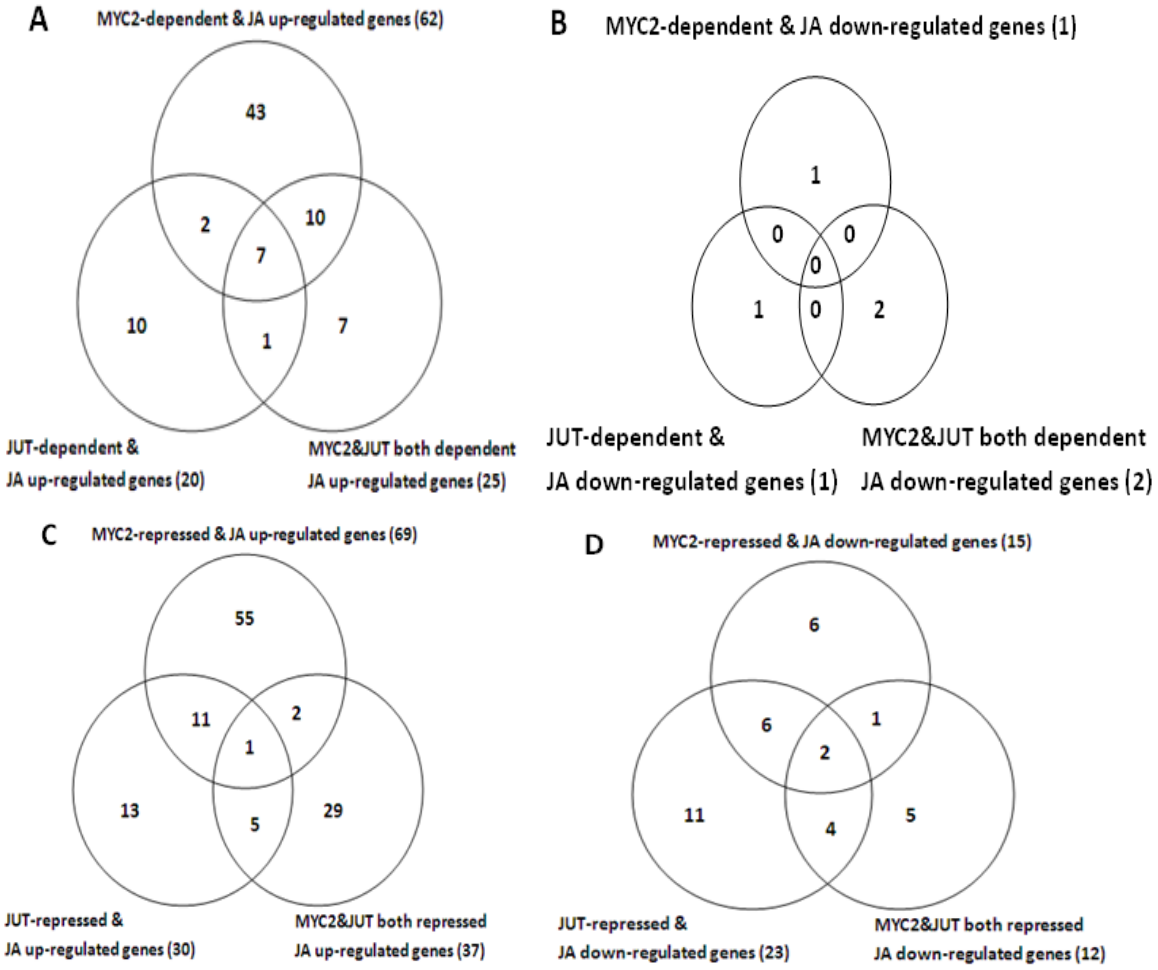


Figure 5.2: Venn diagrams of JA responsive genes regulated by MYC2, JUT and MYC2+JUT.

Table 5.1: JA up-regulated genes that are dependent by MYC2, JUT, and MYC2+JUT.

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	jutMJ/wtMJ	DMMJ/wtMJ	Description of gene product
252265_at	AT3G49620	6.69	0.19	0.23	0.16	similar to 2-oxoacid-dependent dioxygenase.
253060_at	AT4G37710	6.53	0.21	0.44	0.23	VQ motif-containing protein
256376_s_at	AT1G66690	4.27	0.23	0.26	0.25	S-adenosyl-L-methionine:carboxyl methyltransferase
266270_at	AT2G29470	3.04	0.40	0.44	0.41	glutathione transferase
260551_at	AT2G43510	2.45	0.45	0.49	0.46	Member of the defensin-like (DEFL) family.
249675_at	AT5G35940	2.20	0.26	0.28	0.23	jacalin lectin family protein
257365_x_at	AT2G26020	1.87	0.41	0.32	0.45	PDF1.2b (plant defensin 1.2b)

Table 5.2: JA up-regulated genes repressed by MYC2, JUT, and MYC2+JUT.

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	jutMJ/wtMJ	DMMJ/wtMJ	Description of gene product
253502_at	AT4G31940	2.23	1.71	2.03	1.95	member of CYP82C

Table 5.3: JA down-regulated genes repressed by MYC2, JUT, and MYC2+JUT.

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	jutMJ/wtMJ	DMMJ/wtMJ	Description of gene product
256766_at	AT3G22231	0.27	2.72	13.25	3.09	Pathogen and circadian controlled 1 (PCC1)
251705_at	AT3G56400	0.49	2.39	2.61	1.75	member of WRKY Transcription Factor

5.3.6 The Expression of JA Up-regulated Genes in the Wild Type, *jin1*-1, *jut* and *jin1*-1/*jut*

The heat-maps in **Figure 5.3** displays JA responsive genes are differentially expressed in the single mutant *jin1-1*, *jut* and the double mutant *jin1-1/jut*. These JA-responsive genes were selected as the criterion of log2 ratio of "wt (+JA)/wt (mock) > 0.5" (column A in **Table 5.4**). Most of these genes are down-regulated in the mutants in comparison with the wild-type. These results indicate that the MeJA-induced expressions of these JA-responsive genes are largely dependent on the transcription regulation by MYC2 and JUT to response JA.

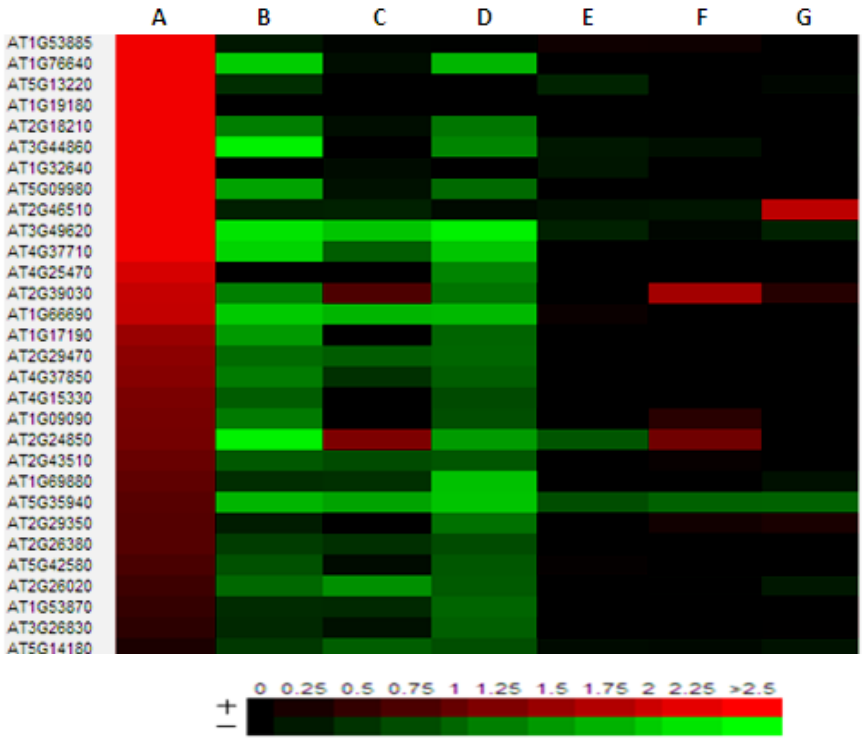


Figure 5.3: Heat-maps of JA up-regulaed genes expression.

JA responsive genes are differentially expressed in wild-type plants, the single mutant *jin1*-1, and *jut*, and the double mutant *jin1*-1/*jut*. Results are transformed from our original microarray data (**Table 5.4**). Scale, log2 ratio from -2.5 to 2.5. The heat-map was transformed by BAR Heatmapper Tool (http://bar.utoronto.ca). A, log2 ratio of "wt (+JA)/wt (mock)"; B, log2 ratio of "*jin1*-1 (+JA)/wt (+JA)"; C, log2 ratio of "*jut* (+JA)/wt (+JA)"; D, log2 ratio of "*jin1*-1/*jut* (+JA) /wt (JA)"; E, log2 ratio of "*jin1*-1 (mock)/wt (mock); F, log2 ratio of "*jut* (mock)/wt (mock)"; G, log2 ratio of "*jin1*-1/*jut* (mock)/wt (mock)".

Table 5.4: Datasheet for Figure 5.3.

Results are transformed from our original microarray data.

A, log2 ratio of "wt (+JA)/wt (mock)"; B, log2 ratio of "*jin1*-1 (+JA)/wt (+JA)"; C, log2 ratio of "*jut* (+JA)/wt (+JA)"; D, log2 ratio of "*jin1*-1/*jut* (+JA) /wt (JA)"; E, log2 ratio of "*jin1*-1 (mock)/wt (mock); F, log2 ratio of "*jut* (mock)/wt (mock)"; G, log2 ratio of "*jin1*-1/*jut* (mock)/wt (mock)".

Affy No.	Gene ID	A	B	C	D	E	F	G
262226_at	AT1G53885	5.89	-0.57	-0.40	-0.39	0.51	0.50	0.19
259866_at	AT1G76640	5.41	-2.18	-0.49	-1.98	0.20	0.00	-0.13
250292_at	AT5G13220	5.14	-0.77	-0.23	-0.31	-0.68	-0.30	-0.43
256017_at	AT1G19180	5.14	-0.13	0.12	-0.10	-0.35	0.02	-0.03
265327_at	AT2G18210	4.64	-1.48	-0.49	-1.41	0.23	0.29	-0.30
246340_s_at	AT3G44860	4.52	-2.51	-0.25	-1.55	-0.57	-0.50	-0.28
261713_at	AT1G32640	3.89	0.09	-0.46	0.09	-0.55	-0.32	-0.16
250455_at	AT5G09980	3.52	-1.81	-0.53	-1.31	-0.22	-0.20	-0.05
265452_at	AT2G46510	2.91	-0.64	-0.66	-0.45	-0.54	-0.56	2.03
252265_at	AT3G49620	2.74	-2.39	-2.11	-2.64	-0.66	-0.42	-0.66
253060_at	AT4G37710	2.71	-2.24	-1.20	-2.11	0.28	0.12	0.02
254075_at	AT4G25470	2.26	-0.22	-0.31	-1.53	-0.34	-0.31	0.20
266142_at	AT2G39030	2.11	-1.50	1.05	-1.40	0.32	1.81	0.70
256376_s_at	AT1G66690	2.10	-2.15	-1.96	-2.00	0.46	0.11	0.08
262516_at	AT1G17190	1.72	-1.73	-0.04	-1.25	0.29	0.27	-0.17
266270_at	AT2G29470	1.60	-1.31	-1.18	-1.27	0.18	0.34	0.23
253070_at	AT4G37850	1.56	-1.45	-0.80	-1.20	-0.04	0.07	0.01
245550_at	AT4G15330	1.44	-1.18	-0.16	-1.02	-0.13	-0.01	-0.01
264647_at	AT1G09090	1.42	-1.44	0.11	-1.06	0.21	0.72	0.13
263539_at	AT2G24850	1.40	-2.51	1.48	-1.74	-1.12	1.36	0.19
260551_at	AT2G43510	1.29	-1.15	-1.04	-1.13	0.01	0.44	0.00
260408_at	AT1G69880	1.21	-0.77	-0.80	-2.07	-0.22	0.32	-0.52
249675_at	AT5G35940	1.14	-1.96	-1.81	-2.11	-1.05	-1.23	-1.24
266292_at	AT2G29350	1.12	-0.62	-0.01	-1.36	0.13	0.53	0.59
245033_at	AT2G26380	1.12	-0.90	-0.78	-1.03	-0.13	-0.09	-0.06
249202_at	AT5G42580	1.01	-1.08	-0.46	-1.14	0.41	-0.01	-0.09
257365_x_at	AT2G26020	0.91	-1.29	-1.64	-1.16	-0.11	-0.37	-0.57
262259_s_at	AT1G53870	0.83	-0.75	-0.73	-1.26	-0.16	-0.05	0.27
258277_at	AT3G26830	0.76	-0.72	-0.52	-1.21	0.22	0.10	0.38
250199_at	AT5G14180	0.61	-0.87	-1.20	-1.06	-0.45	-0.45	-0.54

5.3.7 Selection of A Panel of MYC2 and JUT Differentially Regulated JA Responsive Genes for Quantitative Gene Expression Analyses

In choosing for the subsequent analysis, which would involve measurement of their expression on a time course after treatment, in different genetic backgrounds, a panel of nine genes has been selected for quantitative expression analysis. Two of which would be MYC2 and JUT. This panel is given in **Table 5.5**, and the reasons behind selection of MYC2 and JUT differentially regulated JA responsive genes will be explained in this section.

Table 5.5: A panel of genes for quantitative real-time RT-PCR.

Gene ID	A	B	C	D	E	F	G	Description of gene product
At1g32640	6.03	9.92	3.89	0.09	-0.46	0.09	-2.99	bHLH transcription factor
At2g46510	5.88	8.79	2.91	-0.64	-0.66	-0.45	-3.59	bHLH transcription factor
At2g24850	5.23	6.63	1.4	-2.51	1.48	-1.74	-3.07	tyrosine aminotransferase
At1g19180	6.32	11.46	5.14	-0.13	0.12	-0.1	-4.85	JAZ1
At5g13220	4.7	9.84	5.14	-0.77	-0.23	-0.31	-8.87	JAZ10
At3g49620	4.58	7.32	2.74	-2.39	-2.11	-2.64	-7.85	DIN11
At1g66690	3.79	5.88	2.1	-2.15	-1.96	-2	-0.56	S-adenosyl-L-methionine: carboxyl methyltransferase
At1g53885	4	9.89	5.89	-0.57	-0.4	-0.39	-8.1	senescence-associated protein
At2g26020	5.04	5.95	0.91	-1.29	-1.64	-1.16	-0.12	PDF1.2b (plant defensin 1.2b)
At3g18780	12.3	12.35	0.05	-0.14	-0.03	-0.03	0.09	ACTIN2

A, Gene expression level in wild type (Col-0) background mock;
B, Gene expression level in wild type (Col-0) background after MeJA treatment;
C, log2 ratio of "wt (+JA)/wt (mock)"; D, log2 ratio of "Expression$_{coi1}$/ Expression $_{Col-0}$" without MeJA treatment (referenced from the Genevestigator database) ; E, log2 ratio of "*jin1*-1 (+JA)/wt (+JA)"; F, log2 ratio of "*jut* (+JA)/wt (+JA)";
G, log2 ratio of "*jin1*-1/*jut* (+JA) /wt (+JA)".

Besides MYC2 and JUT, seven JA responsive genes At2g24850 (*TAT3*), At1g19180 (*JAZ1*), At5g13220 (*JAZ10*), At3g49620 (*DIN11*), At1g66690, At1g53885 and At2g26020 (*PDF1.2b*) were selected for qRT-PCR experiments (**Table 5.5**).

The gene At2g24850 (*AtTAT3*) encodes the catalyst tyrosine aminotransferase for the first step of tocopherol biosynthesis (Sandorf and Hollander-Czytko, 2002). Tocopherol (vitamin E) is a JA inducible antioxidant located in the chloroplast (Munne-Bosch, 2005). According to microarray data analyses described here, the expression of *AtTAT3* (At2g24850) was up-regulated at 30min after MeJA treatment (**Figure 5.3.A** & **Table 5.4.A**). The expression of *AtTAT3* was significantly reduced in *jin1*-1 after MeJA treatment in comparison with the wild type Col-0 after MeJA treatment (**Figure 5.3.B** & **Table 5.4.B**). However, this gene was dependent in the single mutant *jut* after MeJA treatment to similar level in wild type (**Figure 5.3.C** & **Table 5.4.C**). This result indicated that MYC2 is required for *AtTAT3* expression in response to MeJA. There was no significant MeJA-induced expression of *AtTAT3* in the double mutant *jin1*-1/*jut* (**Figure 5.3.D** & **Table 5.4.D**). Taken together, in the wild type Col-0 after MeJA treatment, the expression of *AtTAT3* is dependent (positively regulated) by MYC2 but not by JUT. This is also consistent with results which show that the expression of *AtTAT3* is up-regulated in the MYC2 over-expression line (Chini et al., 2007). According to Genvestigator analysis, the expression of *AtTAT3* is also COI1-dependent.

Feed-back regulation arising from interaction between MYC2 and JAZ proteins has been proposed (Chini et al., 2007). The JAZ proteins are the main repressors of MYC2 (**Figure 1.3**). However, it is still not clear how MYC2 regulates JAZs at the transcriptional level in the proposed feedback loop. According to the microarray data, MeJA-induced expression of *JAZ1* was significantly down-regulated in the mutant *jin1*-1 compared to wild type Col-0 (**Figure 5.3.B & Table 5.4.B**). This indicates that the expression of *JAZ1* is positively regulated by MYC2. It was partly down-regulated in the mutant *jut* compared to wild type Col-0 (**Figure 5.3.C & Table 5.4.C**). Genvestigator analysis indicates that the expression

of *JAZ1* is COI1-dependent. Therefore, *JAZ1* was selected for gene expression analyses by qRT-PCR experiments. MeJA-induced expression of *JAZ10* was significantly reduced in *jin1*-1, *jut* and *jin1*-1/*jut* after MeJA treatment, in comparison with the wild-type after MeJA treatment (**B, C and D in Figure 5.3 & Table 5.4**). This indicates that the expression of *JAZ10* is positively regulated by both MYC2 and JUT. According to Genvestigator analysis, the expression of *JAZ10* is COI1-dependent. Therefore, *JAZ10* was selected for gene expression analyses by qRT-PCR experiments.

At3g49620 (*DIN11*) encodes a 2-oxoacid-dependent dioxygenase and its transcript accumulates in senescing tissues. *DIN11* and other senescence- and defence-related genes were significantly up-regulated in the Seedlings Hyper-responsive to Light (*SHL*) over-expression line (Mussig and Altmann, 2003). Light-inducible genes and photosynthesis genes are all repressed by JA (Zhai et al., 2007). Here we show that the transcription of dark-inducible gene *DIN11* is dependent at 30min after MeJA treatment (**Figure 5.3.A & Table 5.4.A**). The expression of *DIN11* was reduced in the *jin1*-1, *jut* and *jin1*-1/*jut* after MeJA treatment in comparison with the wild type Col-0 after MeJA treatment (**B, C and D in Figure 5.3 & Table 5.4**). Apparently therefore, MeJA-induced expression of *DIN11* was dependent by MYC2 and JUT in wild type. According to the Genvestigator analysis, the expression of *DIN11* is COI1-dependent. Therefore, *DIN11* was selected for gene expression analyses by qRT-PCR experiments.

At1g66690 encodes a SAMT-like transferase is one member of the miR163 targets. Gene sequence analyses found that the MIR163 arms aligned to the complementary sequence of At1g66700, At1g66690, At1g66720 and At3g44840 (Allen et al., 2004). According to the microarray data presented here, the expression of At1g66690 was induced by MeJA in wild type seedlings (**Figure 5.3.A & Table 5.4.A**). However, the expression of At1g66690 was dramatically reduced in the single mutant *jin1*-1, *jut* and double mutant *jin1*-1/*jut*, after MeJA treatment in comparison with wild type after MeJA treatment (**B, C and D in Figure 5.3 & Table 5.4**). These results indicate that MeJA-induced expression of At1g66690

requires MYC2 and JUT in wild type background. According to the Genvestigator analysis, At1g66690 was apparently not COI1-dependent. Therefore, At1g66690 was selected for gene expression analyses by qRT-PCR experiments.

At1g53885 encodes a senescence-associated protein, which was annotated by NCBI (http://www.ncbi.nlm.nih.gov/nuccore/NM_104266.2) and GeneCAT Bioinformatics databse (http://genecat.mpg.de/cgi-bin/9867/coexsearch.py). However, TAIR annotation and BLAST analysis show that this gene encodes an unknown function protein (http://www.arabidopsis.org/servlets/TairObject?id=500231497&type=locus). According to the microarray data presented here, the expression of At1g53885 was induced by MeJA in wild type (**Figure 5.3.A & Table 5.4.A**). However, the expression of At1g53885 was dramatically reduced in the single mutant *jin1*-1 and *jut*, as well the double mutant *jin1*-1/*jut* after MeJA treatment in comparison with wild type after MeJA treatment (**B, C and D in Figure 5.3 & Table 5.4**). These results indicate that MeJA-induced expression of At1g53885 requires MYC2 and JUT in wild type background. According to the Genvestigator analysis, the expression of At1g53885 is COI1-dependent. Therefore, At1g53885 was selected for gene expression analyses by qRT-PCR experiments.

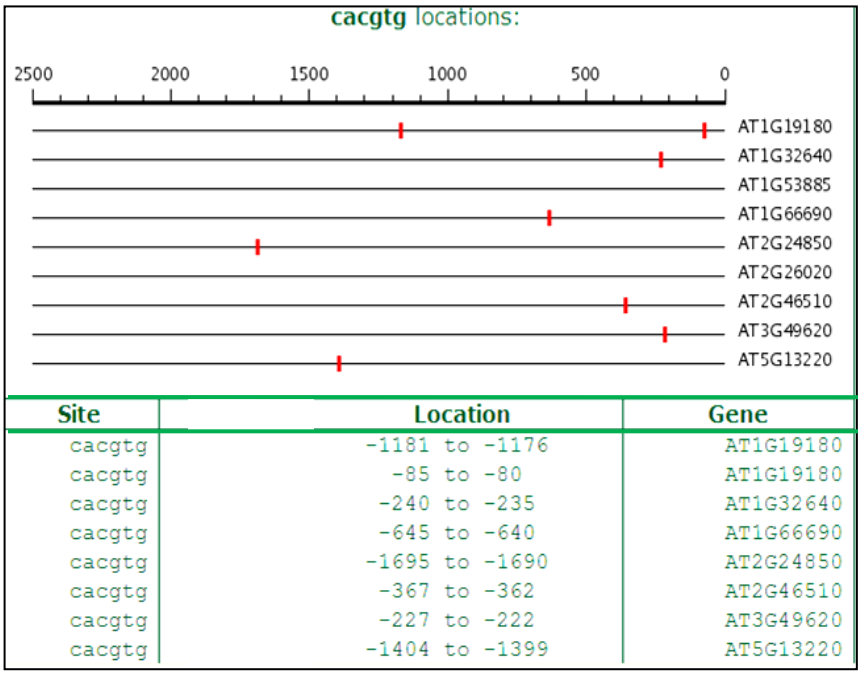
At2g26020 (*PDF1.2b*) is induced by MeJA in wild type up to 8 hours. The earlier and higher expression of *PDF1.2b* has been observed in *gpa1*-1, a loss-of-function mutation in the G alpha subunit. These findings suggest that the alpha subunit of G protein might function as a repressor of the expression of *PDF1.2b* (Okamoto et al., 2009). The genes for *PDF1.1*, *PDF1.2b*, *PDF1.2c* and *PDF1.3* are induced by potassium starvation and JA treatment (Cartieaux et al., 2008). According to the microarray data presented in this chapter, the expression of *PDF1.2b* was less strongly induced by MeJA than JAZ1 and JAZ10 in wild type (**Figure 5.3.A** & **Table 5.4.A**). However, the expression of *PDF1.2b* was dramatically reduced in the single mutant *jin1*-1 and *jut*, as well the double mutant *jin1*-1/*jut* after MeJA treatment in comparison with wild type after MeJA treatment (**B, C and D in Figure 5.3 & Table 5.4**). These results indicate that the MeJA-induced expression

of *PDF1.2b* requires MYC2 and JUT in wild type background. According to the Genvestigator analysis, the expression of *PDF1.2b* is COI1-dependent. Therefore, *PDF1.2b* was selected for gene expression analyses by qRT-PCR experiments.

5.3.8 G-box Motif Analysis for Nine JA Responsive Genes

Based on the microarray data presented in this Chapter, G-box motifs on the putative promoter sequence of nine JA responsive genes were analysed by the software SCOPE as described in **section 2.1.4**. For this 2,500bp upstream of the transcription start site was selected, and the list of genes At1g32640 (*AtMYC2*), At2g46510 (*JUT*), At2g24850 (*AtTAT3*), At1g19180 (*JAZ1*), At5g13220 (*JAZ10*), At3g49620 (*DIN11*), At1g66690, At1g53885 and At2g26020 (*PDF1.2b*) was entered. The G-box motif of "5-CACGTG-3" was identified as the target motif. This G-box motif was identified in the promoter region of At1g19180, At1g32640, At1g66690, At2g24850, At2g46510, At3g49620 and At5g13220. There are five individual G-box motifs were predicted with the localisation 1,000 bp upstream of the transcription start site. However, the promoter region of At1g53885 and At2g26020 did not contain the G-box motif (**Figure 5.4**).

The transcription factor MYC2 should bind to the "G-box" motif (**Figure 1.7**). Apparently therefore the transcription factor MYC2 binds to the promoter of *AtMYC2*. This suggests a possible feed-back regulation between MYC2 and the expression of *AtMYC2*. The different locations of the G-box in promoters for the different genes (**Figure 5.4**) might affect the way in which MYC2 regulates these target genes.



Site	Location	Gene
cacgtg	-1181 to -1176	AT1G19180
cacgtg	-85 to -80	AT1G19180
cacgtg	-240 to -235	AT1G32640
cacgtg	-645 to -640	AT1G66690
cacgtg	-1695 to -1690	AT2G24850
cacgtg	-367 to -362	AT2G46510
cacgtg	-227 to -222	AT3G49620
cacgtg	-1404 to -1399	AT5G13220

Figure 5.4: G-box motif in JA-responsive genes.

Seven of nine JA-responsive genes contain the "G-box" motif (denotes as red bar) in their upstream 2500bp before the transcription start site. The nucleotide location of the motif relative to the transcription start site is given. Two G-box motif are predicted in the promoter region ofAt1g19180 (*JAZ1*); One G-box motif is predicted in the promoter region of At1g32640 (*AtMYC2*), At1g66690, At2g24850 (*AtTAT3*), At2g46510 (*JUT),* At3g48620 (*DIN11*) and At5g13220 (*JAZ10*).

5.3.9 Gene Regulatory Network Predication

StarNet was designed by the VanBuren group as a web-based tool for investigating gene regulatory networks. This program produces hypotheses for correlated networks and supplementary information on gene function (Jupiter and VanBuren, 2008; Jupiter et al., 2009). In this section, StarNet was used to produce a hypothetical gene regulatory network. StarNet analyses are based upon the original microarray data which was collected from the Gene Expression Omnibus of NCBI (National Centre for Biotechnology Information: http://www.ncbi.nlm.nih.gov/) and then normalised by Robust Multichip Average (RMA: http://rmaexpress.bmbolstad.com/) in Bioconductor. This kind of database can be integrated via a StarNet query, which uses Octave (http://www.gnu.org/software/octave/) to generate Pearson Correlation coefficients between the input query gene expression, and the most highly correlated output genes, in all combinations (Jupiter et al., 2009). The main principle of gene regulatory network analyses on StarNet is based on co-expression determined by the analysis of high throughput microarray datasets (**Section 2.1.5** in **Chapter 2**).

In order to investigate the transcriptional regulation in the JA signal pathway, Arabidopsis thaliana was selected as an original cohort of microarray data in advance. Next, the specific Entrez IDs of a group of genes were entered for StarNet tool to produce a hypothetical gene regulatory network. The Entrez ID can be obtained from the tool of Gene ID lookup (http://vanburenlab.tamhsc.edu/gene_lookup.html). These genes are including At1g32640 (*MYC2*), At2g46510 (*JUT*), At2g24850 (*TAT3*) and At3g49620 (*DIN11*), At1g19180 (*JAZ1*), At5g13220 (*JAZ10*) and At2g39940 (*COI1*). However, the genes At1g53885, At1g66690 and At2g26020 (*PDF1.2*) are not yet available on the database of StarNet. Other parameters are set as default values.

Figure 5.5 illustrates the relationships among input genes and gene products, which includes nodes (genes or gene products) and lines (relationship between two nodes). The lines precisely represent the value of the pair-wise Pearson correlation coefficient (Jupiter and VanBuren, 2008; Jupiter et al., 2009). All of these outputs of the StarNet analyses not only establish a group of putative transcription regulatory networks of input JA responsive genes, also indicate the complex pattern of regulatory relationships within the network.

For instance, the JA biosynthesis gene *OPR3* and the JA responsive gene *TAT3* share the significant correlation with At2g46510 (*JUT*). This hypothetical gene regulatory network analysis leads to the hypothesis that AtMYC2 and JUT have important regulatory functions in the JA signal pathway. Additionally, *DIN11* correlated with an ethylene-responsive factor coding gene At1g06160. *COI1* correlated with At5g11700, At5g46410, At5g46760, a transcription factor coding gene (*SHL1*) and a gene ACT Domain Repeat 3 (*ACR3*). Most interestingly, *AtMYC2*, At2g46510 (*JUT*), *JAZ1* and *JAZ2* are significantly correlated; At2g46510 (*JUT*) is closely correlated with *JAZ1*, *JAZ2*, *JAZ5* and *JAZ10*. These outputs indicate that MYC2 and JUT may co-regulate the transcription of *JAZ1* and *JAZ2*. Gene regulatory network prediction by StarNet presented more putative relationships for the further investigation on the transcriptional regulation in the JA signal pathway.

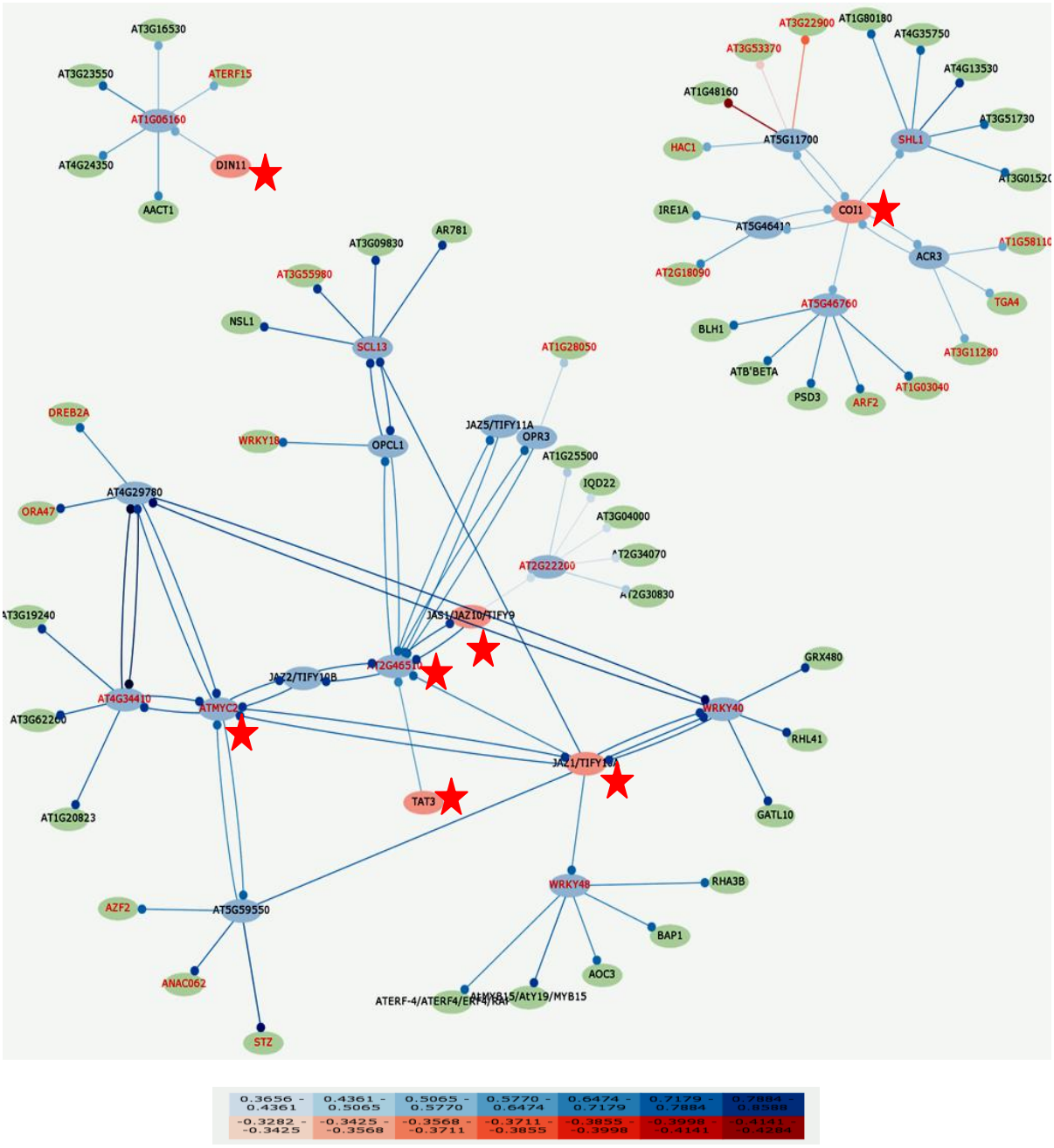


Figure 5.5: Gene regulatory network predication from StarNet.

Scale displays the value of the pair-wise Pearson correlation coefficient. The red 5-ponit stars distinguish the input genes. The gene symbol and description of gene product of StarNet analysis output are shown in **Table 5.6**. The values of Pearson Correlation Coefficient for StarNet output are shown in **Table 5.7**.

Table 5.6: Gene list of StarNet output (Data from the StarNet output webpage).

Entrez ID	Gene Symbol	Description of gene product
815160	OPR3	OPR3 (OPDA-REDUCTASE 3); 12-oxophytodienoate reductase
816258	IRE1A	IRE1A (Yeast endoribonuclease/protein kinase IRE1-like gene)
816321	AT2G18090	PHD finger family protein
816754	AT2G22200	AP2 domain-containing transcription factor
817022	TAT3	TAT3 (TYROSINE AMINOTRANSFERASE 3); transaminase
817194	AR781	AR781
817634	AT2G30830	2-oxoglutarate-dependent dioxygenase, putative
817680	ATERF15	ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 15
817968	AT2G34070	hypothetical protein
818167	BLH1	BLH1 (embryo sac development arrest 29)
818581	COI1	COI1 (CORONATINE INSENSITIVE 1); ubiquitin-protein ligase
819262	AT2G46510	basic helix-loop-helix (bHLH) family protein
819555	AT3G04000	short-chain dehydrogenase/reductase (SDR) family protein
820141	AT3G09830	protein kinase, putative
820146	ATB'BETA	ATB' BETA protein phosphatase type 2A regulator
820299	AT3G11280	myb family transcription factor
820752	ATERF-4	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 4
820901	AT3G16530	legume lectin family protein
821122	AT3G01520	universal stress protein (USP) family protein
821458	AT3G19240	hypothetical protein
821495	AZF2	AZF2 (ARABIDOPSIS ZINC-FINGER PROTEIN 2)
821862	AT3G22900	RNA polymerase Rpb7 N-terminal domain-containing protein
821904	AtMYB15	AtMYB15/AtY19/MYB15 (myb domain protein 15)
821934	AT3G23550	MATE efflux family protein
822169	AOC3	AOC3 (ALLENE OXIDE CYCLASE 3)
822462	GATL10	GATL10 (Galacturonosyltransferase-like 10)
824115	ANAC062	ANAC062 (Arabidopsis NAC domain containing protein 62)
824124	DIN11	DIN11 (DARK INDUCIBLE 11); oxidoreductase
824336	AT3G51730	saposin B domain-containing protein
824505	AT3G53370	DNA-binding S1FA family protein
824764	AT3G55980	zinc finger (CCCH-type) family protein
825291	BAP1	BAP1 (BON ASSOCIATION PROTEIN 1)
825399	AT3G62260	protein phosphatase 2C
826985	AT4G13530	hypothetical protein
827435	SCL13	SCL13 (SCARECROW-LIKE 13); transcription factor
828405	IQD22	IQD22 (IQ-domain 22); calmodulin binding
828538	AT4G24350	phosphorylase family protein
828703	PSD3	calcium ion binding / phosphatidylserine decarboxylase
829100	AT4G29780	hypothetical protein
829308	WRKY18	WRKY18 (WRKY DNA-binding protein 18); transcription factor

Table 5.6: Gene list of StarNet output (continued).

Entrez ID	Gene Symbol	Description of gene product
829591	AT4G34410	AP2 domain-containing transcription factor, putative
829700	RHA3B	RHA3B (RING-H2 finger A3B); protein binding / zinc ion binding
829728	AT4G35750	Rho-GTPase-activating protein-related
830065	SHL1	SHL1 transcription factor
830424	DREB2A	DREB2A (DRE-BINDING PROTEIN 2A)
830866	TGA4	TGA4 (TGACG MOTIF-BINDING FACTOR 4)
831042	AT5G11700	glycine-rich protein
831162	JAS1/JAZ10/TIFY9	JAS1/JAZ10/TIFY9 (JASMONATE-ZIM-DOMAIN PROTEIN 10)
834684	AT5G46410	NLI interacting factor (NIF) family protein
834719	AT5G46760	basic helix-loop-helix (bHLH) family protein
835012	WRKY48	WRKY48 (WRKY DNA-binding protein 48); transcription factor
836074	AT5G59550	zinc finger (C3HC4-type RING finger) family protein
836103	RHL41	RHL41 (RESPONSIVE TO HIGH LIGHT 41) transcription factor
836237	AACT1	AACT1 (ANTHOCYANIN 5-AROMATIC ACYLTRANSFERASE 1); transferase
836321	ARF2	ARF2 (AUXIN RESPONSE FACTOR 2); transcription factor
837125	AT1G06160	ethylene-responsive factor, putative
838310	JAZ5/TIFY11A	JAZ5/TIFY11A (JASMONATE-ZIM-DOMAIN PROTEIN 5)
838501	JAZ1/TIFY10A	JAZ1/TIFY10A (JASMONATE-ZIM-DOMAIN PROTEIN 1)
838639	OPCL1	OPCL1 (OPC-8:0 COA LIGASE1); 4-coumarate-CoA ligase
838674	AT1G20823	zinc finger (C3HC4-type RING finger) family protein
839136	AT1G25500	choline transporter-related
839446	AT1G03040	basic helix-loop-helix (bHLH) family protein
839666	STZ	STZ (SALT TOLERANCE ZINC FINGER) transcription factor
839698	AT1G28050	zinc finger (B-box type) family protein
839735	NSL1	NSL1 (NECROTIC SPOTTED LESIONS 1)
839748	GRX480	GRX480; thiol-disulfide exchange intermediate
840158	ATMYC2	ATMYC2 (JASMONATE INSENSITIVE 1) transcription factor
841235	AT1G48160	signal recognition particle 19 kDa protein
842178	AT1G58110	bZIP family transcription factor
843832	ORA47	ORA47; DNA binding / transcription factor
843834	JAZ2/TIFY10B	JAZ2/TIFY10B (JASMONATE-ZIM-DOMAIN PROTEIN 2)
844035	ACR3	ACR3 (ACT Domain Repeat 3)
844241	HAC1	HAC1 (P300/CBP ACETYLTRANSFERASE-RELATED PROTEIN 2 GENE);
844358	AT1G80180	hypothetical protein
844423	WRKY40	WRKY40 (WRKY DNA-binding protein 40); transcription factor

Table 5.7: Values of Pearson Correlation Coefficient for StarNet output.

Gene pairs	Pearson Corr. Coefficient
COI1 [818581] ---- ACR3 [844035]	0.53633 (n=3249)
COI1 [818581] ---- AT5G46760 [834719]	0.52447 (n=3249)
COI1 [818581] ---- AT5G46410 [834684]	0.52387 (n=3249)
COI1 [818581] ---- SHL1 [830065]	0.52341 (n=3249)
COI1 [818581] ---- AT5G11700 [831042]	0.52210 (n=3249)
JAS1/JAZ10/TIFY9 [831162] ---- AT2G46510 [819262]	0.74788 (n=3249)
JAS1/JAZ10/TIFY9 [831162] ---- AT2G22200 [816754]	0.36559 (n=3249)
JAZ1/TIFY10A [838501] ---- WRKY40 [844423]	0.78551 (n=3249)
JAZ1/TIFY10A [838501] ---- ATMYC2 [840158]	0.75281 (n=3249)
JAZ1/TIFY10A [838501] ---- SCL13 [827435]	0.73614 (n=3249)
JAZ1/TIFY10A [838501] ---- AT2G46510 [819262]	0.65988 (n=3249)
JAZ1/TIFY10A [838501] ---- WRKY48 [835012]	0.65382 (n=3249)
DIN11 [824124] ---- AT1G06160 [837125]	0.53353 (n=3249)
TAT3 [817022] ---- AT2G46510 [819262]	0.63316 (n=3249)
AT2G46510 [819262] ---- JAS1/JAZ10/TIFY9 [831162]	0.74788 (n=3249)
AT2G46510 [819262] ---- JAZ2/TIFY10B [843834]	0.74744 (n=3249)
AT2G46510 [819262] ---- JAZ5/TIFY11A [838310]	0.69281 (n=3249)
AT2G46510 [819262] ---- OPR3 [815160]	0.69084 (n=3249)
AT2G46510 [819262] ---- OPCL1 [838639]	0.67395 (n=3249)
ATMYC2 [840158] ---- JAZ2/TIFY10B [843834]	0.78434 (n=3249)
ATMYC2 [840158] ---- AT4G29780 [829100]	0.76316 (n=3249)
ATMYC2 [840158] ---- AT4G34410 [829591]	0.75931 (n=3249)
ATMYC2 [840158] ---- JAZ1/TIFY10A [838501]	0.75281 (n=3249)
ATMYC2 [840158] ---- AT5G59550 [836074]	0.71400 (n=3249)
ACR3 [844035] ---- TGA4 [830866]	0.57486 (n=3249)
ACR3 [844035] ---- AT1G58110 [842178]	0.56499 (n=3249)
ACR3 [844035] ---- AT3G11280 [820299]	0.54589 (n=3249)
ACR3 [844035] ---- COI1 [818581]	0.53633 (n=3249)
AT5G46760 [834719] ---- ARF2 [836321]	0.69940 (n=3249)
AT5G46760 [834719] ---- AT1G03040 [839446]	0.68037 (n=3249)
AT5G46760 [834719] ---- PSD3 [828703]	0.68011 (n=3249)
AT5G46760 [834719] ---- ATB' BETA [820146]	0.67082 (n=3249)
AT5G46760 [834719] ---- BLH1 [818167]	0.66378 (n=3249)
AT5G46410 [834684] ---- IRE1A [816258]	0.64506 (n=3249)
AT5G46410 [834684] ---- AT2G18090 [816321]	0.61677 (n=3249)
AT5G46410 [834684] ---- COI1 [818581]	0.52387 (n=3249)
SHL1 [830065] ---- AT4G13530 [826985]	0.72563 (n=3249)
SHL1 [830065] ---- AT1G80180 [844358]	0.68635 (n=3249)
SHL1 [830065] ---- AT3G51730 [824336]	0.68295 (n=3249)
SHL1 [830065] ---- AT4G35750 [829728]	0.66506 (n=3249)

Table 5.7: Values of Pearson Correlation Coefficient for StarNet output (continued).

Gene pairs	Pearson Corr. Coefficient
SHL1 [830065] ---- AT3G01520 [821122]	0.66413 (n=3249)
AT5G11700 [831042] ---- HAC1 [844241]	0.57223 (n=3249)
AT5G11700 [831042] ---- COI1 [818581]	0.52210 (n=3249)
AT5G11700 [831042] ---- AT1G48160 [841235]	-0.42838 (n=3249)
AT5G11700 [831042] ---- AT3G22900 [821862]	-0.36905 (n=3249)
AT5G11700 [831042] ---- AT3G53370 [824505]	-0.32822 (n=3249)
AT2G22200 [816754] ---- AT1G25500 [839136]	0.49584 (n=3249)
AT2G22200 [816754] ---- AT2G30830 [817634]	0.45468 (n=3249)
AT2G22200 [816754] ---- AT2G34070 [817968]	0.40602 (n=3249)
AT2G22200 [816754] ---- IQD22 [828405]	0.38595 (n=3249)
AT2G22200 [816754] ---- AT3G04000 [819555]	0.38553 (n=3249)
WRKY40 [844423] ---- AT4G29780 [829100]	0.81395 (n=3249)
WRKY40 [844423] ---- JAZ1/TIFY10A [838501]	0.78551 (n=3249)
WRKY40 [844423] ---- GATL10 [822462]	0.78116 (n=3249)
WRKY40 [844423] ---- GRX480 [839748]	0.77119 (n=3249)
WRKY40 [844423] ---- RHL41 [836103]	0.76732 (n=3249)
SCL13 [827435] ---- OPCL1 [838639]	0.76400 (n=3249)
SCL13 [827435] ---- NSL1 [839735]	0.76052 (n=3249)
SCL13 [827435] ---- AR781 [817194]	0.75721 (n=3249)
SCL13 [827435] ---- AT3G09830 [820141]	0.75677 (n=3249)
SCL13 [827435] ---- AT3G55980 [824764]	0.74694 (n=3249)
WRKY48 [835012] ---- AtMYB15/AtY19/MYB15 [821904]	0.76746 (n=3249)
WRKY48 [835012] ---- AOC3 [822169]	0.69665 (n=3249)
WRKY48 [835012] ---- ATERF-4/ATERF4/ERF4/RAP2.5 [820752]	0.67915 (n=3249)
WRKY48 [835012] ---- RHA3B [829700]	0.67223 (n=3249)
WRKY48 [835012] ---- BAP1 [825291]	0.67160 (n=3249)
AT1G06160 [837125] ---- AT3G23550 [821934]	0.70466 (n=3249)
AT1G06160 [837125] ---- AT4G24350 [828538]	0.64200 (n=3249)
AT1G06160 [837125] ---- AACT1 [836237]	0.61934 (n=3249)
AT1G06160 [837125] ---- AT3G16530 [820901]	0.57177 (n=3249)
AT1G06160 [837125] ---- ATERF15 [817680]	0.56610 (n=3249)
JAZ2/TIFY10B [843834] ---- ATMYC2 [840158]	0.78434 (n=3249)
JAZ2/TIFY10B [843834] ---- AT2G46510 [819262]	0.74744 (n=3249)
JAZ5/TIFY11A [838310] ---- AT2G46510 [819262]	0.69281 (n=3249)
OPR3 [815160] ---- AT2G46510 [819262]	0.69084 (n=3249)
OPR3 [815160] ---- AT1G28050 [839698]	0.48456 (n=3249)
OPCL1 [838639] ---- SCL13 [827435]	0.76400 (n=3249)
OPCL1 [838639] ---- WRKY18 [829308]	0.69473 (n=3249)
OPCL1 [838639] ---- AT2G46510 [819262]	0.67395 (n=3249)
AT4G29780 [829100] ---- AT4G34410 [829591]	0.85885 (n=3249)

Table 5.7: Values of Pearson Correlation Coefficient for StarNet output (continued).

Gene pairs	Pearson Corr. Coefficient
AT4G29780 [829100] ---- WRKY40 [844423]	0.81395 (n=3249)
AT4G29780 [829100] ---- ORA47 [843832]	0.76559 (n=3249)
AT4G29780 [829100] ---- ATMYC2 [840158]	0.76316 (n=3249)
AT4G29780 [829100] ---- DREB2A [830424]	0.71302 (n=3249)
AT4G34410 [829591] ---- AT4G29780 [829100]	0.85885 (n=3249)
AT4G34410 [829591] ---- AT1G20823 [838674]	0.76397 (n=3249)
AT4G34410 [829591] ---- ATMYC2 [840158]	0.75931 (n=3249)
AT4G34410 [829591] ---- AT3G62260 [825399]	0.74706 (n=3249)
AT4G34410 [829591] ---- AT3G19240 [821458]	0.74549 (n=3249)
AT5G59550 [836074] ---- STZ [839666]	0.80965 (n=3249)
AT5G59550 [836074] ---- WRKY40 [844423]	0.75636 (n=3249)
AT5G59550 [836074] ---- ANAC062 [824115]	0.75223 (n=3249)
AT5G59550 [836074] ---- ATMYC2 [840158]	0.71400 (n=3249)
AT5G59550 [836074] ---- AZF2 [821495]	0.69294 (n=3249)

5.4 Discussion

The main goal of this section was to use microarray analysis to identify genes regulated by JA, MYC2, and JUT, that could then be used as a panel to investigate a possible regulatory network controlling their transcription. This would require the transcription of the panel to be assayed quantitatively, with adequate replication, and in a wider variety of genetic backgrounds. With this in mind therefore, and with reference to the relatively high cost of each microarray assay, no replication was employed in the microarray experiments described here.

Figure 5.1 shows the results of the filters of the gene expression data to identify genes up-regulated or down-regulated by MeJA, and differentially regulated in the different mutants compared to wild type. The further analysis in **Figure 5.2** identifies genes in this subset that are regulated similarly in one or more of the different mutant backgrounds. MYC2 and JUT differentially regulate the transcription of JA responsive genes have been analyzed in the result section. These JA responsive genes will be used for quantitative gene expression analyses in **Chapter 6**.

Chapter 6

Transcription Regulation by MYC2 and JUT

6.1 Introduction

SYBR-Green has been extensively used for quantitative RT-PCR (qRT-PCR). The principle of SYBR-Green based qRT-PCR is described in the **section 1.7.3**. It has been reported that transcription measurement by qRT-PCR is about 100 fold more sensitive than microarray hybridisation. For example, the transcripts of all transcription factors could be detected by qRT-PCR in yeast, however, most were undetectable using microarray (Holland, 2002; Horak and Snyder, 2002). After increasing the specificity of primers, the transcription profiles of over 1,400 Arabidopsis transcription factors have been identified by SYBR-Green based qRT-PCR. However, less than 55% of the transcription factors transcripts could be detected in the same samples by the typical Arabidopsis Affymetrix chip (22k). Most importantly, 52 shoot-specific TFs and 35 root-specific TFs have been studied by optimised SYBR-Green based qRT-PCR as the method of choice (Czechowski et al., 2004).

In this chapter, SYBR-Green qRT-PCR was used to quantitatively measure the expression level of a panel of transcription factors and JA-responsive genes in seven different genetic backgrounds, which are wild-type Col-0, the single mutants *jin1*-1, *jut*, *coi1*-16, *jai3*-1, the double mutant *jin1*-1/*jut* and JUTOE, with and without MeJA treatment. High-specific primers (designed and optimised by Primerdesign®) were used to enhance the specificity of SYBR-Green qRT-PCR in these experiments. For testing the specificity of SYBR-Green assays, the dissociation curves were generated as 95°C for 15 seconds, 60°C for 60 seconds and 95°C for 15seconds. The principle and experimental methods for SYBR-Green qRT-PCR were described in **section 2.3.11**.

6.2 Results and Discussions

6.2.1 Expression Analyses of *AtMYC2*

The expression of *AtMYC2* was rapidly induced by MeJA (**Figure 6.1.A**). MeJA-induced expression of *AtMYC2* in *jin1*-1 was higher than in the wild type background, at 1h after MeJA treatment (**Figure 6.1.B**). This indicates that MYC2 negative regulates its own expression. There was no obvious difference in *AtMYC2* expression in wild type Col-0, the single mutant *jut* and the double mutant *jin1*-1/*jut* (**Figure 6.1.C** & **Figure 6.1.D**). These results are consistent with the Northern blotting results of *AtMYC2* expression in the *jut* background (**Figure 4.3** in **Chapter 4**) and indicate that JUT is not required for the initial MeJA-induced *AtMYC2* expression. There was no MeJA-induced *AtMYC2* expression in *coi1*-16 (**Figure 6.1.E**), which is consistent with the Northern blotting result indicating that COI1 is required for MeJA-induced expression of *AtMYC2* (**Figure 4.2**). There was reduced expression of *AtMYC2* in *jai3*-1 compared to wild type after MeJA treatment (**Figure 6.1.F**). It has been reported that the truncated protein of JAI3-1 in mutant *jai3*-1 continues binding the SCF^{COI1} complex and blocks the activity of SCF^{COI1} (Chini et al., 2007). As a consequence, JAZ proteins could not be degraded by the ubiquintin system, and then redundantly repress the activity of MYC2. This provides an explanation why MYC2-regulated JA-responsive genes are not activated by MeJA in this mutant. This explanation is consistent with the lower expression of *AtMYC2* in *jai3*-1 than wild type Col-0 after MeJA treatment (**Figure 6.1.F** & **Figure 6.1.A**). The expression of MYC2 was similarly induced by MeJA in Col-0 and *jut*, indicating that JUT is not required for this response. However, the expression of MYC2 was partially repressed in JUTOE, indicating that constitutive expression of *JUT* represses MeJA-induced expression of MYC2 (**Figure 6.1.G**).

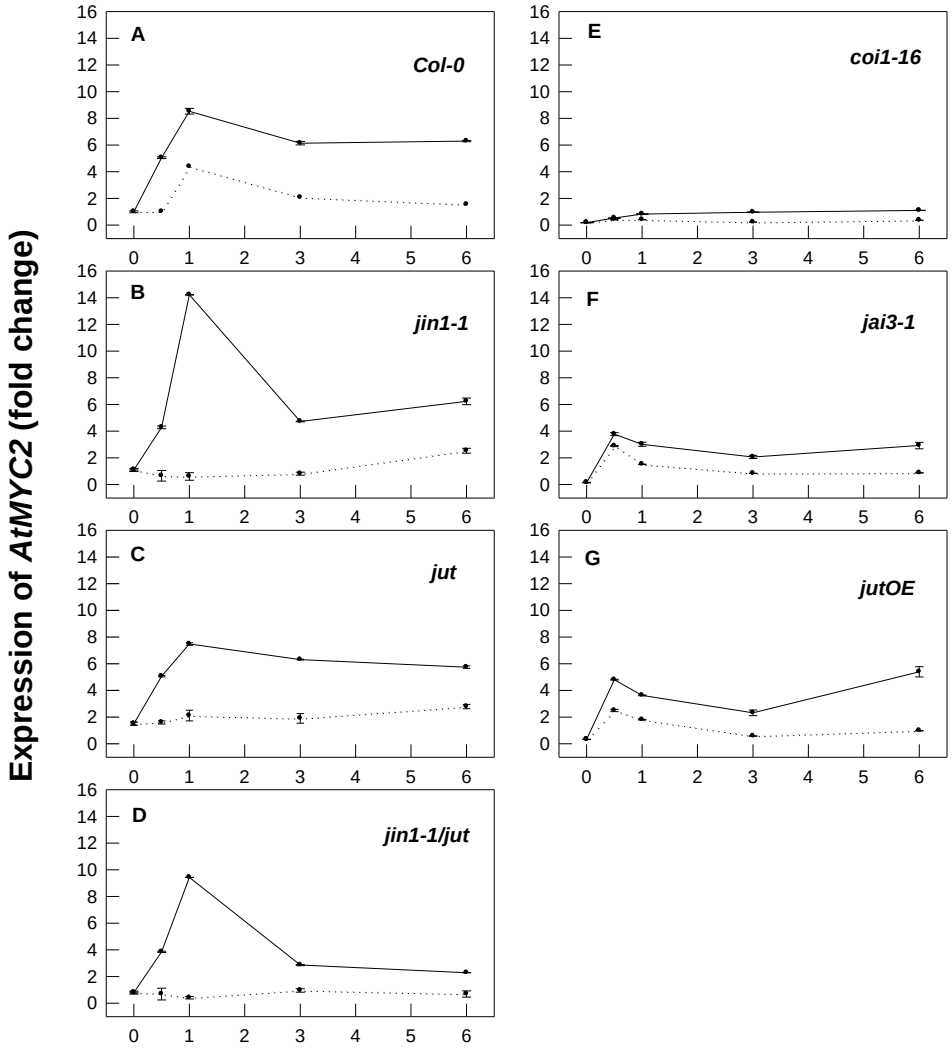


Figure 6.1: Expression of *AtMYC2* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *AtMYC2* (At1g32640) after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *AtMYC2* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *AtMYC2* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.2 Expression Analyses of *JUT*

The expression of *JUT* was rapidly induced by MeJA (**Figure 6.2.A**). This was slightly decreased in the single mutant *jin1*-1 compared to the wild type background, at 0.5h after MeJA treatment (**Figure 6.2.B**). This result indicates that MYC2 positively regulates MeJA-induced expression of *JUT*, which is consistent with the Northern blotting results of the expression of *JUT* in the *jin1*-1 background (**Figure 4.3**). The expression of *JUT* was detectable in the single mutant *jut* and the double mutant *jin1*-1*/jut* (**Figure 6.2.C** & **Figure 6.2.D**). There was no MeJA-induced *JUT* expression in *coi1*-16 (**Figure 6.2.E**). This result indicates that COI1 is required for MeJA-induced expression of *JUT*. After MeJA treatment, the expression of *JUT* was lower in *jai3*-1 than in the wild type (**Figure 6.2.F**). An explanation for this result might be the non-degraded JAZ proteins repressing the activity of MYC2, suppressing MeJA-induced expression of *JUT* in *jai3*-1. Additionally, MeJA-induced expression was repressed in JUTOE, indicating that constitutive expression of *JUT* represses its own expression (**Figure 6.2.G**).

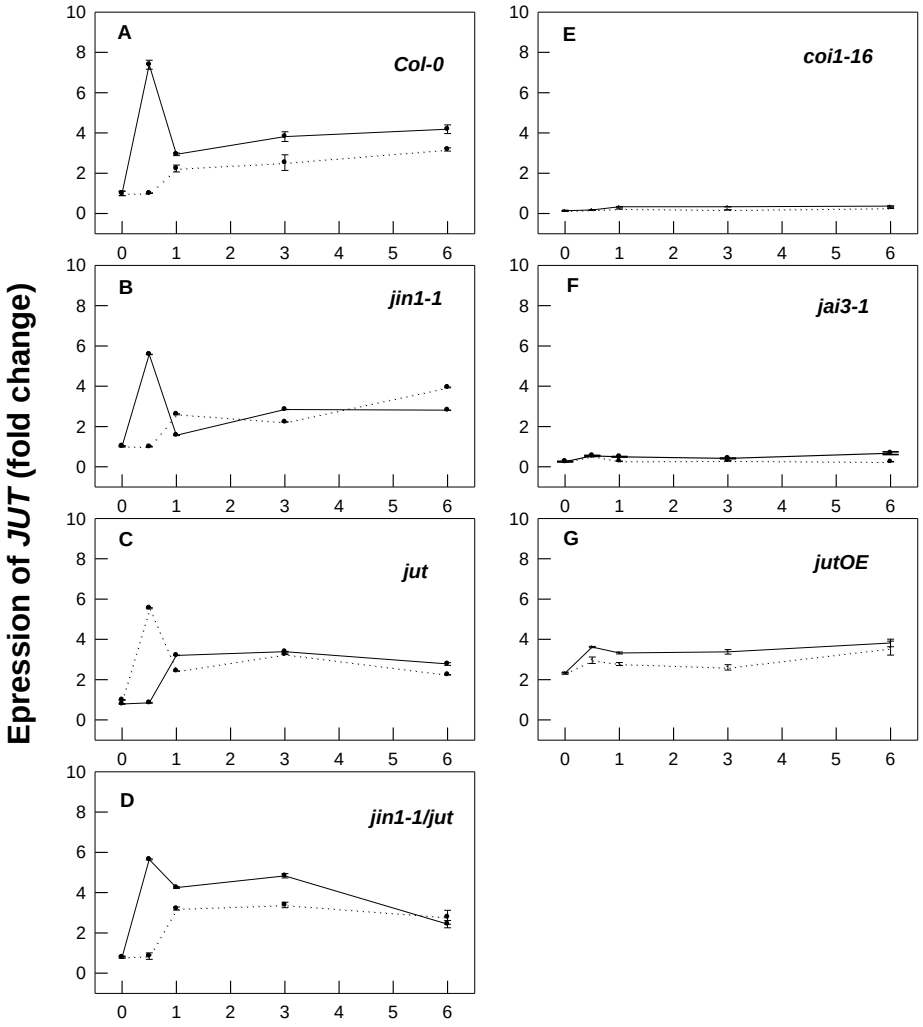


Figure 6.2: Expression of *JUT* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *JUT* (At2g46510) after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *JUT* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *JUT* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.3 Expression Analyses of *AtTAT3*

The expression of *AtTAT3* was rapidly induced by MeJA (**Figure 6.3.A**) and was reduced in *jin1*-1 compared to the wild type background at 0.5h after MeJA treatment (**Figure 6.3.B**). This indicates that MYC2 positively regulates MeJA-induced *AtTAT3* expression. There is no obvious expression change in *jut* compared to the wild type after MeJA treatment (**Figure 6.3.C**). Reduced expression of *AtTAT3* was detected in the double mutant *jin1*-1*/jut* (**Figure 6.3.D**). These results are consistent with MYC2, but not JUT, as a positive regulator of the expression of *AtTAT3*. There was no MeJA-induced expression of *AtTAT3* in *coi1*-16 (**Figure 6.3.E**). This result indicates that COI1 is required for MeJA-induced expression of *AtTAT3*. There was reduced expression of *AtTAT3* in *jai3*-1 compared to the wild type after MeJA treatment (**Figure 6.3.F**). An explanation for this result might be the non-degraded JAZ proteins repressing the activity of MYC2, and then suppressing MeJA-induced expression of *AtTAT3* in *jai3*-1 (**Figure 6.3.F** & **Figure 6.3.A**). In the line JUTOE, the expression of *AtTAT3* was increased by treatment with MeJA, compared to mock. This increase was lower than in wild-type at 0.5h, and 6h, but higher than wild-type at 1h, and 3h after MeJA treatment (**Figure 6.3.G)**. This indicates that constitutive expression of *JUT* represses the initial MeJA-induced expression of *AtTAT3*, but activates MeJA-induced expression of *AtTAT3* at 1h and 3h after MeJA treatment.

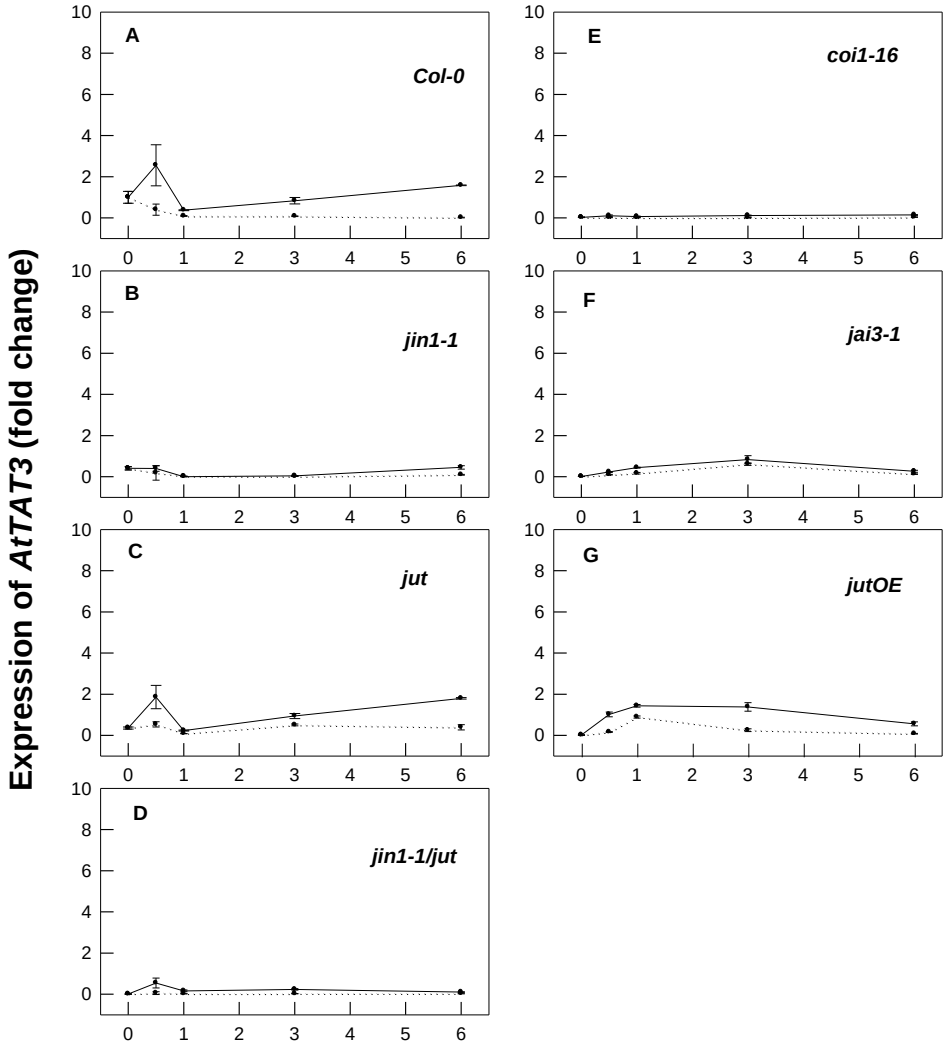


Figure 6.3: Expression of *AtTAT3* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *AtTAT3* (At2g24850) after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *AtTAT3* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *AtTAT3* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.4 Expression Analyses of *JAZ1*

The expression of *JAZ1* was rapidly induced in wild type Col-0 at 0.5h after MeJA treatment (**Figure 6.4.A**) and was reduced in *jin1*-1 compared to the wild type background at 0.5h after MeJA treatment (**Figure 6.4.B**). This indicates that MYC2 positively regulates MeJA-induced expression of *JAZ1*. Increased expression of *JAZ1* was identified in the single mutant *jut* compared to wild type at 0.5h after MeJA treatment (**Figure 6.4.C**). This result suggests that JUT negatively regulates MeJA-induced expression of *JAZ1*. Increased expression of *JAZ1* was identified in the double-mutant *jin1*-1/*jut*, indicating that the suppression of JUT overweights the activation of MYC2 (**Figure 6.4.D**). There was no MeJA-induced expression of *JAZ1* in *coi1*-16 (**Figure 6.4.E**). This indicates that COI1 is required for MeJA-induced expression of *JAZ1*. The expression of *JAZ1* was lower in *jai3*-1 compared to the wild type at 0.5h after MeJA treatment (**Figure 6.4.F**). An explanation for this result might be the non-degraded JAZ proteins repressing the activity of MYC2, and then suppressing MeJA-induced expression of *JAZ1* in *jai3*-1. MeJA-induced expression of *JAZ1* was reduced in JUTOE compared to the wild type at 0.5h after MeJA treatment (**Figure 6.4.G**). This result indicates that constitutive expression of *JUT* negatively regulates MeJA-induced expression of *JAZ1*.

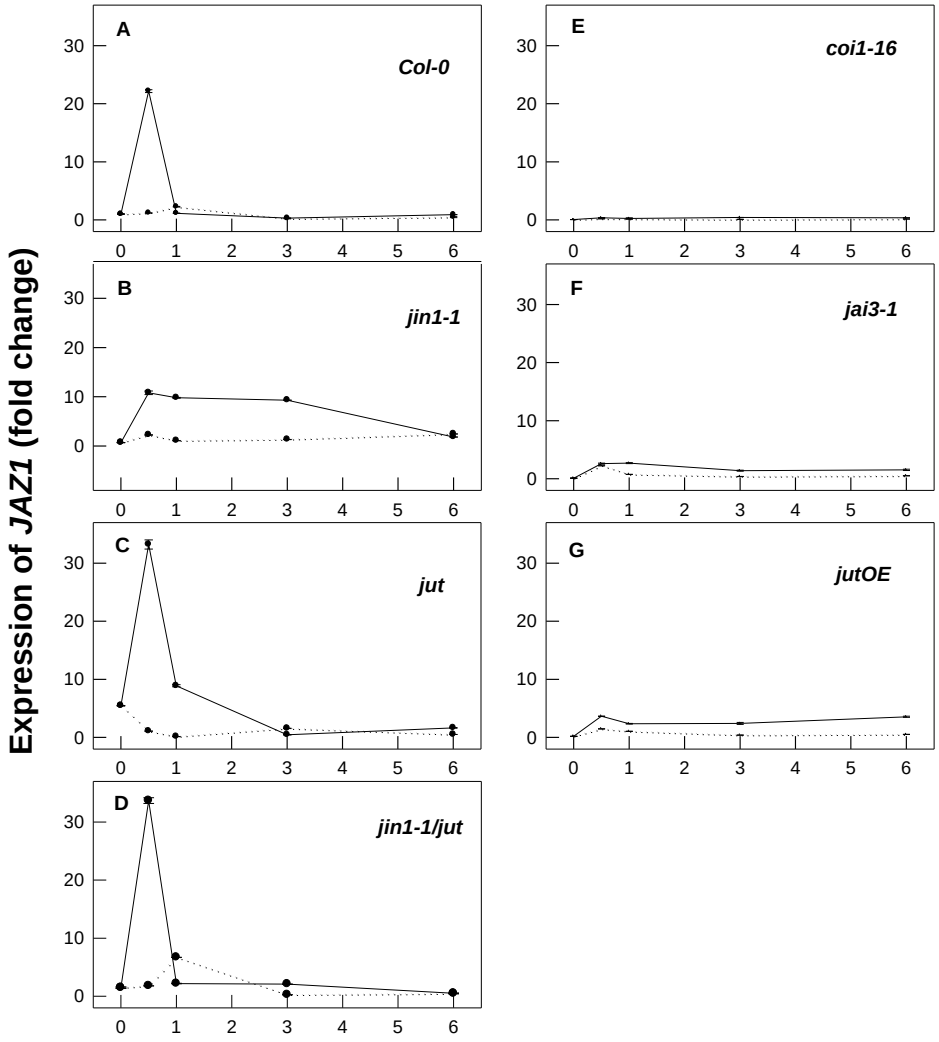


Figure 6.4: Expression of *JAZ1* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *JAZ1* (At1g19180) after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *JAZ1* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *JAZ1* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.5 Expression Analyses of *JAZ10*

The expression of *JAZ10* was rapidly induced in wild type Col-0 at 0.5h after MeJA treatment (**Figure 6.5.A**) and was reduced in *jin1*-1 compared to the wild type background at 0.5h and 1h after MeJA treatment (**Figure 6.5.B**). This indicates that MYC2 positively regulates MeJA-induced expression of *JAZ10*. Increased expression of *JAZ10* was identified in *jut* compared to the wild type at 0.5h after MeJA treatment (**Figure 6.5.C**). This result suggests that JUT negatively regulates MeJA-induced expression of *JAZ10*. A similar expression of *JAZ10* was identified in the double-mutant *jin1*-1*/jut* compared to in the single mutant *jin1*-1, indicating that the activation by MYC2 overweights the suppression by JUT (**Figure 6.5.D**). There was no MeJA-induced expression of *JAZ10* in *coi1*-16 (**Figure 6.5.E**). This indicates that COI1 is required for MeJA-induced expression of *JAZ10*. The expression of *JAZ10* was lower in *jai3-1* compared to the wild type at 0.5h, 1h, 3h and 6h after MeJA treatment (**Figure 6.5.F**). An explanation for this result might be the non-degraded JAZ proteins repressing the activity of MYC2, and then suppressing MeJA-induced expression of *JAZ10* in *jai3*-1. MeJA-induced expression of *JAZ1* was reduced in JUTOE than in the wild type at 0.5h, 1h, 3h and 6h after MeJA treatment (**Figure 6.5.G**). This result indicates that constitutive expression of *JUT* negatively regulates MeJA-induced expression of *JAZ10*.

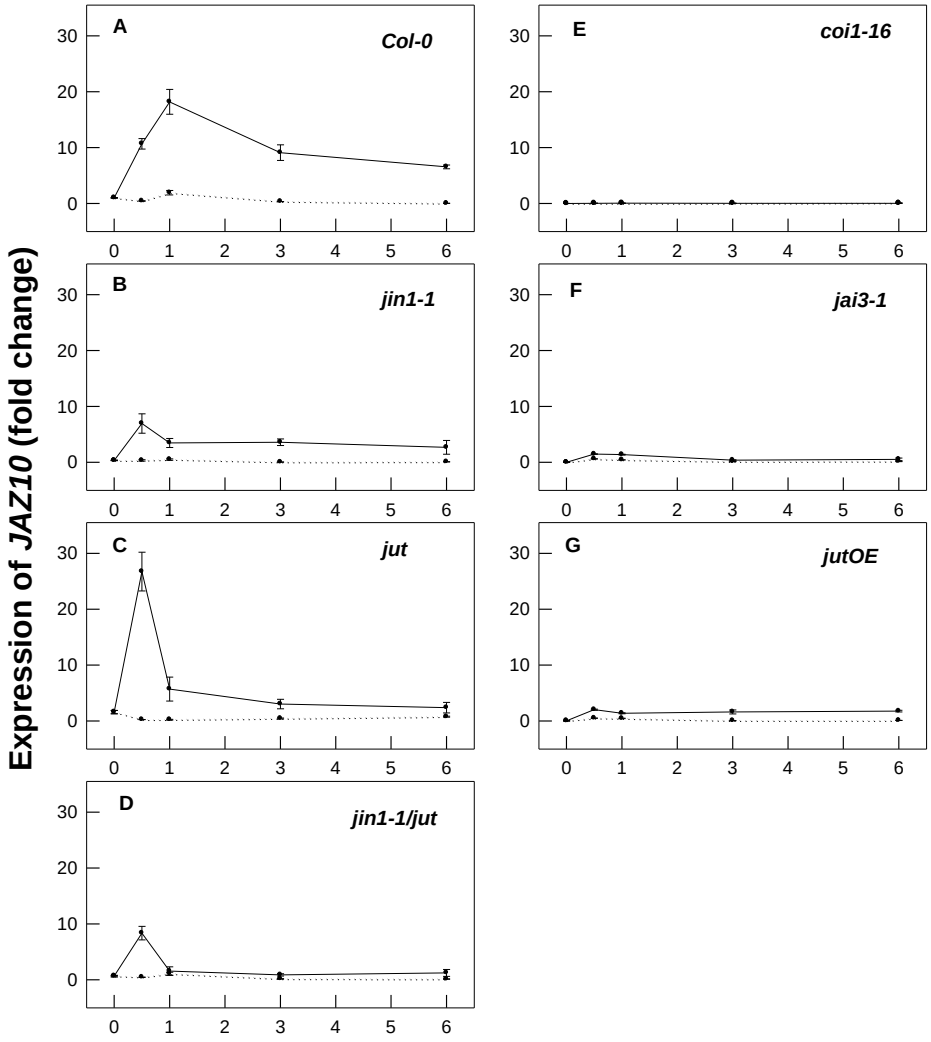


Figure 6.5: Expression of *JAZ10* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *JAZ10* (At5g13220) after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *JAZ10* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *JAZ10* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.6 Expression Analyses of *DIN11*

The expression of *DIN11* was rapidly induced in wild type Col-0 at 0.5h after MeJA treatment (**Figure 6.6.A**) and was reduced in *jin1*-1, *jut* and *jin1*-1/*jut* compared to the wild type background at 0.5h after MeJA treatment (**B, C &D in Figure 6.6**). These results indicate that MYC2 and JUT positively regulate MeJA-induced expression of *DIN11*. There was no MeJA-induced expression of *DIN11* in *coi1*-16 (**Figure 6.6.E**). This indicates that COI1 is required for MeJA-induced expression of *DIN11*. There was no MeJA-induced *DIN11* expression in *jai3*-1 at 0.5h after MeJA treatment (**Figure 6.6.F**). An explanation for the result might be the non-degraded JAZ proteins repressing the activity of MYC2, and then suppressing MeJA-induced expression of *DIN11* in *jai3*-1. MeJA-induced expression of *DIN11* was reduced in JUTOE compared to the wild type at 0.5h and 1h after MeJA treatment, and was increased in JUTOE compared to the wild type at 3h and 6h after MeJA treatment (**Figure 6.6.G**). This result indicates that constitutive expression of *JUT* negatively regulates the initial MeJA-induced expression of *DIN11* at 0.5h and 1h after MeJA treatment, while positively regulates the late MeJA-induced expression of *DIN11* at 3h and 6h after MeJA treatment.

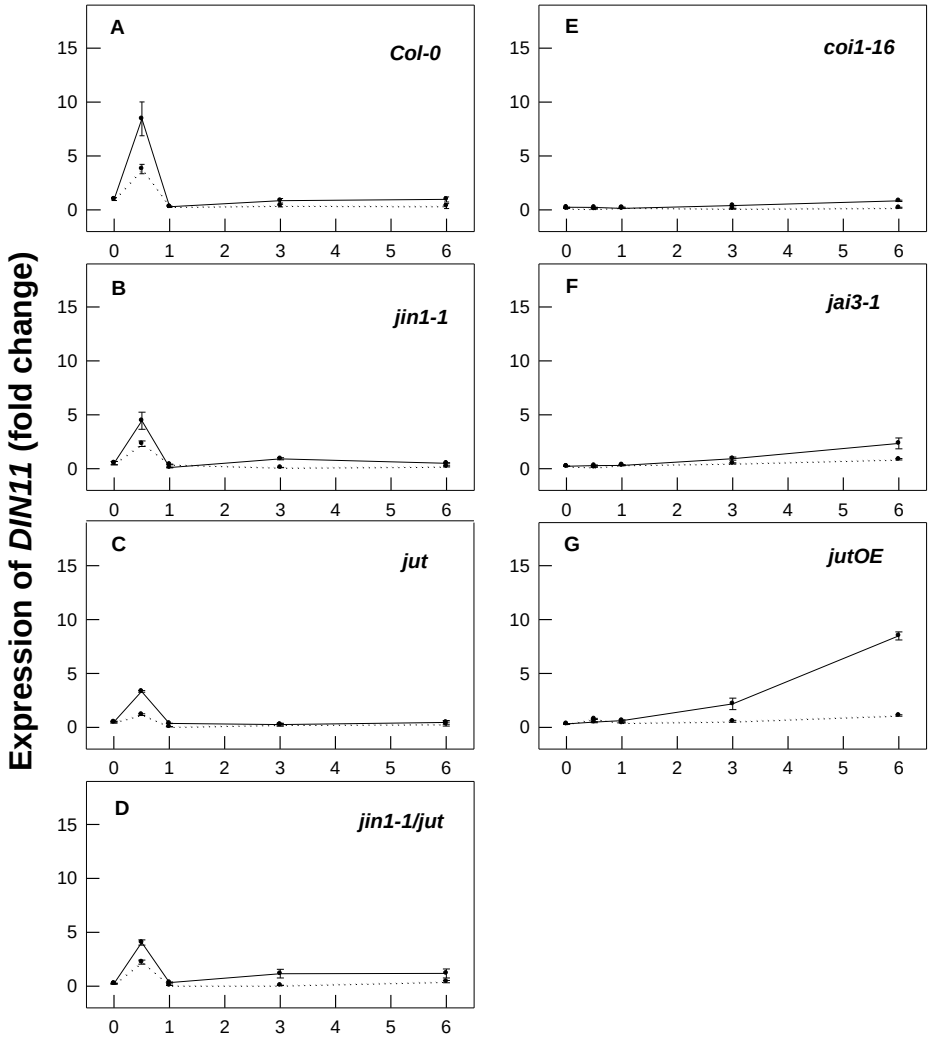


Figure 6.6: Expression of *DIN11* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *DIN11* (At3g49260) after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *DIN11* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *DIN11* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.7 Expression Analyses of *At1g66690*

The expression of *At1g66690* was rapidly induced in wild type Col-0 at 0.5h, 1h, 3h and 6h after MeJA treatment (**Figure 6.7.A**) and was reduced in *jin1*-1, *jut* and *jin1*-1/*jut* compared to the wild type background at 1h, 3h and 6h after MeJA treatment (**B, C &D in Figure 6.7**). These results indicate that MYC2 and JUT positively regulate MeJA-induced expression of *At1g66690*. There was no MeJA-induced expression of *At1g66690* in *coi1*-16 (**Figure 6.7.E**). This indicates that COI1 is required for MeJA-induced expression of *At1g66690*. There was no MeJA-induced expression of *At1g66690* in *jai3*-1 after MeJA treatment (**Figure 6.7.F**). An explanation for this result might be the non-degraded JAZ proteins repressing the activity of MYC2, and then suppressing MeJA-induced expression of *At1g66690* in *jai3*-1. MeJA-induced expression of *At1g66690* was reduced in JUTOE compared to the wild type at 0.5h, 1h, after MeJA treatment (**Figure 6.7.G**). This result indicates that constitutive expression of *JUT* negatively regulates the initial MeJA-induced expression of *At1g66690*.

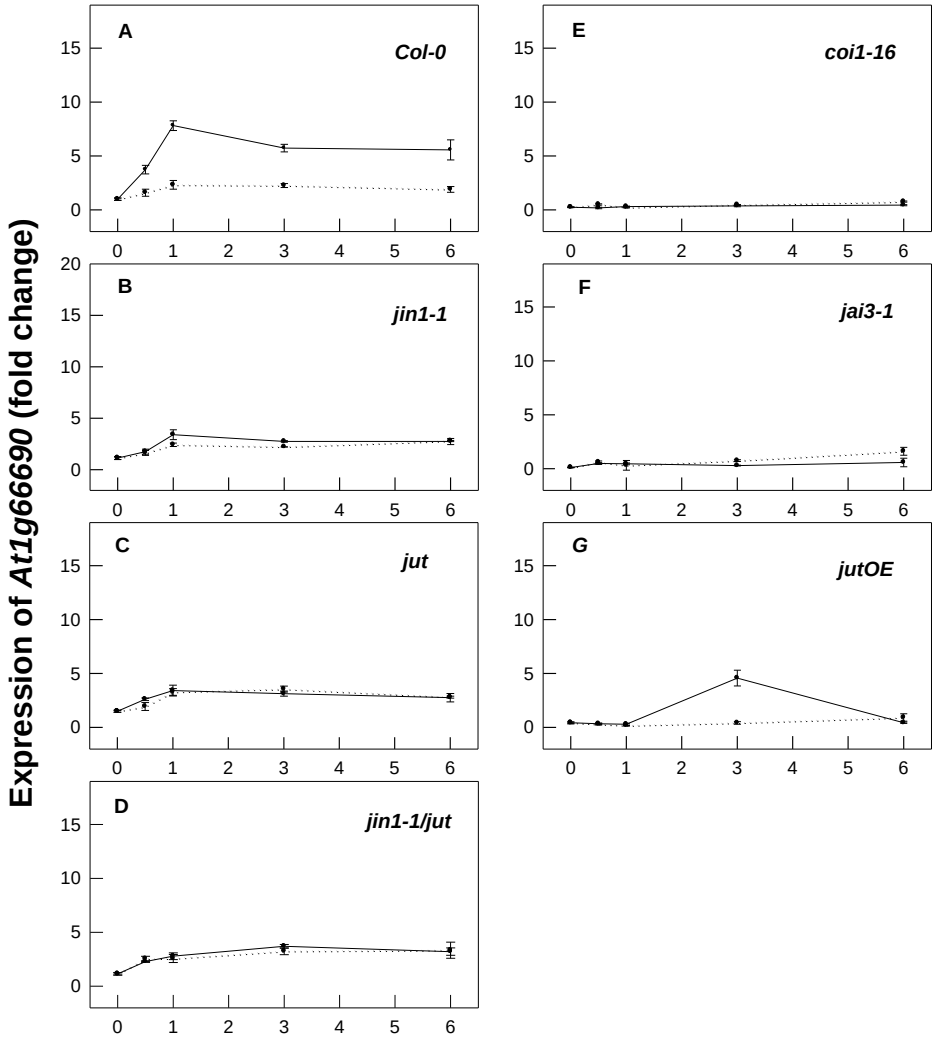


Figure 6.7: Expression of *At1g66690* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *At1g66690* after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *At1g66690* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *At1g66690* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours).Each solid black circle at the in dicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.8 Expression Analyses of *At1g53885*

The expression of *At1g53885* was rapidly induced in wild type Col-0 at 0.5h after MeJA treatment (**Figure 6.8.A**) and was reduced in *jin1*-1, *jut* and *jin1*-1/*jut* compared to the wild type background at 0.5h and 1h after MeJA treatment (**B, C &D in Figure 6.8**). These results indicate that MYC2 and JUT positively regulate MeJA-induced expression of *At1g53885*. There was no MeJA-induced expression of *At1g53885* in *coi1*-16 (**Figure 6.8.E**). This indicates that COI1 is required for MeJA-induced expression of *At1g53885*. MeJA-induced expression of *At1g53885* was lower in *jai3*-1 compared to the wild type Col-0 at 0.5h, 1h 3h and 6h after MeJA treatment (**Figure 6.8.F**). An explanation for this result might be the non-degraded JAZ proteins repressing the activity of MYC2, and then suppressing MeJA-induced expression of *At1g53885* in *jai3*-1. MeJA-induced expression of *At1g53885* was reduced in JUTOE compared to the wild type at 0.5h, 1h after MeJA treatment (**Figure 6.8.G**). This result indicates that constitutive expression of *JUT* negatively regulates the initial MeJA-induced expression of *At1g53885*.

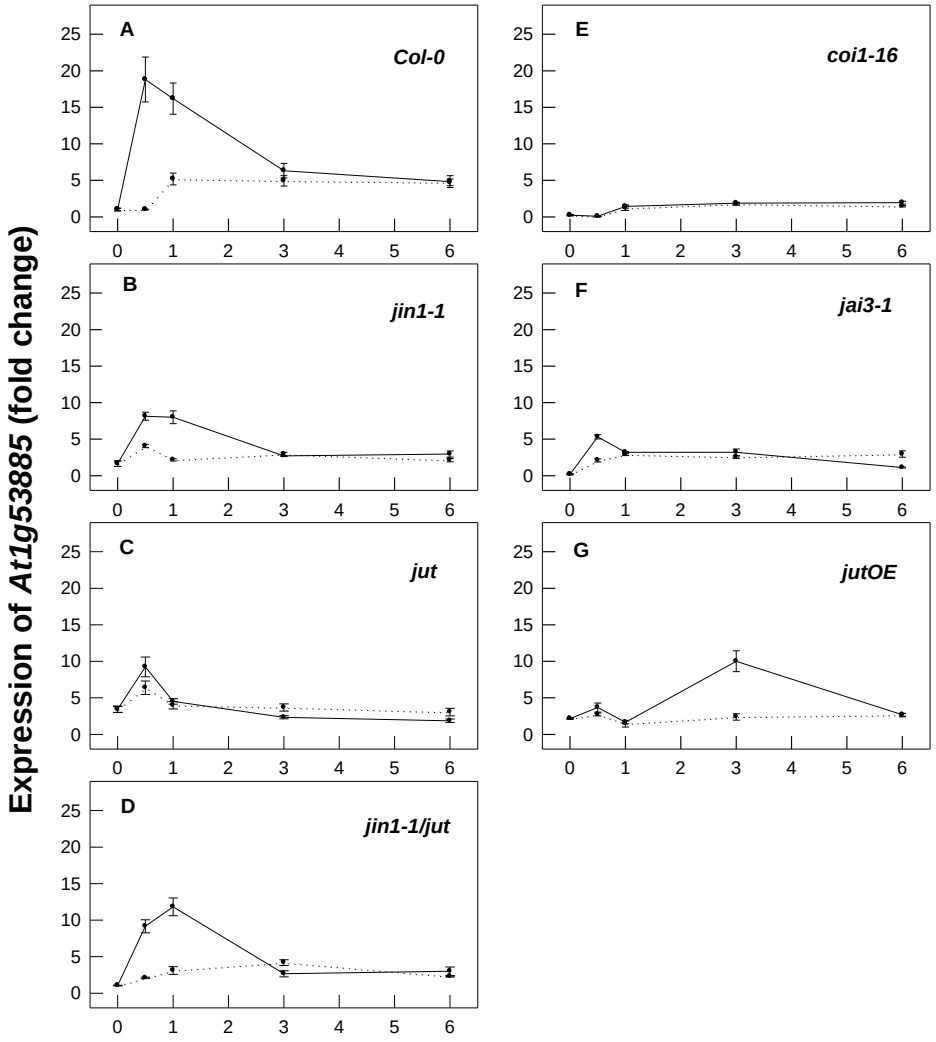


Figure 6.8: Expression of *At1g53885* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *At1g53885* after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *At1g53885* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *At1g53885* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

6.2.9 Expression Analyses of *PDF1.2b*

The expression of *PDF1.2b* was rapidly induced at 0.5h after MeJA treatment and the high expression level was maintained at 3h and 6h after MeJA treatment (**Figure 6.9.A**). The expression of *PDF1.2b* was reduced in *jin1*-1 and *jin1*-1/*jut* compared to the wild type background at 0.5h after MeJA treatment (**B** & **D** in **Figure 6.9**). These results indicate that MYC2 positively regulates the initial MeJA-induced expression of *PDF1.2b*. The expression of *PDF1.2b* was reduced in *jut* and *jin1*-1/*jut* compared to the wild type background at 6h after MeJA treatment (**C** & **D** in **Figure 6.9**). These results indicate that JUT positively regulate the late MeJA-induced expression of *PDF1.2b*. There was no MeJA-induced expression of *PDF1.2b* in *coi1*-16 (**Figure 6.9.E**). This indicates that COI1 is required for MeJA-induced expression of *PDF1.2b*. There was no MeJA-induced expression of *PDF1.2b* in *jai3*-1 after MeJA treatment (**Figure 6.9.F**). An explanation for this result might be the non-degraded JAZ proteins repressing the activity of MYC2, and then suppressing MeJA-induced expression of *PDF1.2b* in *jai3*-1. MeJA-induced expression of *PDF1.2b* was reduced in JUTOE compared to the wild type at 0.5h, 1h, 3h and 6h after MeJA treatment (**Figure 6.9.G**). This result indicates that constitutive expression of *JUT* negatively regulates MeJA-induced expression of *PDF1.2b*.

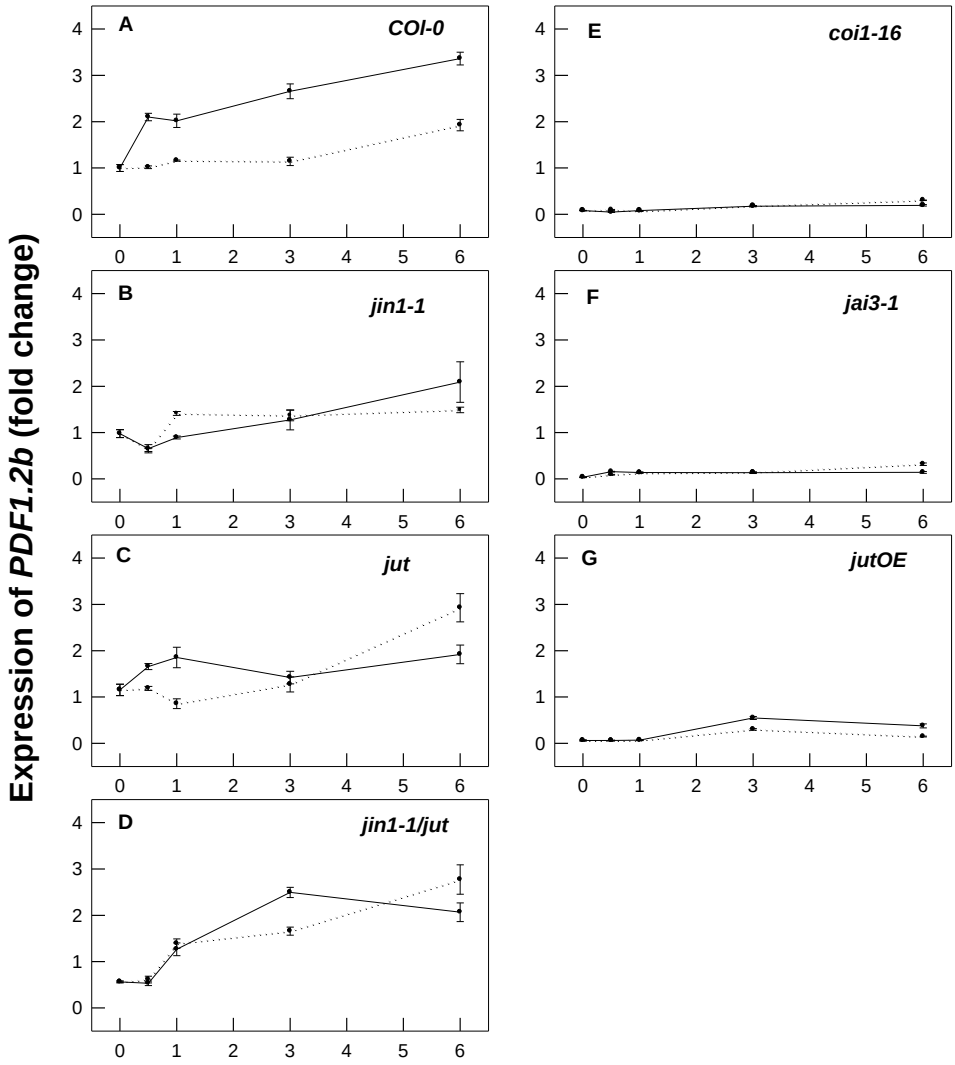


Figure 6.5: Expression of *PDF1.2b* (fold change) in different backgrounds.
qRT-PCR investigated the expression of *PDF1.2b* (At2g26020) after 20μM MeJA or mock treatment in the wild type Col-0, the mutants *jin1*-1, *jut*, *jin1*-1/*jut*, *coi1*-16, *jai3*-1 and the over-expression line JUTOE. *ACTIN2* (At3g18780) was selected as the normalizer for the equal cDNA loading in all qRT-PCR experiments. Solid line displays the expression of *PDF1.2b* (fold change) at 0, 0.5h, 1h, 3h and 6h after 20μM MeJA treatment. The dotted line represents the expression of *PDF1.2b* (fold change) at 0, 0.5h, 1h, 3h and 6h mock treatment. Fold changes relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Each solid black circle at the indicated time point displays the mean value of three biological replicates. Fourteen two-week-old seedlings were pooled for each biological replicate. Error bars illustrate the value of Standard Error.

Chapter 7
General Discussion

The main objective in my thesis was to identify new components in the JA signal pathway. Expression of one bHLH transcription factor coding gene (At2g46510) was rapidly induced by MeJA and wounding treatments and dependent on COI1. This transcription factor, therefore, was named as Jasmonate Up-regulated Transcription factor (JUT). In addition, a T-DNA insertion mutant in *JUT* (N874647) displayed a reduced responsiveness to MeJA-induced root growth inhibition and wound-induced shoot growth inhibition compared to the wild type Col-0. Furthermore, global gene expression and quantitative gene expression analyses revealed that JUT co-operates in different ways with MYC2 to regulate the expression of a panel of JA responsive genes. These discoveries lead to a novel regulatory model for the JA signal pathway.

Gene expression profile analyses of publically available information indicated that one predicted transcription factor coding gene At2g46510 was up-regulated by wounding and MeJA, compared to the untreated control (**Figure 3.1** & **Figure 3.2**). Moreover, At2g46510 (*JUT*) had a similar expression profile to *AtMYC2* after wounding and MeJA treatments, as well as at different development stages (**Figure 3.3**). The gene product of At2g46510 was previously identified as a positive regulator in the ABA signal pathway, and its expression was up-regulated after ABA treatment (Li et al., 2007). The expression profile on Genevestigator also revealed that the expression of *JUT* and *AtMYC2* was significantly reduced in the *coi1* mutant compared to wild type (**Table 3.1**). MYC2 functions as an important transcription regulator in the JA signal pathway and its expression is COI1-dependent (Lorenzo et al., 2004). So far, at least fifteen COI1-dependent transcription factors have been identified. Most of these are JA- and wounding- responsive genes, for example WRKY18 (Wang et al., 2008). JUT, therefore, was predicted to be a novel COI1-dependent transcription factor. In order to identify the role of JUT in regulation of the JA response, a homozygous T-DNA insertion mutant, *jut* (N874647), was confirmed by PCR

and DNA sequence analysis (**Figure 3.4** & **Figure 3.7**).

A T-DNA insertion mutant in *JUT* (N874647) displayed a reduced responsiveness to MeJA-induced root growth inhibition in comparison with wild type (Col-0) (**Figure 3.10**). Wounding reduces Arabidopsis leaf growth, and requires genes for JA biosynthesis (*AOS*, *OPR3*) and JA signalling (*COI1*, *JIN1*, *JAI3*), but not JAR1 (Zhang and Turner, 2008). In this study, *jut* displayed a reduced responsiveness to wound-induced shoot growth inhibition in comparison with wild type (Col-0) (**Figure 3.13**). This result indicated that wound-induced leaf growth inhibition also requires the function of JUT. Thus, JUT functions in the regulation of shoot growth by wound-induced endogenous JA. The reduced shoot growth arises from a reduction in mitosis. This implies that MYC2 and JUT may regulate mitosis related genes.

MYC2 and JUT share significant homology in their predicted bHLH, DNA-binding domains (**Figure 3.16)**. The N-terminal of MYC2 interacts with the C-terminal domain of JAI3/JAZ3 (Chini et al., 2007). It may be significant therefore that the N-terminal sequences of JUT and MYC2 have high similarity (**Figure 3.16**). Possibly therefore, JUT also interacts with JAZ proteins via this motif. According to the Pearson Correlation Coefficient (r-value) analysis on GeneCAT database (Mutwil et al., 2008), *AtMYC2* and *JUT* are co-expressed with each other and several other JA responsive genes, such as *JAZ2* and *JAZ5* (**Figure 3.18**). According to the gene expression profile analysis by the cluster cutting tool on the *AtGenExpress* database (Goda et al., 2008), *JUT* is co-expressed with *JAZ10*, *JAZ2*, *JAZ9*, *JAZ5*, *AtMYC2* and *JAZ6* (**Figure 3.19**). These analyses described in Chapter 3 indicated that *JUT* is regulated similarly to, and co-expressed with *AtMYC2*. This suggests that JUT is a component in the JA signal pathway.

Chapter 7: General Discussion

In **Chapter 4**, the expression of *AtMYC2* and *JUT* in wild type (Col-0) and in mutants of the JA signal pathway was investigated. Northern analysis confirmed that *JUT* was induced within 15 min by MeJA or wounding. MeJA-induced expression of *JUT* was maintained at a high level from 15min till 6h (**Figure 4.1**). Northern blotting analyses demonstrated that the expression of *JUT* and *AtMYC2* was not increased after MeJA treatment in *coi1*-16 compared to the wild type Col-0 (**Figure 4.2**). Taken together, these results indicated that COI1 was required for MeJA-induced expression of *JUT*. In this sense, therefore, JUT is functionally a COI1-dependent transcription factor.

In the *jai3*-1 mutant, the truncated protein JAI3-1 binds the SCF^{COI1} complex and blocks the activity of SCF^{COI1}. JAZ proteins repress the activity of MYC2 (Chini et al., 2007). The majority of JA responsive genes were down-regulated in the mutant *jai3*-1 compared to the wild type after 2h and 6h of 50μM JA treatment (Chini et al., 2007). Therefore, there would be less MeJA-induced expression of *AtMYC2* and *JUT* in *jai3*-1 compared to the wild type (Col-0). Unexpectedly, the expression of *AtMYC2* and *JUT* measured by Northern blotting analysis was increased at 15min after MeJA treatment in *jai3*-1 compared to the wild type (Col-0) (**Figure 4.2**). However, the expression of *AtMYC2* and *JUT* measured by qRT-PCR indicated was reduced at 30min and subsequent time points in the *jai3*-1 mutant compared to wild type (Col-0) (plot **F** in **Figure 6.1** & **Figure 6.2**). One possible explanation for the initial (15min) MeJA-induced expression of *AtMYC2* and *JUT* in *jai3*-1 is that the JAI3 mutant protein may take some time to 'block' the activity of COI1 protein (as described in **section 1.4.2** in **Chapter 1**), and that *AtMYC2* and *JUT* expression are therefore increased for a short period after MeJA treatment. Certainly, the MeJA-induced expression of JA responsive genes was suppressed in *jai3*-1 at 30min and later time points after MeJA treatment (plot **F** in **Figure 6.1** to **Figure 6.9**).

Northern analysis also demonstrated that JUT is not required for the initial MeJA-induced expression of *AtMYC2*, and MYC2 is not required for the initial MeJA-induced expression of *JUT* (**Figure 4.3**). However, MYC2 is apparently required for maintaining the high level

expression of *JUT* at 45min, 1h, 3h and 6h after MeJA treatment (**Figure 4.3**). This indicates therefore that MYC2 partially regulates the expression of *JUT*. The double mutant *jin1*-1/*jut* displayed a reduced responsiveness to MeJA-induced root growth inhibition than *jut*, but an increased responsiveness than *jin1*-1. This indicates that the interaction between MYC2 and JUT in regulation of root growth inhibition by MeJA is not simply additive. Both JUTOE and MYC2OE had increased responsiveness to inhibition by MeJA than wild type (**Figure 4.10**). Taken together, these results confirm that JUT, with MYC2, regulates plant growth in response to MeJA.

It was recently shown that JA regulates flowering time, and that the *coi1* mutant flowers earlier than wild type (Robson et al., 2010). I therefore compared flowering time in wild type (Col-0), single mutants (*jin1*-1, *jut, jai3*-1 and *coi1*-16), double mutant *jin1*-1/*jut*, and MYC2OE and JUTOE (**section 4.2.4.4**). The results in **Figure 4.11** indicated that *jin1*-1/*jut*, *jin1*-1 and *jut* flowered earlier than wild type (Col-0). In agreement, MYC2OE and JUTOE displayed delayed flowering time compared to wild type (Col-0). These results indicate that MYC2 and JUT function in the JA signal pathway as negative regulators of flowering time.

In **Chapter 5**, Arabidopsis ATH1 Genome Arrays were applied to investigate the global transcription profiling of wild type (Col-0), single mutants *jin1*-1, *jut* and double mutant *jin1*-1/*jut* in both untreated control and MeJA-treated samples. The identification of MeJA, MYC2 and JUT regulated genes is described in the **section 5.3**. Pie charts (**Figure 5.1**), Venn diagrams (**Figure 5.2**) and Heat-maps (**Figure 5.3**) were used to interpret microarray data analyses in visualised formats. Based on the expression of JA-responsive genes in the wild type (Col-0), single mutant (*jin1*-1, *jut*) and double mutant *jin1*-1/*jut*, a panel of genes were selected from the microarray data analyses for the quantitative measurement of expression in **Chapter 6**. This panel of genes includes two transcription factors (MYC2 and JUT), and seven JA responsive genes (*AtTAT3*, *JAZ1*, *JAZ10*, *DIN11*, At1g66690, At1g53885 and *PDF1.2b*).

Chapter 7: General Discussion

In **Chapter 6**, SYBR-Green qRT-PCR was used to quantitatively measure the expression level of the panel of JA responsive genes, in seven genetic backgrounds, which included wild type (Col-0), the single mutants *jin1*-1, *jut*, *jai3*-1, *coi1*-16 and the double mutant *jin1*-1/*jut*, and JUTOE, with and without MeJA treatment. Gene expression levels (Fold changes) relative to wild type Col-0 untreated (time zero) sample are plotted in time course (hours). Firstly, the expression analyses of a panel of JA responsive genes within an indicated time course (0, 0.5h, 1h, 3h and 6h) in wild type background indicated that this panel of genes were up-regulated by MeJA in comparison with the untreated control (plot **A** in **Figure 6.1** to **Figure 6.9**). Next, the expression analyses of this panel of genes in *jin1*-1, *jut* and *jin1*-1/*jut* indicated that JUT co-operates in different ways with MYC2 to regulate MeJA-induced expression of a panel of JA responsive genes (plot **B**, **C** and **D** in **Figure 6.1** to **Figure 6.9**). For instance, MYC2 positively regulated MeJA-induced expression of *TAT3*, *PDF1.2b*, *DIN11*, At1g53885, At1g66690, *JAZ10* and *JAZ1*, and negatively regulated MeJA-induced expression of *AtMYC2*. JUT positively regulated MeJA-induced expression of *PDF1.2b*, *DIN11*, At1g53885 and At1g66690, and negatively regulated MeJA-induced expression of *JUT*, *JAZ1* and *JAZ10*. The expression analyses of this panel of genes in *coi1*-16 suggested that COI1 was required for their MeJA-induced expression (plot **E** in **Figure 6.1** to **Figure 6.9**). In addition, the expression analyses of this panel of genes in the mutant *jai3*-1 revealed low MeJA-induced expression of this panel of genes possibly because the truncated protein JAZ3-1 led to the repression of MYC2 by non-degraded JAZ proteins (plot **F** in **Figure 6.1** to **Figure 6.9**). The expression analyses of this panel of genes in JUTOE indicated that constitutive expression of *JUT* repressed the initial MeJA-induced expression of this panel of genes (plot **G** in **Figure 6.1** to **Figure 6.9**).

Chapter 7: General Discussion

Current understanding of the JA signal pathway is that in response to a biologically active JA signal (JA-Ile), JAZ proteins bind to COI1 and are then degraded by ubiquitin and destroyed by the 26S proteasome. MYC2 is therefore relieved from the JAZ suppression and activates the transcription of JA responsive genes (Staswick, 2008; Chini et al., 2009a; Fonseca et al., 2009a; Frankowski et al., 2009; Gfeller et al., 2010). However, the transcription regulatory model of the JA signal pathway has not been well-identified. The results in this thesis establish JUT as a novel transcription regulator in the JA signal pathway. *JUT* is co-expressed with *AtMYC2*. JUT, like MYC2, is required for in response to MeJA-induced growth inhibition, wound-induced growth inhibition and regulation of flowering time. JUT also co-operates in different ways with MYC2 to regulate the expression of a panel of JA responsive genes. A novel regulatory model of the JA signal pathway is illustrated in **Figure 7.1**, which combines published knowledge of the JA signal pathway and results presented here on transcriptional regulation of a panel of JA responsive genes by MYC2 and JUT.

Chapter 7: General Discussion

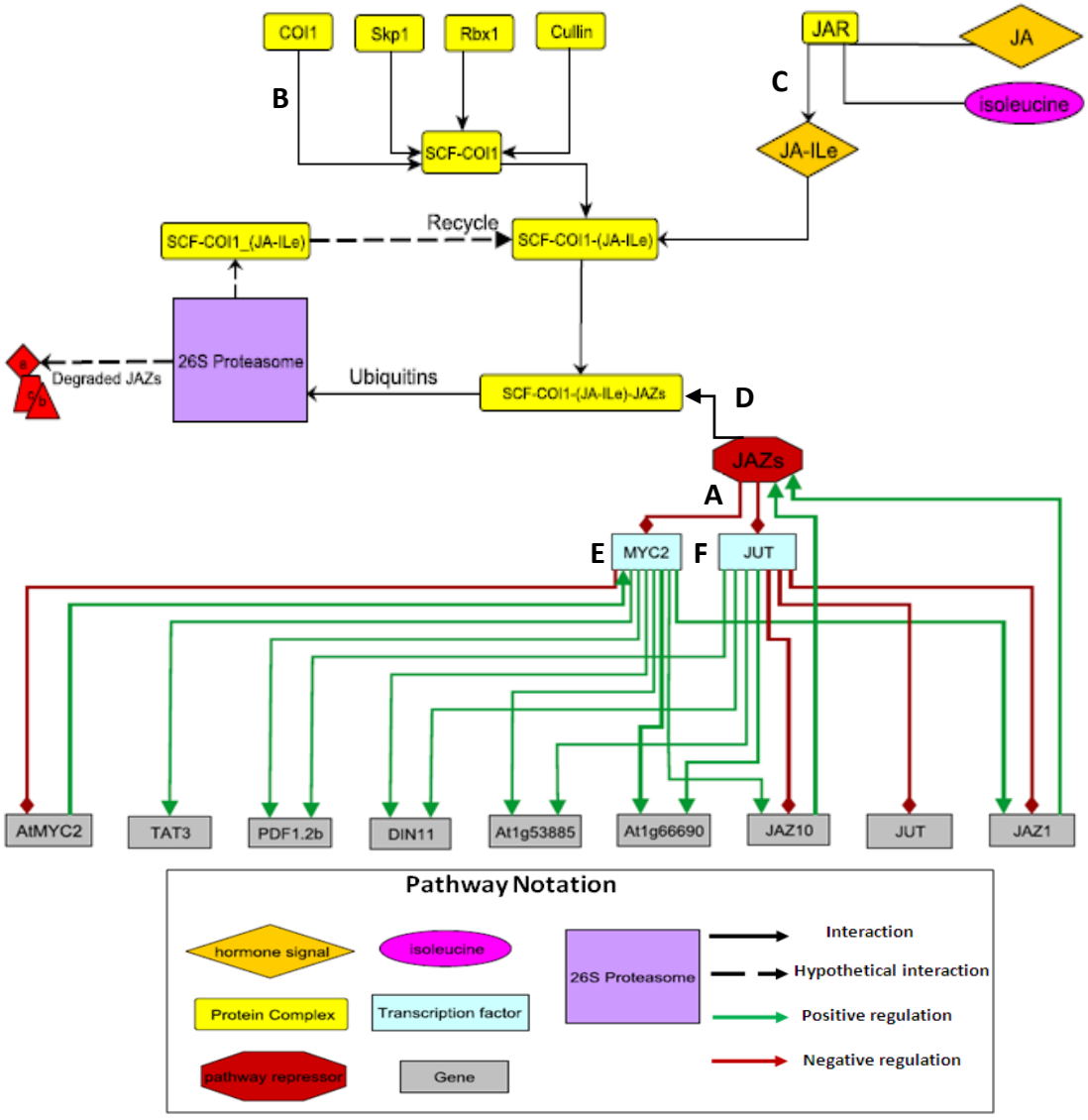


Figure 7.1: A regulatory model of the JA signal pathway.

A. JAZs repress the transcription activity of MYC2 and JUT. **B.** COI1 associates with SKP1, Rbx1 and Cullin to form SCF-COI1 complex. **C.** JA-Ile is synthesised by a jasmonate-amino acid synthetase (JAR1). **D.** In response to the JA-Ile signal, JAZ proteins bind to SCF-COI1 complex and are then degraded by ubiquitin and 26S proteasome. MYC2 and JUT are then free from the repression of JAZs. **E.** MYC2 positively regulates the expression of *TAT3*, *PDF1.2b*, *DIN11*, At1g53885, At1g66690, *JAZ10* and *JAZ1*, and negatively regulates the expression of *AtMYC2*. **F.** JUT positively regulates the expression of *PDF1.2b*, *DIN11*, At1g53885 and At1g66690, and negatively regulates the expression of *JUT*, *JAZ1* and *JAZ10*.

Chapter 7: General Discussion

Future questions that should be addressed include further characterisation of transcription factors that regulate JA responses. For instance, in Chapter 3, the T-DNA insertion mutants in two MYB transcription factor coding genes (MYB70 and MYB77) were insensitive to MeJA-induced root growth inhibition. The regulatory mechanism of these two transcription factors in the JA signal pathway needs future investigation. A T-DNA insertion mutant *jut* (N874647) has been investigated in this research, which displaying a reduced responsiveness to MeJA-induced growth inhibition and early flowering time in comparison with the wild type Col-0. Consistently, JUTOE line displayed a delayed flowering time and increased responsiveness to MeJA treatment in comparison with the wild type (Col-0) and *jut* (N874647). These results indicating that loss of JUT function induces a reduced responsiveness to MeJA-induced growth inhibition, while constitutive expression of *JUT* displays an increased responsiveness to MeJA-induced growth inhibition. However, only one mutant of *JUT* was used in this study, more aspects of phenotypic effects of other independent mutants on *JUT* need to be investigated in future study. That would indicate much more full understandings about the biological function of JUT in JA signal pathway.

A major challenge is also to identify the direct target genes that MYC2 and JUT regulate. This could be approached by Chromatin Immuno-precipitation (ChIP) to investigate the direct DNA binding sequences of MYC2 & JUT. It also seems likely that JUT could bind JAZ proteins and this could be tested by using yeast-two hybrid assays or direct binding arrays. Finally, the functions of the panel of JA responsive genes (as shown in **Figure 7.1**) which are regulated by MYC2 and JUT should be studied. For instance, the regulation of these genes on the responses of MeJA- and wound-induced growth inhibition could be investigated in knock-out mutants of these genes. All these future studies could establish a transcription regulatory model of MYC2 and JUT and help to develop a systematic understanding of JA signal pathway in Arabidopsis.

TableA.1: Arabidopsis bHLH transcription factors

Gene ID	Description of gene product
AT1G01260	basic helix-loop-helix (bHLH) family protein
AT1G02340	HFR1 (LONG HYPOCOTYL IN FAR-RED)
AT1G03040	basic helix-loop-helix (bHLH) family protein
AT1G05805	basic helix-loop-helix (bHLH) family protein
AT1G06170	basic helix-loop-helix (bHLH) family protein
AT1G09530	PAP3/PIF3/POC1 (PHYTOCHROME INTERACTING FACTOR 3)
AT1G10120	DNA binding / transcription factor
AT1G10585	transcription factor
AT1G10586	transcription regulator
AT1G10610	DNA binding / transcription factor
AT1G12540	basic helix-loop-helix (bHLH) family protein
AT1G12860	basic helix-loop-helix (bHLH) family protein
AT1G18400	BEE1 (BR ENHANCED EXPRESSION 1); transcription factor
AT1G22490	basic helix-loop-helix (bHLH) family protein
AT1G25310	MEE8 (maternal effect embryo arrest 8)
AT1G25330	basic helix-loop-helix (bHLH) family protein
AT1G26260	basic helix-loop-helix (bHLH) family protein
AT1G27740	basic helix-loop-helix (bHLH) family protein
AT1G30670	basic helix-loop-helix (bHLH) family protein
AT1G32640	ATMYC2 (JASMONATE INSENSITIVE 1)
AT1G35460	basic helix-loop-helix (bHLH) family protein
AT1G49770	basic helix-loop-helix (bHLH) family protein
AT1G51070	basic helix-loop-helix (bHLH) family protein
AT1G51140	basic helix-loop-helix (bHLH) family protein

TableA.1: Arabidopsis bHLH transcription factors (continued)

Gene ID	Description of gene product
AT1G59640	ZCW32 (BIGPETAL, BIGPETALUB)
AT1G61660	basic helix-loop-helix (bHLH) family protein
AT1G62975	basic helix-loop-helix (bHLH) family protein (bHLH125)
AT1G63650	EGL3 (ENHANCER OF GLABRA3)
AT1G66470	basic helix-loop-helix (bHLH) family protein
AT1G68240	transcription factor
AT1G68810	basic helix-loop-helix (bHLH) family protein
AT1G68920	basic helix-loop-helix (bHLH) family protein
AT1G69010	BIM2 (BES1-INTERACTING MYC-LIKE PROTEIN 2)
AT1G71200	basic helix-loop-helix (bHLH) family protein
AT1G72210	basic helix-loop-helix (bHLH) family protein (bHLH096)
AT1G73830	BEE3 (BR ENHANCED EXPRESSION 3)
AT1G74500	bHLH family protein
AT2G14760	basic helix-loop-helix protein / bHLH protein
AT2G16910	AMS (ABORTED MICROSPORES)
AT2G18300	basic helix-loop-helix (bHLH) family protein
AT2G20180	PIL5 (PHYTOCHROME INTERACTING FACTOR 3-LIKE 5)
AT2G22750	basic helix-loop-helix (bHLH) family protein
AT2G22760	basic helix-loop-helix (bHLH) family protein
AT2G22770	NAI1; DNA binding / transcription factor
AT2G24260	basic helix-loop-helix (bHLH) family protein
AT2G28160	ATBHLH029/BHLH029/FIT1/FRU
AT2G31210	basic helix-loop-helix (bHLH) family protein
AT2G31215	basic helix-loop-helix (bHLH) family protein

TableA.1: Arabidopsis bHLH transcription factors (continued)

Gene ID	Description of gene product
AT2G31220	basic helix-loop-helix (bHLH) family protein
AT2G34820	basic helix-loop-helix (bHLH) family protein
AT2G40200	basic helix-loop-helix (bHLH) family protein
AT2G41130	basic helix-loop-helix (bHLH) family protein
AT2G41240	BHLH100; DNA binding / transcription factor
AT2G42280	basic helix-loop-helix (bHLH) family protein
AT2G42300	basic helix-loop-helix (bHLH) family protein
AT2G43010	PIF4 (PHYTOCHROME INTERACTING FACTOR 4)
AT2G43140	DNA binding / transcription factor
AT2G46510	basic helix-loop-helix (bHLH) family protein
AT2G46810	basic helix-loop-helix (bHLH) family protein
AT2G46970	PIL1 (PHYTOCHROME INTERACTING FACTOR 3-LIKE 1);
AT3G06120	MUTE (MUTE); DNA binding / transcription factor
AT3G07340	basic helix-loop-helix (bHLH) family protein
AT3G19860	basic helix-loop-helix (bHLH) family protein
AT3G21330	basic helix-loop-helix (bHLH) family protein
AT3G22100	basic helix-loop-helix (bHLH) family protein
AT3G23210	basic helix-loop-helix (bHLH) family protein
AT3G23690	basic helix-loop-helix (bHLH) family protein
AT3G24140	FMA (FAMA); DNA binding / transcription activator
AT3G25710	basic helix-loop-helix (bHLH) family protein
AT3G26744	ICE1 (INDUCER OF CBF EXPRESSION 1)
AT3G47640	basic helix-loop-helix (bHLH) family protein
AT3G50330	HEC2 (HECATE 2); DNA binding / transcription factor

TableA.1: Arabidopsis bHLH transcription factors (continued)

Gene ID	Description of gene product
AT3G56770	basic helix-loop-helix (bHLH) family protein
AT3G56970	BHLH038/ORG2 (OBP3-RESPONSIVE GENE 2)
AT3G56980	BHLH039/ORG3 (OBP3-RESPONSIVE GENE 3)
AT3G57800	basic helix-loop-helix (bHLH) family protein
AT3G59060	PIL6 (PHYTOCHROME-INTERACTING FACTOR 5)
AT3G61950	basic helix-loop-helix (bHLH) family protein
AT3G62090	PIL2 (PHYTOCHROME INTERACTING FACTOR 3-LIKE 2)
AT4G00050	UNE10 (unfertilized embryo sac 10)
AT4G00120	EDA33/IND (INDEHISCENT)
AT4G00480	ATMYC1 (Arabidopsis thaliana myc-related transcription factor 1)
AT4G00870	basic helix-loop-helix (bHLH) family protein
AT4G01460	basic helix-loop-helix (bHLH) family protein
AT4G02590	UNE12 (unfertilized embryo sac 12)
AT4G05170	DNA binding / transcription factor
AT4G09180	basic helix-loop-helix (bHLH) family protein
AT4G09820	TT8 (TRANSPARENT TESTA 8)
AT4G14410	basic helix-loop-helix (bHLH) family protein
AT4G16430	basic helix-loop-helix (bHLH) family protein
AT4G17880	basic helix-loop-helix (bHLH) family protein
AT4G20970	basic helix-loop-helix (bHLH) family protein
AT4G21330	DYT1 (DYSFUNCTIONAL TAPETUM 1)
AT4G25400	basix helix-loop-helix (bHLH) family protein
AT4G25410	basix helix-loop-helix (bHLH) family protein
AT4G28790	basic helix-loop-helix (bHLH) family protein

TableA.1: Arabidopsis bHLH transcription factors (continued)

Gene ID	Description of gene product
AT4G28800	transcription factor
AT4G28811	transcription regulator
AT4G28815	transcription regulator
AT4G29930	basic helix-loop-helix (bHLH) family protein
AT4G30980	basic helix-loop-helix (bHLH) family protein
AT4G33880	basic helix-loop-helix (bHLH) family protein
AT4G34530	basic helix-loop-helix (bHLH) family protein
AT4G36060	basic helix-loop-helix (bHLH) family protein
AT4G36540	BEE2 (BR ENHANCED EXPRESSION 2)
AT4G36930	SPT (SPATULA); DNA binding / transcription factor
AT4G37850	basic helix-loop-helix (bHLH) family protein
AT4G38070	bHLH family protein
AT5G01310	basic helix-loop-helix (bHLH) family protein
AT5G04150	BHLH101; DNA binding / transcription factor
AT5G08130	BIM1 (BES1-interacting Myc-like protein 1)
AT5G09750	HEC3 (HECATE 3); DNA binding / transcription factor
AT5G10570	basic helix-loop-helix (bHLH) family protein
AT5G37800	basic helix-loop-helix (bHLH) family protein
AT5G38860	BIM3 (BES1-INTERACTING MYC-LIKE PROTEIN 3)
AT5G41315	GL3 (GLABRA 3); transcription factor
AT5G43175	basic helix-loop-helix (bHLH) family protein
AT5G43650	basic helix-loop-helix (bHLH) family protein
AT5G46690	BHLH071 (BETA HLH PROTEIN 71)
AT5G46760	basic helix-loop-helix (bHLH) family protein

TableA.1: Arabidopsis bHLH transcription factors (continued)

Gene ID	Description of gene product
AT5G46830	basic helix-loop-helix (bHLH) family protein
AT5G48560	basic helix-loop-helix (bHLH) family protein
AT5G50915	basic helix-loop-helix (bHLH) family protein
AT5G51780	basic helix-loop-helix (bHLH) family protein
AT5G51790	basic helix-loop-helix (bHLH) family protein
AT5G53210	SPCH (SPEECHLESS)
AT5G54680	ILR3 (IAA-LEUCINE RESISTANT3)
AT5G56960	basic helix-loop-helix (bHLH) family protein
AT5G57150	basic helix-loop-helix (bHLH) family protein
AT5G58010	basic helix-loop-helix (bHLH) family protein
AT5G61270	PIF7 (PHYTOCHROME-INTERACTING FACTOR7)
AT5G62610	basic helix-loop-helix (bHLH) family protein
AT5G65320	basic helix-loop-helix (bHLH) family protein
AT5G67060	HEC1 (HECATE 1); transcription factor
AT5G67110	ALC (ALCATRAZ); DNA binding / transcription factor

Table A.2: Arabidopsis MYB transcription factors

Gene ID	Description of gene product
AT1G06180	ATMYB13 (myb domain protein 13)
AT1G08810	MYB60 (myb domain protein 60)
AT1G09540	ATMYB61/MYB61 (MYB DOMAIN PROTEIN 61)
AT1G09770	ATCDC5 (ARABIDOPSIS THALIANA HOMOLOG OF CDC5)
AT1G14350	FLP (FOUR LIPS); DNA binding / transcription factor

Table A.2: Arabidopsis MYB transcription factors (continued)

Gene ID	Description of gene product
AT1G17950	MYB52 (myb domain protein 52)
AT1G18570	MYB51 (MYB DOMAIN PROTEIN 51)
AT1G18710	AtMYB47 (myb domain protein 47)
AT1G22640	MYB3 (myb domain protein 3)
AT1G25340	MYB116 (myb domain protein 116)
AT1G26780	MYB117 (myb domain protein 117)
AT1G35515	HOS10 (HIGH RESPONSE TO OSMOTIC STRESS 10)
AT1G48000	MYB112 (myb domain protein 112)
AT1G49010	myb family transcription factor
AT1G56160	AtMYB72/MYB72 (myb domain protein 72)
AT1G56650	PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT 1)
AT1G57560	AtMYB50 (myb domain protein 50)
AT1G63910	ATMYB103 (MYB DOMAIN PROTEIN 103)
AT1G66230	MYB20 (myb domain protein 20)
AT1G66370	MYB113 (myb domain protein 113)
AT1G66380	MYB114 (myb domain protein 114)
AT1G66390	PAP2 (PRODUCTION OF ANTHOCYANIN PIGMENT 2)
AT1G68320	MYB62 (myb domain protein 62)
AT1G69560	MYB105 (myb domain protein 105)
AT1G73410	MYB54 (myb domain protein 54)
AT1G74080	MYB122 (myb domain protein 122)
AT1G74430	MYB95 (myb domain protein 95)
AT1G74650	AtMYB31/AtY13 (myb domain protein 31)
AT1G79180	AtMYB63 (myb domain protein 63)

Table A.2: Arabidopsis MYB transcription factors (continued)

Gene ID	Description of gene product
AT2G02820	MYB88 (myb domain protein 88)
AT2G16720	MYB7 (myb domain protein 7)
AT2G23290	AtMYB70 (myb domain protein 70)
AT2G25230	MYB100 (myb domain protein 100)
AT2G26950	AtMYB104 (myb domain protein 104)
AT2G26960	AtMYB81 (myb domain protein 81)
AT2G31180	AtMYB14/Myb14at (myb domain protein 14)
AT2G32460	AtM1/AtMYB101/MYB101 (myb domain protein 101)
AT2G36890	ATMYB38/MYB38/RAX2 (myb domain protein 38)
AT2G37630	AS1/ATMYB91/ATPHAN/MYB91
AT2G38090	myb family transcription factor
AT2G39880	MYB25 (myb domain protein 25)
AT2G47190	MYB2 (myb domain protein 2)
AT2G47460	ATMYB12/MYB12 (MYB DOMAIN PROTEIN 12)
AT3G01140	MYB106 (myb domain protein 106)
AT3G01530	AtMYB57 (myb domain protein 57)
AT3G02940	MYB107 (myb domain protein 107)
AT3G06490	MYB108 (MYB DOMAIN PROTEIN 108)
AT3G08500	MYB83 (myb domain protein 83)
AT3G09230	ATMYB1 (MYB DOMAIN PROTEIN 1)
AT3G09370	MYB3R-3 (myb domain protein 3R-3)
AT3G10590	myb family transcription factor
AT3G10595	myb family transcription factor
AT3G11280	myb family transcription factor

Table A.2: Arabidopsis MYB transcription factors (continued)

Gene ID	Description of gene product
AT3G11440	ATMYB65 (myb domain protein 65)
AT3G11450	DNAJ heat shock N-terminal domain-containing protein
AT3G12720	AtMYB67/AtY53 (myb domain protein 67)
AT3G12820	AtMYB10 (myb domain protein 10)
AT3G13540	ATMYB5 (MYB DOMAIN PROTEIN 5)
AT3G13890	MYB26 (myb domain protein 26)
AT3G18100	MYB4R1 (myb domain protein 4R1)
AT3G23250	AtMYB15/AtY19/MYB15 (myb domain protein 15)
AT3G24310	MYB305 (myb domain protein 305)
AT3G27785	MYB118 (myb domain protein 118)
AT3G27810	ATMYB21 (MYB DOMAIN PROTEIN 21)
AT3G27920	GL1 (GLABRA 1)
AT3G28470	AtMYB35 (myb domain protein 35)
AT3G28910	MYB30 (myb domain protein 30)
AT3G29020	MYB110 (myb domain protein 110)
AT3G30210	MYB121 (myb domain protein 121)
AT3G46130	MYB111 (myb domain protein 111)
AT3G47600	MYB94 (myb domain protein 94)
AT3G48920	AtMYB45 (myb domain protein 45)
AT3G49690	ATMYB84/MYB84/RAX3 (myb domain protein 84)
AT3G50060	MYB77; DNA binding / transcription factor
AT3G52250	DNA binding / transcription factor
AT3G53200	AtMYB27 (myb domain protein 27)
AT3G55730	MYB109 (myb domain protein 109)

Table A.2: Arabidopsis MYB transcription factors (continued)

Gene ID	Description of gene product
AT3G60460	DUO1 (DUO POLLEN 1)
AT3G61250	AtMYB17 (myb domain protein 17)
AT3G62610	AtMYB11 (myb domain protein 11)
AT4G00540	ATMYB3R2/MYB3R-2/PC-MYB2
AT4G01680	MYB55 (myb domain protein 55)
AT4G05100	AtMYB74 (myb domain protein 74)
AT4G09460	ATMYB6 (MYB DOMAIN PROTEIN 6)
AT4G13480	AtMYB79 (myb domain protein 79)
AT4G17785	MYB39 (MYB DOMAIN PROTEIN 39)
AT4G18770	MYB98 (myb domain protein 98)
AT4G21440	ATM4/ATMYB102 (ARABIDOPSIS MYB-LIKE 102)
AT4G22680	MYB85 (myb domain protein 85)
AT4G25560	AtMYB18 (myb domain protein 18)
AT4G26930	MYB97 (myb domain protein 97)
AT4G28110	AtMYB41 (myb domain protein 41)
AT4G32730	PC-MYB1 (myb domain protein 3R1)
AT4G33450	AtMYB69 (myb domain protein 69)
AT4G34990	AtMYB32 (myb domain protein 32)
AT4G37260	AtMYB73/MYB73 (myb domain protein 73)
AT4G37780	AtMYB87/MYB87 (myb domain protein 87)
AT4G38620	MYB4 (myb domain protein 4)
AT5G01200	myb family transcription factor
AT5G02320	MYB3R-5 (myb domain protein 3R-5)
AT5G04760	myb family transcription factor

Table A.2: Arabidopsis MYB transcription factors (continued)

Gene ID	Description of gene product
AT5G05790	myb family transcription factor
AT5G06100	ATMYB33/MYB33 (myb domain protein 33)
AT5G06110	DNAJ heat shock N-terminal domain-containing protein
AT5G07690	MYB29 (myb domain protein 29)
AT5G07700	MYB76 (myb domain protein 76)
AT5G08520	myb family transcription factor
AT5G10280	MYB92 (myb domain protein 92)
AT5G11050	MYB64 (myb domain protein 64)
AT5G11510	MYB3R-4
AT5G12870	AtMYB46/MYB46
AT5G14340	AtMYB40 (myb domain protein 40)
AT5G14750	ATMYB66/WER/WER1 (WEREWOLF 1)
AT5G15310	AtMIXTA/AtMYB16 (myb domain protein 16)
AT5G16600	MYB43 (myb domain protein 43)
AT5G16770	AtMYB9 (myb domain protein 9)
AT5G17800	AtMYB56 (myb domain protein 56)
AT5G23000	ATMYB37/MYB37/RAX1 (myb domain protein 37)
AT5G23650	myb family transcription factor
AT5G26660	ATMYB4 (myb domain protein 4)
AT5G35550	TT2 (TRANSPARENT TESTA 2)
AT5G39700	MYB89 (myb domain protein 89)
AT5G40330	MYB23 (myb domain protein 23)
AT5G40350	MYB24 (myb domain protein 24)
AT5G40360	MYB115 (myb domain protein 115)

Table A.2: Arabidopsis MYB transcription factors (continued)

Gene ID	Description of gene product
AT5G40430	MYB22 (myb domain protein 22)
AT5G45420	myb family transcription factor
AT5G49330	AtMYB111 (myb domain protein 111)
AT5G49620	AtMYB78 (myb domain protein 78)
AT5G52260	AtMYB19 (myb domain protein 19)
AT5G52600	AtMYB82 (myb domain protein 82)
AT5G54230	MYB49 (myb domain protein 49)
AT5G55020	MYB120 (myb domain protein 120)
AT5G56110	ATMYB103/ATMYB80/MS188/MYB103
AT5G57620	MYB36 (myb domain protein 36)
AT5G58850	MYB119 (myb domain protein 119)
AT5G58900	myb family transcription factor
AT5G60890	ATMYB34/ATR1/MYB34
AT5G62320	MYB99 (myb domain protein 99)
AT5G62470	MYB96 (myb domain protein 96)
AT5G65230	AtMYB53 (myb domain protein 53)
AT5G65790	MYB68 (myb domain protein 68)
AT5G67300	ATMYB44/ATMYBR1/MYBR1 (MYB DOMAIN PROTEIN 44)

Table A.3: Arabidopsis AP2-EREBP transcription factors

Gene ID	Description of gene product
AT1G01250	AP2 domain-containing transcription factor
AT1G03800	ATERF10/ERF10 (ERF domain protein 10)
AT1G04370	ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 14
AT1G06160	ethylene-responsive factor
AT1G12610	DDF1 (DWARF AND DELAYED FLOWERING 1)
AT1G12630	DNA binding / transcription activator/ transcription factor
AT1G12890	AP2 domain-containing transcription factor
AT1G12980	ESR1 (ENHANCER OF SHOOT REGENERATION 1)
AT1G13260	RAV1 (Related to ABI3/VP1 1)
AT1G15360	SHN1/WIN1 (SHINE1)
AT1G16060	ovule development protein
AT1G19210	AP2 domain-containing transcription factor
AT1G21910	AP2 domain-containing transcription factor family protein
AT1G22190	AP2 domain-containing transcription factor
AT1G22810	AP2 domain-containing transcription factor
AT1G22985	AP2 domain-containing transcription factor
AT1G24590	DRNL (DORNROSCHEN-LIKE)
AT1G25470	AP2 domain-containing transcription factor
AT1G25560	AP2 domain-containing transcription factor
AT1G28160	ethylene-responsive element-binding family
AT1G28360	ATERF12/ERF12 (ERF domain protein 12)
AT1G28370	ATERF11/ERF11 (ERF domain protein 11)
AT1G33760	AP2 domain-containing transcription factor
AT1G36060	AP2 domain-containing transcription factor

Table A.3: Arabidopsis AP2-EREBP transcription factors (continued)

Gene ID	Description of gene product
AT1G43160	RAP2.6 (related to AP2 6)
AT1G44830	AP2 domain-containing transcription factor TINY
AT1G46768	RAP2.1 (related to AP2 1)
AT1G49120	AP2 domain-containing transcription factor
AT1G50640	ATERF3/ERF3
AT1G50680	AP2 domain-containing transcription factor
AT1G51120	AP2 domain-containing transcription factor
AT1G51190	PLT2 (PLETHORA 2)
AT1G53170	ATERF-8/ATERF8
AT1G53910	RAP2.12; DNA binding / transcription factor
AT1G63030	DDF2 (DWARF AND DELAYED FLOWERING 2)
AT1G64380	AP2 domain-containing transcription factor
AT1G68550	AP2 domain-containing transcription factor
AT1G68840	RAV2
AT1G71130	AP2 domain-containing transcription factor
AT1G71450	AP2 domain-containing transcription factor
AT1G71520	AP2 domain-containing transcription factor
AT1G72360	ethylene-responsive element-binding protein
AT1G72570	DNA binding / transcription factor
AT1G74930	ORA47; DNA binding / transcription factor
AT1G75490	DNA binding / transcription factor
AT1G77200	AP2 domain-containing transcription factor TINY
AT1G77640	AP2 domain-containing transcription factor
AT1G78080	RAP2.4 (related to AP2 4); DNA binding / transcription factor

Table A.3: Arabidopsis AP2-EREBP transcription factors (continued)

Gene ID	Description of gene product
AT1G79700	ovule development protein
AT1G80580	ethylene-responsive element-binding family protein
AT2G20350	AP2 domain-containing transcription factor
AT2G20880	AP2 domain-containing transcription factor
AT2G22200	AP2 domain-containing transcription factor
AT2G23340	AP2 domain-containing transcription factor
AT2G25820	transcription factor
AT2G28550	RAP2.7/TOE1 (TARGET OF EAT1 1)
AT2G31230	ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 15
AT2G33710	AP2 domain-containing transcription factor family protein
AT2G35700	AP2 domain-containing transcription factor
AT2G36450	HRD (HARDY)
AT2G38340	AP2 domain-containing transcription factor
AT2G39250	SNZ (SCHNARCHZAPFEN)
AT2G40220	ABI4 (ABA INSENSITIVE 4)
AT2G40340	AP2 domain-containing transcription factor
AT2G40350	DNA binding / transcription factor
AT2G41710	ovule development protein, putative
AT2G44840	ATERF13/EREBP
AT2G44940	AP2 domain-containing transcription factor TINY
AT2G46310	CRF5 (CYTOKININ RESPONSE FACTOR 5)
AT2G47520	AP2 domain-containing transcription factor
AT3G11020	DREB2B (DRE-binding protein 2B)
AT3G14230	RAP2.2; DNA binding / transcription factor AP2

Table A.3: Arabidopsis AP2-EREBP transcription factors (continued)

Gene ID	Description of gene product
AT3G15210	ATERF-4/ATERF4/ERF4/RAP2.5
AT3G16280	DNA binding / transcription factor
AT3G16770	ATEBP/ERF72/RAP2.3 (RELATED TO AP2 3)
AT3G20310	ATERF-7/ATERF7/ERF7 (ETHYLINE RESPONSE FACTOR7)
AT3G20840	PLT1 (PLETHORA 1); transcription factor
AT3G23220	DNA binding / transcription factor
AT3G23230	ethylene-responsive factor, putative
AT3G23240	ATERF1/ERF1 (ETHYLENE RESPONSE FACTOR 1)
AT3G25730	AP2 domain-containing transcription factor
AT3G25890	AP2 domain-containing transcription factor
AT3G50260	COOPERATIVELY REGULATED BY ETHYLENE AND JASMONATE
AT3G54320	WRI1 (WRINKLED 1)
AT3G54990	SMZ (SCHLAFMUTZE)
AT3G57600	AP2 domain-containing transcription factor
AT3G60490	AP2 domain-containing transcription factor TINY
AT3G61630	CRF6 (CYTOKININ RESPONSE FACTOR 6)
AT4G06746	RAP2.9 (related to AP2 9)
AT4G11140	CRF1 (CYTOKININ RESPONSE FACTOR 1)
AT4G13040	AP2 domain-containing transcription factor family protein
AT4G13620	AP2 domain-containing transcription factor
AT4G16750	DRE-binding transcription factor
AT4G17490	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 6
AT4G17500	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 1
AT4G18450	ethylene-responsive factor

Table A.3: Arabidopsis AP2-EREBP transcription factors (continued)

Gene ID	Description of gene product
AT4G23750	CRF2 (CYTOKININ RESPONSE FACTOR 2)
AT4G25470	CBF2 (FREEZING TOLERANCE QTL 4)
AT4G25480	DREB1A (DEHYDRATION RESPONSE ELEMENT B1A)
AT4G25490	CBF1 (C-REPEAT/DRE BINDING FACTOR 1)
AT4G27950	CRF4 (CYTOKININ RESPONSE FACTOR 4)
AT4G28140	AP2 domain-containing transcription factor
AT4G31060	AP2 domain-containing transcription factor
AT4G32800	AP2 domain-containing transcription factor TINY
AT4G34410	AP2 domain-containing transcription factor
AT4G36900	RAP2.10 (related to AP2 10)
AT4G36920	AP2 (APETALA 2)
AT4G37750	ANT (AINTEGUMENTA)
AT4G39780	AP2 domain-containing transcription factor
AT5G05410	DREB2A (DRE-BINDING PROTEIN 2A)
AT5G07310	AP2 domain-containing transcription factor
AT5G07580	DNA binding / transcription factor
AT5G10510	AIL6 (AINTEGUMENTA-LIKE 6)
AT5G11190	SHN3 (SHINE3)
AT5G11590	TINY2 (TINY2)
AT5G13330	RAP2.6L (related to AP2 6L)
AT5G13910	LEP (LEAFY PETIOLE)
AT5G17430	DNA binding / transcription factor
AT5G18450	AP2 domain-containing transcription factor
AT5G18560	PUCHI; DNA binding / transcription factor

Table A.3: Arabidopsis AP2-EREBP transcription factors (continued)

Gene ID	Description of gene product
AT5G19790	RAP2.11 (related to AP2 11)
AT5G21960	AP2 domain-containing transcription factor
AT5G25190	ethylene-responsive element-binding protein
AT5G25390	SHN2 (SHINE2)
AT5G25810	TNY (TINY)
AT5G43410	ethylene-responsive factor, putative
AT5G44210	ATERF-9/ATERF9/ERF9 (ERF domain protein 9)
AT5G47220	ATERF-2/ATERF2/ERF2 (ETHYLENE RESPONSE FACTOR 2)
AT5G47230	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 5
AT5G50080	DNA binding / transcription factor
AT5G51190	AP2 domain-containing transcription factor
AT5G51990	CBF4/DREB1D (C- REPEAT-BINDING FACTOR 4)
AT5G52020	AP2 domain-containing protein
AT5G53290	CRF3 (CYTOKININ RESPONSE FACTOR 3)
AT5G57390	AIL5 (AINTEGUMENTA-LIKE 5)
AT5G60120	TOE2 (TARGET OF EAT1 2)
AT5G61590	AP2 domain-containing transcription factor family protein
AT5G61600	ethylene-responsive element-binding family protein
AT5G61890	AP2 domain-containing transcription factor family protein
AT5G64750	ABR1 (ABA REPRESSOR1); DNA binding / transcription factor
AT5G65130	AP2 domain-containing transcription factor
AT5G65510	AIL7 (AINTEGUMENTA-LIKE 7)
AT5G67000	DNA binding / transcription factor
AT5G67010	AP2 domain-containing transcription factor

Table A.4: JA up-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)>10"

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
266901_at	AT2G34600	102.20	JAZ7
253259_at	AT4G34410	93.00	a member of the ERF
259879_at	AT1G76650	78.70	calcium-binding EF hand family protein
262226_at	AT1G53885	59.42	senescence-associated protein-related
253832_at	AT4G27654	53.35	unknown protein
247431_at	AT5G62520	52.79	a protein with similarity to RCD1
261648_at	AT1G27730	46.67	related to Cys2/His2-type zinc-finger proteins
261033_at	AT1G17380	43.38	JAZ5
255937_at	AT1G12610	42.62	a member of the DREB
259866_at	AT1G76640	42.56	calmodulin-related protein
261443_at	AT1G28480	40.99	glutaredoxin family protein
256159_at	AT1G30135	37.34	JAZ8
245209_at	AT5G12340	37.05	similar to unknown protein
252368_at	AT3G48520	37.00	member of CYP94B
261892_at	AT1G80840	35.51	pathogen-induced transcription factor
250292_at	AT5G13220	35.34	JAZ10
256017_at	AT1G19180	35.25	JAZ1
258947_at	AT3G01830	34.02	calmodulin-related protein
260399_at	AT1G72520	29.00	lipoxygenase, putative
265327_at	AT2G18210	24.96	similar to unknown protein
246340_s_at	AT3G44860	22.99	farnesoic acid carboxyl-O-methyltransferase
245041_at	AT2G26530	21.24	unknown function
253643_at	AT4G29780	20.22	similar to unknown protein
253859_at	AT4G27657	20.18	similar to unknown protein

Table A.4: JA up-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)>10" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
264758_at	AT1G61340	19.66	F-box family protein
262211_at	AT1G74930	19.22	a member of the DREB
261037_at	AT1G17420	17.64	Lipoxygenase
260744_at	AT1G15010	17.49	similar to unknown protein
264217_at	AT1G60190	16.74	armadillo/beta-catenin repeat family protein
261470_at	AT1G28370	16.57	a member of the ERF
249522_at	AT5G38700	16.51	similar to unknown protein
253830_at	AT4G27652	16.46	similar to unknown protein
267411_at	AT2G34930	15.68	disease resistance family protein
251950_at	AT3G53600	15.68	zinc finger (C2H2 type) family protein
266246_at	AT2G27690	15.35	member of CYP94C
254326_at	AT4G22610	15.26	protease inhibitor/seed storage/lipid transfer protein (LTP)
266455_at	AT2G22760	14.92	basic helix-loop-helix (bHLH) family protein
259518_at	AT1G20510	14.86	OPCL1 (OPC-8:0 COA LIGASE1)
261713_at	AT1G32640	14.85	a 68 kD MYC-related transcriptional activator
245677_at	AT1G56660	13.76	similar to unknown protein
263182_at	AT1G05575	13.36	similar to unknown protein
257262_at	AT3G21890	13.29	zinc finger (B-box type) family protein
249928_at	AT5G22250	12.80	CCR4-NOT transcription complex protein
266799_at	AT2G22860	12.70	Phytosulfokine 2 precursor
251745_at	AT3G55980	12.62	zinc finger (CCCH-type) family protein
247177_at	AT5G65300	12.55	similar to Avr9/Cf-9 rapidly elicited protein 75
265618_at	AT2G25460	12.44	similar to unknown protein
247360_at	AT5G63450	12.39	member of CYP94B

Table A.4: JA up-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)>10" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
264145_at	AT1G79310	12.38	latex-abundant protein
266821_at	AT2G44840	12.36	a member of the ERF
254120_at	AT4G24570	12.34	mitochondrial substrate carrier family protein
265530_at	AT2G06050	12.16	a 12-oxophytodienoate reductase
257644_at	AT3G25780	12.09	allene oxide cyclase
264153_at	AT1G65390	12.06	ATPP2-A5; transmembrane receptor
248400_at	AT5G52020	12.03	a member of the DREB
256442_at	AT3G10930	11.87	unknown protein
266545_at	AT2G35290	11.80	similar to Os03g0170100
256763_at	AT3G16860	11.76	phytochelatin synthetase-related
264289_at	AT1G61890	11.54	MATE efflux family protein
250455_at	AT5G09980	11.48	PROPEP4 (Elicitor peptide 4 precursor)
262171_at	AT1G74950	11.42	JAZ2
262164_at	AT1G78070	11.22	WD-40 repeat family protein
248353_at	AT5G52320	11.15	member of CYP96A
257919_at	AT3G23250	10.95	Member of the R2R3 factor gene family.
264000_at	AT2G22500	10.95	mitochondrial substrate carrier family protein
258941_at	AT3G09940	10.89	MDHAR (MONODEHYDROASCORBATE REDUCTASE)
257022_at	AT3G19580	10.69	zinc finger protein
264661_at	AT1G09950	10.55	transcription factor-related
260237_at	AT1G74430	10.54	transcription factor (MYB95).
251336_at	AT3G61190	10.54	protein with a C2 domain
245250_at	AT4G17490	10.37	a member of the ERF
252114_at	AT3G51450	10.13	strictosidine synthase family protein

Table A.4: JA up-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)>10" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
250098_at	AT5G17350	10.05	similar to unknown protein
266800_at	AT2G22880	10.04	VQ motif-containing protein
260227_at	AT1G74450	10.03	similar to unknown protein

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5"

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
245692_at	AT5G04150	0.12	basic helix-loop-helix (bHLH) family protein
260070_at	AT1G73830	0.12	BEE3 (BR ENHANCED EXPRESSION 3)
265724_at	AT2G32100	0.13	ATOFP16/OFP16 (Arabidopsis thaliana ovate family protein 16)
251625_at	AT3G57260	0.14	beta 1,3-glucanase
265387_at	AT2G20670	0.14	similar to unknown protein
267389_at	AT2G44460	0.15	glycosyl hydrolase family 1 protein
258487_at	AT3G02550	0.16	LOB domain protein 41
251677_at	AT3G56980	0.16	ORG3 (OBP3-responsive gene 3)
255807_at	AT4G10270	0.16	wound-responsive family protein
247474_at	AT5G62280	0.16	similar to unknown protein
248676_at	AT5G48850	0.17	male sterility MS5 family protein
246917_at	AT5G25280	0.18	serine-rich protein-related
266070_at	AT2G18660	0.18	expansin family protein (EXPR3)
246884_at	AT5G26220	0.18	ChaC-like family protein
249752_at	AT5G24660	0.19	similar to unknown protein
253510_at	AT4G31730	0.19	glutamine dumper1
260116_at	AT1G33960	0.19	induced by avirulence gene avrRpt2 and RPS2
247540_at	AT5G61590	0.21	a member of the ERF

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
260037_at	AT1G68840	0.21	Rav2 is part of a complex
249008_at	AT5G44680	0.22	methyladenine glycosylase family protein
246001_at	AT5G20790	0.23	similar to unknown protein
254954_at	AT4G10910	0.23	unknown protein
260126_at	AT1G36370	0.24	a putative serine hydroxymethyltransferase.
257615_at	AT3G26510	0.24	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein
262170_at	AT1G74940	0.25	senescence-associated protein-related
251443_at	AT3G59940	0.25	kelch repeat-containing F-box family protein
253812_at	AT4G28240	0.25	wound-responsive protein-related
249467_at	AT5G39610	0.26	transcription factor.
266745_at	AT2G02950	0.26	a basic soluble protein
256766_at	AT3G22231	0.27	Pathogen and Circadian Controlled 1 (PCC1)
247524_at	AT5G61440	0.27	thioredoxin family protein
253207_at	AT4G34770	0.27	auxin-responsive family protein
261684_at	AT1G47400	0.28	similar to unknown protein
245696_at	AT5G04190	0.28	phytochrome kinase substrate 4
251169_at	AT3G63210	0.28	a novel zinc-finger protein
262986_at	AT1G23390	0.28	kelch repeat-containing F-box family protein
260869_at	AT1G43800	0.29	acyl-(acyl-carrier-protein) desaturase
266150_s_at	AT2G12290	0.29	similar to protein binding / zinc ion binding
260266_at	AT1G68520	0.30	zinc finger (B-box type) family protein
250582_at	AT5G07580	0.30	a member of the ERF
251017_at	AT5G02760	0.30	protein phosphatase 2C family protein

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
246522_at	AT5G15830	0.31	bZIP transcription factor family protein
252340_at	AT3G48920	0.32	Member of the R2R3 factor gene family.
260770_at	AT1G49200	0.32	zinc finger (C3HC4-type RING finger) family protein
265208_at	AT2G36690	0.32	oxidoreductase, 2OG-Fe(II) oxygenase family protein
251196_at	AT3G62950	0.32	glutaredoxin family protein
265342_at	AT2G18300	0.32	basic helix-loop-helix (bHLH) family protein
248385_at	AT5G51910	0.32	TCP family transcription factor
245136_at	AT2G45210	0.32	auxin-responsive protein-related
256598_at	AT3G30180	0.32	a cytochrome p450 enzyme
246071_at	AT5G20150	0.32	SPX (SYG1/Pho81/XPR1) domain-containing protein
266364_at	AT2G41230	0.32	similar to ARL (ARGOS-LIKE)
250828_at	AT5G05250	0.33	similar to unknown protein
260230_at	AT1G74500	0.33	bHLH family protein
248564_at	AT5G49700	0.33	DNA-binding protein-related
261768_at	AT1G15550	0.34	Involved in later steps of the gibberellic acid biosynthetic pathway.
258935_at	AT3G10120	0.34	similar to unknown protein
252417_at	AT3G47480	0.34	calcium-binding EF hand family protein
256914_at	AT3G23880	0.35	F-box family protein
257373_at	AT2G43140	0.35	DNA binding / transcription factor
258419_at	AT3G16670	0.35	similar to unknown protein
249065_at	AT5G44260	0.35	zinc finger (CCCH-type) family protein
259373_at	AT1G69160	0.35	similar to unknown protein
266578_at	AT2G23910	0.35	cinnamoyl-CoA reductase-related

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
264616_at	AT2G17740	0.35	DC1 domain-containing protein
261881_at	AT1G80760	0.35	Similar to gb:D17443 major intrinsic protein from Oryza sativa
250396_at	AT5G10970	0.36	zinc finger (C2H2 type) family protein
261177_at	AT1G04770	0.36	male sterility MS5 family protein
267238_at	AT2G44130	0.36	kelch repeat-containing F-box family protein
245362_at	AT4G17460	0.36	homeobox protein HAT1
259822_at	AT1G66230	0.36	a putative transcription factor (MYB20)
267628_at	AT2G42280	0.36	basic helix-loop-helix (bHLH) family protein
266479_at	AT2G31160	0.36	similar to unknown protein
251436_at	AT3G59900	0.36	Auxin-inducible gene ARGOS controls lateral organ size
255742_at	AT1G25560	0.37	AP2 domain-containing transcription factor
262396_at	AT1G49470	0.37	similar to unknown protein
249493_at	AT5G39080	0.37	transferase family protein
263231_at	AT1G05680	0.37	UDP-glucoronosyl/UDP-glucosyl transferase family protein
246580_at	AT1G31770	0.37	ABC transporter family protein
257062_at	AT3G18290	0.37	EMB2454 (EMBRYO DEFECTIVE 2454)
251321_at	AT3G61460	0.37	a novel ring finger protein
254056_at	AT4G25250	0.38	invertase/pectin methylesterase inhibitor family protein
254343_at	AT4G21990	0.38	a protein disulfide isomerase-like (PDIL) protein
253806_at	AT4G28270	0.39	zinc finger (C3HC4-type RING finger) family protein
248801_at	AT5G47370	0.39	homeobox-leucine zipper genes induced by auxin
263953_at	AT2G36050	0.39	ATOFP15/OFP15 (Arabidopsis thaliana ovate family protein 15)
265117_at	AT1G62500	0.39	protease inhibitor/seed storage/lipid transfer (LTP) family protein
264788_at	AT2G17880	0.39	DNAJ heat shock protein

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
266656_at	AT2G25900	0.39	putative Cys3His zinc finger protein (ATCTH) mRNA, complete
260287_at	AT1G80440	0.39	kelch repeat-containing F-box family protein
250152_at	AT5G15120	0.39	similar to unknown protein
264745_at	AT1G62180	0.39	a protein disulfide isomerase-like (PDIL) protein
253317_at	AT4G33960	0.40	similar to unknown protein
251857_at	AT3G54770	0.40	similar to RNA recognition motif (RRM)-containing protein
260077_at	AT1G73620	0.40	thaumatin-like protein
258570_at	AT3G04530	0.40	a second Arabidopsis phosphoenolpyruvate carboxylase kinase
261265_at	AT1G26800	0.40	zinc finger (C3HC4-type RING finger) family protein
250327_at	AT5G12050	0.40	similar to unknown protein
246034_at	AT5G08350	0.40	GRAM domain-containing protein / ABA-responsive protein-related
265249_at	AT2G01940	0.40	involved in an early event in shoot gravitropism
263151_at	AT1G54120	0.41	similar to unknown protein
262656_at	AT1G14200	0.41	zinc finger (C3HC4-type RING finger) family protein
261567_at	AT1G33055	0.41	unknown protein
247991_at	AT5G56320	0.41	member of Alpha-Expansin Gene Family
247585_at	AT5G60680	0.41	similar to unknown protein
265584_at	AT2G20180	0.41	A basic helix-loop-helix transcription factor
248183_at	AT5G54040	0.41	DC1 domain-containing protein
256098_at	AT1G13700	0.41	glucosamine/galactosamine-6-phosphate isomerase family protein
257467_at	AT1G31320	0.41	lateral organ boundaries domain protein 4 (LBD4)

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
266209_at	AT2G27550	0.42	a protein similar to TFL1
245090_at	AT2G40900	0.42	nodulin MtN21 family protein
256828_at	AT3G22970	0.42	similar to unknown protein
246911_at	AT5G25810	0.42	a member of the DREB
245296_at	AT4G16370	0.42	oligopeptide transporter
265837_at	AT2G14560	0.42	similar to unknown protein
248062_at	AT5G55450	0.42	protease inhibitor/seed storage/lipid transfer protein (LTP)
248270_at	AT5G53450	0.42	ORG1 (OBP3-RESPONSIVE GENE 1)
253515_at	AT4G31320	0.43	auxin-responsive protein
257421_at	AT1G12030	0.43	similar to unknown protein
265028_at	AT1G24530	0.43	transducin family protein / WD-40 repeat family protein
259365_at	AT1G13300	0.43	myb family transcription factor
253400_at	AT4G32860	0.43	similar to conserved hypothetical protein
252078_at	AT3G51740	0.43	a leucine-repeat receptor kinase
262811_at	AT1G11700	0.43	similar to unknown protein
263194_at	AT1G36060	0.43	a member of the DREB
263373_at	AT2G20515	0.43	similar to Os04g0517000
258133_at	AT3G24500	0.43	One of three genes in A. thaliana encoding multiprotein bridging factor 1
262281_at	AT1G68570	0.44	proton-dependent oligopeptide transport (POT) family protein
250891_at	AT5G04530	0.44	beta-ketoacyl-CoA synthase family protein
256626_at	AT3G20015	0.44	pepsin A; similar to aspartyl protease family protein
251400_at	AT3G60420	0.44	similar to unknown protein
247337_at	AT5G63660	0.44	LCR74/PDF2.5 (Low-molecular-weight cysteine-rich 74)

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
259723_at	AT1G60960	0.44	member of Fe(II) transporter isolog family
265048_at	AT1G52050	0.44	jacalin lectin family protein
246968_at	AT5G24870	0.44	zinc finger (C3HC4-type RING finger) family protein
246231_at	AT4G37080	0.44	similar to unknown protein
257203_at	AT3G23730	0.44	xyloglucan:xyloglucosyl transferase
255284_at	AT4G04610	0.44	a protein disulfide isomerase-like (PDIL) protein
261023_at	AT1G12200	0.45	flavin-containing monooxygenase family protein / FMO family protein
248306_at	AT5G52830	0.45	member of WRKY Transcription Factor; Group II-e
267457_at	AT2G33790	0.45	pollen Ole e 1 allergen protein containing 14.6% proline residues
253227_at	AT4G35030	0.45	protein kinase family protein
262911_s_at	AT1G07400	0.45	17.8 kDa class I heat shock protein (HSP17.8-CI)
261663_at	AT1G18330	0.45	EARLY-PHYTOCHROME-RESPONSIVE1
259632_at	AT1G56430	0.45	nicotianamine synthase
260226_at	AT1G74660	0.45	Constitutive overexpression of MIF1
266278_at	AT2G29300	0.45	tropinone reductase
263981_at	AT2G42870	0.46	similar to unknown protein
249211_at	AT5G42680	0.46	similar to unknown protein
254377_at	AT4G21650	0.46	subtilase family protein
262238_at	AT1G48300	0.46	similar to soluble diacylglycerol acyltransferase
258856_at	AT3G02040	0.46	SRG3 (SENESCENCE-RELATED GENE 3)
245925_at	AT5G28770	0.46	bZIP protein BZO2H3 mRNA, partial cds
248177_at	AT5G54630	0.46	zinc finger protein-related
254424_at	AT4G21510	0.46	F-box family protein

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
259329_at	AT3G16360	0.46	AHP4
251373_at	AT3G60530	0.47	zinc finger (GATA type) family protein
251183_at	AT3G62630	0.47	similar to calmodulin-binding protein
256099_at	AT1G13710	0.47	member of CYP78A
263402_at	AT2G04050	0.47	MATE efflux family protein
249885_at	AT5G22940	0.47	Homolog of FRA8 (AT2G28110)
267230_at	AT2G44080	0.47	ARL, a gene similar to ARGOS involved in cell expansion-dependent organ growth
250445_at	AT5G10760	0.47	aspartyl protease family protein
251174_at	AT3G63200	0.47	PLA IIIB/PLP9 (Patatin-like protein 9)
264635_at	AT1G65500	0.47	similar to unknown protein
257398_at	AT2G01990	0.47	similar to unknown protein
265160_at	AT1G31050	0.47	transcription factor
263536_at	AT2G25000	0.47	Pathogen-induced transcription factor
260030_at	AT1G68880	0.47	bZIP transcription factor family protein
259103_at	AT3G11690	0.47	similar to unknown protein
258252_at	AT3G15720	0.47	glycoside hydrolase family 28 protein
260919_at	NA	0.47	NA
258059_at	AT3G29035	0.47	a protein with transcription factor activity
263836_at	AT2G40330	0.47	Bet v I allergen family protein
246229_at	AT4G37160	0.48	SKS15 (SKU5 Similar 15)
256332_at	AT1G76890	0.48	a plant trihelix DNA-binding protein
264521_at	AT1G10020	0.48	similar to unknown protein
257964_at	AT3G19850	0.48	phototropic-responsive NPH3 family protein

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
250002_at	AT5G18690	0.48	AGP25/ATAGP25 (ARABINOGALACTAN PROTEINS 25)
254832_at	AT4G12490	0.48	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
259996_at	AT1G67910	0.48	similar to unknown protein
261845_at	AT1G15960	0.48	member of Nramp2 family
254631_at	AT4G18610	0.48	similar to unknown protein
266946_at	AT2G18890	0.48	kinase; similar to ATP binding
257080_at	AT3G15240	0.48	similar to unknown protein
253483_at	AT4G31910	0.48	transferase family protein
258091_at	AT3G14560	0.49	unknown protein
249869_at	AT5G23050	0.49	acyl-activating enzyme 17 (AAE17)
246932_at	AT5G25190	0.49	a member of the ERF
255417_at	AT4G03190	0.49	an F box protein
256097_at	AT1G13670	0.49	similar to unknown protein
259507_at	AT1G43910	0.49	AAA-type ATPase family protein
256262_at	AT3G12150	0.49	similar to unnamed protein product
258493_at	AT3G02555	0.49	similar to unknown protein
249823_s_at	AT5G23350	0.49	GRAM domain-containing protein / ABA-responsive protein-related
251705_at	AT3G56400	0.49	member of WRKY Transcription Factor (WRKY 70)
259561_at	AT1G21250	0.49	cell wall-associated kinase
261070_at	AT1G07390	0.49	protein binding
258468_at	AT3G06070	0.49	similar to unknown protein
246275_at	AT4G36540	0.49	BEE2 (BR ENHANCED EXPRESSION 2)

Table A.5: JA down-regulated genes sorted by the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	Description of gene product
247134_at	AT5G66230	0.49	similar to unknown protein
263852_at	AT2G04450	0.49	ATNUDT6 (Arabidopsis thaliana Nudix hydrolase homolog 6)
267557_at	AT2G32710	0.49	Kip-related protein (KRP) gene
250083_at	AT5G17220	0.49	glutathione transferase
253340_s_at	AT4G33260	0.49	putative cdc20 protein (CDC20.2)

Table A.6: MYC2-dependent and JA up-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5"

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
259866_at	AT1G76640	42.56	0.22	calmodulin-related protein
265327_at	AT2G18210	24.96	0.36	similar to unknown protein
246340_s_at	AT3G44860	22.99	0.18	a farnesoic acid carboxyl-O-methyltransferase.
254326_at	AT4G22610	15.26	0.32	protease inhibitor/seed storage/lipid transfer protein
266799_at	AT2G22860	12.70	0.50	Phytosulfokine 2 precursor
264145_at	AT1G79310	12.38	0.44	latex-abundant protein, putative (AMC4)
250455_at	AT5G09980	11.48	0.28	PROPEP4 (Elicitor peptide 4 precursor)
258941_at	AT3G09940	10.89	0.40	MONODEHYDROASCORBATE REDUCTASE
254385_s_at	AT4G21830	9.95	0.48	methionine sulfoxide reductase domain-containing protein
266271_at	AT2G29440	9.69	0.40	glutathione transferase
249971_at	AT5G19110	9.16	0.37	extracellular dermal glycoprotein-related
252265_at	AT3G49620	6.69	0.19	similar to 2-oxoacid-dependent dioxygenase.
253060_at	AT4G37710	6.53	0.21	VQ motif-containing protein
259432_at	AT1G01520	5.53	0.49	myb family transcription factor
261450_s_at	AT1G21110	5.42	0.48	O-methyltransferase
255795_at	AT2G33380	4.52	0.27	a calcium binding protein
266142_at	AT2G39030	4.32	0.35	GCN5-related N-acetyltransferase (GNAT) family protein
256376_s_at	AT1G66690	4.27	0.23	S-adenosyl-L-methionine:carboxyl methyltransferase protein
247723_at	AT5G59220	4.16	0.32	protein phosphatase 2C, putative / PP2C
251770_at	AT3G55970	3.35	0.28	oxidoreductase, 2OG-Fe(II) oxygenase family protein
262516_at	AT1G17190	3.30	0.30	glutathione transferase
245928_s_at	AT5G24770	3.25	0.43	protein with acid phosphatase activity
250648_at	AT5G06760	3.07	0.49	late embryogenesis abundant protein

Table A.6: MYC2-dependent and JA up-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
266270_at	AT2G29470	3.04	0.40	glutathione transferase
253070_at	AT4G37850	2.94	0.36	basic helix-loop-helix (bHLH) family protein
245550_at	AT4G15330	2.72	0.44	a member of the cytochrome P450 family
264647_at	AT1G09090	2.67	0.37	RESPIRATORY BURST OXIDASE HOMOLOG B
263539_at	AT2G24850	2.63	0.18	a tyrosine aminotransferase
260551_at	AT2G43510	2.45	0.45	Member of the defensin-like (DEFL) family
247718_at	AT5G59310	2.39	0.43	a member of the lipid transfer protein family
253684_at	AT4G29690	2.33	0.30	type I phosphodiesterase/nucleotide pyrophosphatase protein
248727_at	AT5G47990	2.21	0.35	member of CYP705A
249675_at	AT5G35940	2.20	0.26	jacalin lectin family protein
245713_at	AT5G04370	2.16	0.48	A member of the Arabidopsis SABATH methyltransferase
259878_at	AT1G76790	2.14	0.49	O-methyltransferase family 2 protein
260727_at	AT1G48100	2.10	0.49	glycoside hydrolase family 28 protein
249202_at	AT5G42580	2.01	0.47	a member of the cytochrome P450 family
246481_s_at	AT5G15960	1.99	0.48	cold and ABA inducible protein kin1
267459_at	AT2G33850	1.99	0.07	similar to unknown protein
263480_at	AT2G04032	1.95	0.27	ZIP7 (ZINC TRANSPORTER 7 PRECURSOR)
266503_at	AT2G47780	1.88	0.29	rubber elongation factor (REF) protein-related
257365_x_at	AT2G26020	1.87	0.41	PDF1.2b (plant defensin 1.2b)
264774_at	AT1G22890	1.87	0.38	similar to unknown protein
249814_at	AT5G23840	1.86	0.45	MD-2-related lipid recognition domain-containing protein
249812_at	AT5G23830	1.80	0.48	MD-2-related lipid recognition domain-containing protein
264160_at	AT1G65450	1.80	0.15	transferase family protein

Table A.6: MYC2-dependent and JA up-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
260166_at	AT1G79840	1.77	0.32	Glabra 2, a homeodomain protein
256878_at	AT3G26460	1.76	0.49	major latex protein-related
265058_s_at	AT1G52030	1.74	0.30	Similar to myrosinase binding proteins
264147_at	AT1G02205	1.74	0.38	Expression of the CER1 gene
265053_at	AT1G52000	1.74	0.43	jacalin lectin family protein
249408_at	AT5G40330	1.70	0.25	a MYB transcription factor
245319_at	AT4G16146	1.68	0.49	similar to unknown protein
252437_at	AT3G47380	1.67	0.46	invertase/pectin methylesterase inhibitor family protein
251420_at	AT3G60490	1.64	0.37	a member of the DREB
256601_s_at	AT3G28290	1.63	0.48	AT14A-like
246687_at	AT5G33370	1.62	0.12	GDSL-motif lipase/hydrolase family protein
263098_at	AT2G16005	1.62	0.20	MD-2-related lipid recognition domain-containing protein
248729_at	AT5G48010	1.61	0.45	an oxidosqualene cyclase
251509_at	AT3G59010	1.58	0.49	pectinesterase family protein
262128_at	AT1G52690	1.52	0.35	late embryogenesis abundant protein
259429_at	AT1G01600	1.51	0.35	a member of the CYP86A
264146_at	AT1G02205	1.49	0.12	Expression of the CER1 gene
260948_at	AT1G06100	1.49	0.32	fatty acid desaturase family protein

Table A.7: MYC2-dependent and JA down-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)<0.5"

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
250083_at	AT5G17220	0.49	0.46	glutathione transferase

Table A.8: MYC2-repressed and JA up-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5"

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
258947_at	AT3G01830	34.02	1.54	calmodulin-related protein
253643_at	AT4G29780	20.22	1.81	similar to unknown protein
249522_at	AT5G38700	16.51	1.79	similar to unknown protein
266821_at	AT2G44840	12.36	1.78	a member of the ERF
254120_at	AT4G24570	12.34	2.13	mitochondrial substrate carrier family protein
251336_at	AT3G61190	10.54	1.53	a protein with a C2 domain
245250_at	AT4G17490	10.37	1.67	a member of the ERF
258606_at	AT3G02840	9.54	1.75	immediate-early fungal elicitor family protein
254158_at	AT4G24380	9.26	1.58	hydrolase, acting on ester bonds
264617_at	AT2G17660	9.03	1.72	nitrate-responsive NOI protein
262382_at	AT1G72920	7.56	1.97	disease resistance protein (TIR-NBS class)
249197_at	AT5G42380	7.32	1.68	calmodulin-related protein
267028_at	AT2G38470	7.29	1.64	Member of the plant WRKY transcription factor
256356_s_at	AT1G66500	6.18	1.53	zinc finger (C2H2-type) family protein
248964_at	AT5G45340	5.81	1.75	a protein with ABA 8'-hydroxylase activity
262383_at	AT1G72940	5.56	2.22	disease resistance protein (TIR-NBS class)

Table A.8: MYC2-repressed and JA up-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
252679_at	AT3G44260	5.46	1.71	CCR4-NOT transcription complex protein
252131_at	AT3G50930	4.86	2.78	AAA-type ATPase family protein
265670_s_at	AT2G32190	4.78	1.75	similar to unknown protein
256526_at	AT1G66090	4.74	2.15	disease resistance protein (TIR-NBS class)
248611_at	AT5G49520	4.65	1.51	member of WRKY Transcription Factor
252908_at	AT4G39670	4.44	1.51	ACCELERATED CELL DEATH 11
245119_at	AT2G41640	4.43	1.55	similar to unknown protein
265723_at	AT2G32140	4.38	1.94	transmembrane receptor
254231_at	AT4G23810	4.05	1.51	member of WRKY Transcription Factor
265725_at	AT2G32030	4.05	1.68	GCN5-related N-acetyltransferase (GNAT) protein
257840_at	AT3G25250	3.91	1.85	Arabidopsis protein kinase
266010_at	AT2G37430	3.86	1.56	zinc finger (C2H2 type) family protein (ZAT11)
263931_at	AT2G36220	3.51	1.50	similar to unknown protein
263783_at	AT2G46400	3.40	2.34	member of WRKY Transcription Factor
256633_at	AT3G28340	3.24	1.76	protein with galacturonosyltransferase activity
253915_at	AT4G27280	3.12	1.65	calcium-binding EF hand family protein
245777_at	AT1G73540	2.90	1.51	Arabidopsis thaliana Nudix hydrolase homolog 21
248448_at	AT5G51190	2.88	2.37	a member of the ERF
260211_at	AT1G74440	2.82	2.00	similar to unknown protein
265732_at	AT2G01300	2.71	1.59	similar to unknown protein
247240_at	AT5G64660	2.64	1.74	U-box domain-containing protein
251774_at	AT3G55840	2.51	1.54	similar to unknown protein

Table A.8: MYC2-repressed and JA up-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
248799_at	AT5G47230	2.51	1.86	a member of the ERF
245840_at	AT1G58420	2.50	1.67	similar to unknown protein
263379_at	AT2G40140	2.50	1.77	CZF1/ZFAR1
257999_at	AT3G27540	2.46	1.54	glycosyl transferase family 17 protein
255654_at	AT4G00970	2.36	1.59	protein kinase family protein
258537_at	AT3G04210	2.36	2.17	disease resistance protein (TIR-NBS class)
253502_at	AT4G31940	2.23	1.71	member of CYP82C
251507_at	AT3G59080	2.23	1.53	similar to aspartyl protease family protein
257925_at	AT3G23170	2.18	1.93	similar to ATBET12
256185_at	AT1G51700	2.08	1.63	dof zinc finger protein (adof1).
257918_at	AT3G23230	2.07	2.27	a member of the ERF
253140_at	AT4G35480	2.03	1.55	a putative RING-H2 finger protein RHA3b.
265668_at	AT2G32020	1.98	1.61	GCN5-related N-acetyltransferase (GNAT) protein
262085_at	AT1G56060	1.92	1.60	similar to unknown protein
261984_at	AT1G33760	1.81	2.61	a member of the DREB
259325_at	AT3G05320	1.81	1.58	similar to protein-O-fucosyltransferase 1
264866_at	AT1G24140	1.75	1.64	matrixin family protein
249264_s_at	AT5G41740	1.69	2.15	disease resistance protein (TIR-NBS-LRR class)
248164_at	AT5G54490	1.67	2.18	a PINOID (PID)-binding protein
258436_at	AT3G16720	1.66	2.32	RING-H2 protein
256576_at	AT3G28210	1.61	1.95	putative zinc finger protein (PMZ)
254408_at	AT4G21390	1.61	1.60	B120; protein kinase/ sugar binding
257697_at	AT3G12700	1.57	1.70	aspartyl protease family protein

Table A.8: MYC2-repressed and JA up-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
257751_at	AT3G18690	1.56	1.51	a nuclear-localized
259992_at	AT1G67970	1.55	1.63	member of Heat Stress Transcription Factor (Hsf)
248191_at	AT5G54130	1.54	1.54	calcium ion binding
260023_at	AT1G30040	1.54	1.57	gibberellin 2-oxidase
249139_at	AT5G43170	1.53	1.60	zinc finger protein
255532_at	AT4G02170	1.52	1.55	similar to unknown protein
247729_at	AT5G59530	1.51	1.52	2-oxoglutarate-dependent dioxygenase

Table A.9: MYC2-repressed and JA down-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)<0.5"

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
251625_at	AT3G57260	0.14	3.25	beta 1,3-glucanase
266070_at	AT2G18660	0.18	1.97	expansin family protein (EXPR3)
260116_at	AT1G33960	0.19	1.82	induced by avirulence gene avrRpt2 and RPS2
256766_at	AT3G22231	0.27	2.72	Pathogen and Circadian Controlled 1 (PCC1)
263231_at	AT1G05680	0.37	1.56	UDP-glucoronosyl/UDP-glucosyl transferase family protein
265117_at	AT1G62500	0.39	1.52	protease inhibitor/seed storage/lipid transfer protein (LTP)
266209_at	AT2G27550	0.42	1.52	a protein similar to TFL1
252078_at	AT3G51740	0.43	1.62	a leucine-repeat receptor kinase
258133_at	AT3G24500	0.43	1.53	A. thaliana encoding multiprotein bridging factor 1

Table A.9: MYC2-repressed and JA down-regulated genes are listed with the ratio of "(jin1-1+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)<0.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jin1MJ/wtMJ	Description of gene product
251400_at	AT3G60420	0.44	2.14	similar to unknown protein
259723_at	AT1G60960	0.44	1.59	member of Fe(II) transporter isolog family
250445_at	AT5G10760	0.47	1.92	aspartyl protease family protein
264635_at	AT1G65500	0.47	1.89	similar to unknown protein
254832_at	AT4G12490	0.48	2.38	protease inhibitor/seed storage/lipid transfer protein (LTP)
251705_at	AT3G56400	0.49	2.39	member of WRKY Transcription Factor (WRKY 70)

Table A.10: JUT-dependent and JA up-regulated genes are listed with the ratio of "(jut+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5"

Affy No.	Gene ID	wtMJ/wtmock	jutMJ/wtMJ	Description of gene product
253859_at	AT4G27657	20.18	0.41	similar to unknown protein
257280_at	AT3G14440	8.35	0.39	9-cis-epoxycarotenoid dioxygenase
252265_at	AT3G49620	6.69	0.23	similar to 2-oxoacid-dependent dioxygenase.
253060_at	AT4G37710	6.53	0.44	VQ motif-containing protein
261450_s_at	AT1G21110	5.42	0.36	O-methyltransferase, putative
256376_s_at	AT1G66690	4.27	0.26	S-adenosyl-L-methionine:carboxyl methyltransferase
256324_at	AT1G66760	3.48	0.43	MATE efflux family protein
266270_at	AT2G29470	3.04	0.44	glutathione transferase belonging to the tau class of GSTs
263948_at	AT2G35980	2.54	0.50	similar to tobacco hairpin-induced gene (HIN1)
260551_at	AT2G43510	2.45	0.49	Member of the defensin-like (DEFL) family
249675_at	AT5G35940	2.20	0.28	jacalin lectin family protein
260727_at	AT1G48100	2.10	0.48	glycoside hydrolase family 28 protein

Table A.10: JUT-dependent and JA up-regulated genes are listed with the ratio of "(jut+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jutMJ/wtMJ	Description of gene product
250541_at	AT5G09520	2.00	0.41	hydroxyproline-rich glycoprotein family protein
257365_x_at	AT2G26020	1.87	0.32	PDF1.2b (plant defensin 1.2b)
250500_at	AT5G09530	1.81	0.40	hydroxyproline-rich glycoprotein family protein
249205_at	AT5G42600	1.76	0.48	MRN (MARNERAL SYNTHASE)
250474_at	AT5G10230	1.71	0.40	a calcium-binding protein annexin (AnnAt7)
261021_at	AT1G26380	1.68	0.49	FAD-binding domain-containing protein
250199_at	AT5G14180	1.53	0.44	lipase family protein
252381_s_at	AT3G47750	1.51	0.48	member of ATH subfamily

Table A.11: JUT-dependented and JA down-regulated genes are listed with the ratio of "(jut+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)<0.5"

Affy No.	Gene ID	wtMJ/wtmock	jutMJ/wtMJ	Description of gene product
266393_at	AT2G41260	0.45	0.39	Late-embryogenesis-abundant gene

Table A.12: JUT-repressed and JA up-regulated genes are listed with the ratio of "(jut+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5"

Affy No.	Gene ID	wtMJ/wtmock	jutMJ/wtMJ	Description of gene product
256159_at	AT1G30135	37.34	1.61	similar to unknown protein
248400_at	AT5G52020	12.03	1.78	a member of the DREB
248964_at	AT5G45340	5.81	1.56	a protein with ABA 8'-hydroxylase activity
262383_at	AT1G72940	5.56	1.56	disease resistance protein (TIR-NBS class)
262381_at	AT1G72900	4.33	1.71	disease resistance protein (TIR-NBS class)

Table A.12: JUT-repressed and JA up-regulated genes are listed with the ratio of "(jut+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jutMJ/wtMJ	Description of gene product
266142_at	AT2G39030	4.32	2.07	GCN5-related N-acetyltransferase (GNAT) family protein
263497_at	AT2G42540	4.25	1.56	A cold-regulated gene
245567_at	AT4G14630	3.44	2.51	germin-like protein
263783_at	AT2G46400	3.40	1.96	member of WRKY Transcription Factor
262698_at	AT1G75960	3.13	1.51	AMP-binding protein, putative
245777_at	AT1G73540	2.90	1.60	AArabidopsis thaliana Nudix hydrolase homolog 21
248448_at	AT5G51190	2.88	1.58	a member of the ERF
261020_at	AT1G26390	2.68	1.53	FAD-binding domain-containing protein
263539_at	AT2G24850	2.63	2.79	Encodes a tyrosine aminotransferase
258537_at	AT3G04210	2.36	2.10	disease resistance protein (TIR-NBS class)
253502_at	AT4G31940	2.23	2.03	member of CYP82C
254550_at	AT4G19690	2.16	2.02	Fe(II) transport protein (IRT1)
253301_at	AT4G33720	2.04	3.52	pathogenesis-related protein, putative
254232_at	AT4G23600	2.04	1.87	cystine lyase
267409_at	AT2G34910	1.97	1.56	similar to unknown protein
263480_at	AT2G04032	1.95	1.62	ZIP7 (ZINC TRANSPORTER 7 PRECURSOR)
247778_at	AT5G58750	1.82	1.56	wound-responsive protein-related
261984_at	AT1G33760	1.81	2.10	a member of the DREB

Table A.12: JUT-repressed and JA up-regulated genes are listed with the ratio of "(jut+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	jutMJ/wtMJ	Description of gene product
249408_at	AT5G40330	1.70	1.62	a MYB transcription factor
249264_s_at	AT5G41740	1.69	1.92	disease resistance protein (TIR-NBS-LRR class)
255923_at	AT1G22180	1.65	1.50	SEC14 cytosolic factor family protein
256576_at	AT3G28210	1.61	1.90	a putative zinc finger protein (PMZ)
257697_at	AT3G12700	1.57	1.60	aspartyl protease family protein
259992_at	AT1G67970	1.55	1.61	member of Heat Stress Transcription Factor (Hsf) family
262128_at	AT1G52690	1.52	3.37	late embryogenesis abundant protein

Table A.13: JUT-repressed and JA down-regulated genes are listed with the ratio of "(jut+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)<0.5"

Affy No.	Gene ID	wtMJ/wtmock	jutMJ/wtMJ	Description of gene product
245692_at	AT5G04150	0.12	1.72	basic helix-loop-helix (bHLH) family protein
265724_at	AT2G32100	0.13	1.86	ATOFP16/OFP16
251625_at	AT3G57260	0.14	6.69	beta 1,3-glucanase
251677_at	AT3G56980	0.16	1.89	ORG3 (OBP3-responsive gene 3)
266070_at	AT2G18660	0.18	4.10	expansin family protein (EXPR3)
260116_at	AT1G33960	0.19	2.20	induced by avirulence gene avrRpt2 and RPS2
246001_at	AT5G20790	0.23	1.56	similar to unknown protein
256766_at	AT3G22231	0.27	13.25	Pathogen and circadian controlled 1 (PCC1)
261684_at	AT1G47400	0.28	1.90	similar to unknown protein
250582_at	AT5G07580	0.30	1.60	a member of the ERF
265342_at	AT2G18300	0.32	1.62	basic helix-loop-helix (bHLH) family protein
260230_at	AT1G74500	0.33	1.55	bHLH family protein
252417_at	AT3G47480	0.34	1.72	calcium-binding EF hand family protein
254056_at	AT4G25250	0.38	1.52	invertase/pectin methylesterase inhibitor
266209_at	AT2G27550	0.42	1.58	a protein similar to TFL1
265837_at	AT2G14560	0.42	2.08	similar to unknown protein
251400_at	AT3G60420	0.44	2.73	similar to unknown protein
265048_at	AT1G52050	0.44	1.85	jacalin lectin family protein
264635_at	AT1G65500	0.47	1.55	similar to unknown protein
260919_at	NA	0.47	2.63	NA
256097_at	AT1G13670	0.49	1.51	similar to unknown protein
251705_at	AT3G56400	0.49	2.61	member of WRKY Transcription Factor (WRKY 70)
259561_at	AT1G21250	0.49	3.05	cell wall-associated kinase

Table A.14: JA up-regulated genes that are dependent by both MYC2 and JUT are listed with the ratio of "(DM+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5"

Affy No.	Gene ID	wtMJ/wtmock	DMMJ/wtMJ	Description of gene product
259866_at	AT1G76640	42.56	0.25	calmodulin-related protein
265327_at	AT2G18210	24.96	0.38	similar to unknown protein
246340_s_at	AT3G44860	22.99	0.34	a farnesoic acid carboxyl-O-methyltransferase
250455_at	AT5G09980	11.48	0.40	Elicitor peptide 4 precursor
252265_at	AT3G49620	6.69	0.16	similar to 2-oxoacid-dependent dioxygenase
253060_at	AT4G37710	6.53	0.23	VQ motif-containing protein
254075_at	AT4G25470	4.79	0.35	a member of the DREB
266142_at	AT2G39030	4.32	0.38	GCN5-related N-acetyltransferase (GNAT)
256376_s_at	AT1G66690	4.27	0.25	S-adenosyl-L-methionine:carboxyl methyltransferase
262516_at	AT1G17190	3.30	0.42	glutathione transferase
266270_at	AT2G29470	3.04	0.41	glutathione transferase
253070_at	AT4G37850	2.94	0.44	basic helix-loop-helix (bHLH) family protein
245550_at	AT4G15330	2.72	0.49	a member of the cytochrome P450 family
264647_at	AT1G09090	2.67	0.48	RESPIRATORY BURST OXIDASE HOMOLOG B
263539_at	AT2G24850	2.63	0.30	a tyrosine aminotransferase
260551_at	AT2G43510	2.45	0.46	Member of the defensin-like (DEFL) family.
260408_at	AT1G69880	2.32	0.24	ATH8 (thioredoxin H-type 8)
249675_at	AT5G35940	2.20	0.23	jacalin lectin family protein
266292_at	AT2G29350	2.17	0.39	a short-chain alcohol dehydrogenase
245033_at	AT2G26380	2.17	0.49	disease resistance protein-related / LRR protein-related
249202_at	AT5G42580	2.01	0.45	a member of the cytochrome P450 family
257365_x_at	AT2G26020	1.87	0.45	PDF1.2b (plant defensin 1.2b)
262259_s_at	AT1G53870	1.78	0.42	similar to unknown protein

Table A.14: JA up-regulated genes that are dependent by both MYC2 and JUT are listed with the ratio of "(DM+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5" (continued)

Affy No.	Gene ID	wtMJ/wtmock	DMMJ/wtMJ	Description of gene product
258277_at	AT3G26830	1.70	0.43	a cytochrome P450 enzyme
250199_at	AT5G14180	1.53	0.48	lipase family protein

Table A.15: JA down-regulated genes that are dependent by both MYC2 and JUT are listed with the ratio of "(DM+MJ)/(wt+MJ)<0.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)<0.5"

Affy No.	Gene ID	wtMJ/wtmock	DMMJ/wtMJ	Description of gene product
251017_at	AT5G02760	0.30	0.49	protein phosphatase 2C family protein
266364_at	AT2G41230	0.32	0.47	similar to ARL (ARGOS-LIKE)

Table A.16: JA up-regulated genes that are repressed by both MYC2 and JUT are listed with the ratio of "(DM+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1.5"

Affy No.	Gene ID	wtMJ/wtmock	DMMJ/wtMJ	Description of gene product
248353_at	AT5G52320	11.15	1.63	member of CYP96A
261431_at	AT1G18710	9.44	1.82	member of the R2R3 factor gene family
245711_at	AT5G04340	5.30	1.55	putative c2h2 zinc finger transcription factor
247478_at	AT5G62360	4.50	1.60	invertase/pectin methylesterase inhibitor
255604_at	AT4G01080	4.28	2.33	similar to unknown protein
263497_at	AT2G42540	4.25	1.91	A cold-regulated gene
247723_at	AT5G59220	4.16	1.77	protein phosphatase 2C
245628_at	AT1G56650	4.06	1.67	MYB domain containing transcription factor
256489_at	AT1G31550	3.89	1.54	GDSL-motif lipase
250648_at	AT5G06760	3.07	1.69	late embryogenesis abundant protein
256529_at	AT1G33260	2.80	1.57	protein kinase family protein
255132_at	AT4G08170	2.32	1.53	inositol 1,3,4-trisphosphate 5/6-kinase
267010_at	AT2G39250	2.23	1.55	a AP2 domain transcription factor
253502_at	AT4G31940	2.23	1.95	member of CYP82C
251054_at	AT5G01540	2.23	1.57	lectin protein kinase
254550_at	AT4G19690	2.16	1.86	Fe(II) transport protein (IRT1)
249191_at	AT5G42760	2.14	1.62	similar to hypothetical protein
253301_at	AT4G33720	2.04	2.07	pathogenesis-related protein
254232_at	AT4G23600	2.04	1.80	cystine lyase
256577_at	AT3G28220	1.98	1.77	meprin and TRAF homology domain protein
261449_at	AT1G21120	1.97	1.55	O-methyltransferase, putative
245627_at	AT1G56600	1.87	1.79	GALACTINOL SYNTHASE 2
251725_at	AT3G56260	1.83	1.92	similar to unknown protein

Table A.16: JA up-regulated genes that are repressed by both MYC2 and JUT are listed with the ratio of "(DM+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)>1" (continued)

Affy No.	Gene ID	wtMJ/wtmock	DMMJ/wtMJ	Description of gene product
251811_at	AT3G54990	1.82	1.54	a AP2 domain transcription factor
264866_at	AT1G24140	1.75	1.62	matrixin family protein
265058_s_at	AT1G52030	1.74	1.52	Similar to myrosinase binding proteins
264147_at	AT1G02205	1.74	1.86	expression of the CER1 gene
265053_at	AT1G52000	1.74	1.89	jacalin lectin family protein
252437_at	AT3G47380	1.67	1.71	invertase/pectin methylesterase inhibitor
267588_at	AT2G42060	1.60	1.56	CHP-rich zinc finger protein
248190_at	AT5G54130	1.59	1.69	calcium ion binding
259526_at	AT1G12570	1.55	1.51	glucose-methanol-choline (GMC) oxidoreductase
258003_at	AT3G29030	1.55	1.51	an expansin.
248191_at	AT5G54130	1.54	1.78	calcium ion binding
262128_at	AT1G52690	1.52	3.27	late embryogenesis abundant protein
249770_at	AT5G24110	1.51	1.64	member of WRKY Transcription Factor; Group III
258901_at	AT3G05640	1.51	1.66	protein phosphatase 2C

Table A.17: JA down-regulated genes that are repressed by both MYC2&JUT are listed with the ratio of "(DM+MJ)/(wt+MJ)>1.5", and then sorted with the ratio of "(wt+MJ)/(wtmock)<0.5"

Affy No.	Gene ID	wtMJ/wtmock	DMMJ/wtMJ	Description of gene product
245692_at	AT5G04150	0.12	1.69	basic helix-loop-helix (bHLH) family protein
265724_at	AT2G32100	0.13	1.63	Arabidopsis thaliana ovate family protein 16
251677_at	AT3G56980	0.16	1.73	ORG3 (OBP3-responsive gene 3)
246884_at	AT5G26220	0.18	1.60	ChaC-like family protein
256766_at	AT3G22231	0.27	3.09	Pathogen and circadian controlled 1 (PCC1)
261684_at	AT1G47400	0.28	2.35	similar to unknown protein
264745_at	AT1G62180	0.39	1.55	a protein disulfide isomerase-like (PDIL)
245296_at	AT4G16370	0.42	1.53	oligopeptide transporter
248270_at	AT5G53450	0.42	1.69	ORG1 (OBP3-RESPONSIVE GENE 1)
258133_at	AT3G24500	0.43	1.86	multiprotein bridging factor 1
262281_at	AT1G68570	0.44	1.52	proton-dependent oligopeptide transport (POT)
251705_at	AT3G56400	0.49	1.75	member of WRKY Transcription Factor (WRKY70)

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